Weathering Pathways and Protocols for Environmentally Relevant Microplastics and Nanoplastics: What Are We Missing?

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Abstract

To date, most studies of microplastics have been carried out with pristine particles. However, most plastics in the environment will be aged to some extent; hence, understanding the effects of weathering and accurately mimicking weathering processes are crucial. By using microplastics that lack environmental relevance, we are unable to fully assess the risks associated with microplastic pollution in the environment. Emerging studies advocate for harmonization of experimental methods, however, the subject of reliable weathering protocols for realistic assessment has not been addressed. In this work, we critically analysed the current knowledge regarding protocols used for generating environmentally relevant microplastics and leachates for effects studies. We present the expected and overlooked weathering pathways that plastics will undergo throughout their lifecycle. International standard weathering protocols developed for polymers were critically analysed for their appropriateness for use in microplastics research. We show that most studies using weathered microplastics involve sorption experiments followed by toxicity assays. The most frequently reported weathered plastic types in the literature are polystyrene>polyethylene>polypropylene>polyvinyl chloride, which does not reflect the global plastic production and plastic types detected globally. Only ~10% of published effect studies have used aged microplastics and of these, only 12 use aged nanoplastics. This highlights the need to embrace the use of environmentally relevant microplastics and to pay critical attention to the appropriateness of the weathering methods adopted moving forward. We advocate for quality reporting of weathering protocols and characterisation for harmonization and reproducibility across different research efforts.

Keywords: risk assessment, aging, leachate, ecotoxicity, ASTM standard, quality criteria

1.0 Introduction

Plastic pollution in the environment has received considerable attention over the last decade. The projected rate of global plastic production has been estimated to outweigh current and predicted future efforts aimed at reducing plastic pollution (Borrelle et al., 2020) and plastic debris already accumulated in the environment are persistent. Hence the environmental impacts of plastics may not decrease for the next decade even with new legislation and initiatives. The smaller fragments, known as microplastics and nanoplastics are even more worrisome due to their reported and potential adverse effects (Carbery et al., 2018; Gigault et al., 2021; Jeong et al., 2017). Microplastics form as a result of fragmentation of bulk plastics due to environmental weathering, referred to as secondary microplastics, or are intentionally manufactured, known as primary microplastics (Andrady, 2011; Cole et al., 2011). A vast majority of plastics in the environment are of secondary origin, while between 15–31% of plastics in the environment is estimated to be primary (Boucher and Friot, 2017).

Although some microplastics will be pristine (as manufactured) at the point of release into the environment, those that come from water or wastewater treatment plants would have undergone some degree of weathering before release into the environment. Even though these processes can remove up to 95% of microplastics, the biosolids streams (i.e., dewatered or stabilized sludge) of these facilities can still end up in the environment (via land application). Hence, the contribution of the pathways occurring in these systems to the physicochemical changes of plastics during their lifecycle should not be ignored.

Plastics may undergo various physical, chemical and biological transformations, before release into the environment, some of which lead to the production of micro- or nanoplastics. These include: hydrolysis (Sarno et al., 2020), photooxidation (Cai et al., 2018b), chemical oxidation (Bianchetti et al., 2015), natural organic matter (NOM) adsorption/attachment and flocculant aggregation (Lapointe et al., 2020; Lowry et al., 2012), etc. Upon release into the natural environment, plastic particles may further undergo photodegradation, hydrolysis, chemical oxidation, biodegradation, mechanical stress, *etc.* (Alimi et al., 2018; Andrady, 2011; Shah et al., 2008). Plastics will encounter one or more

of these weathering pathways during their lifecycle either simultaneously or sequentially, however, most microplastics studies only explore a few of these processes in isolation when mimicking environmentally relevant systems. To mimic microplastics that are representative of those found in the environment, weathering studies must consider the processes that occur both before and after release into the environment.

The time it takes to observe noticeable physicochemical changes in weathered plastics may range from a few weeks to several years (Chamas et al., 2020), hence the weathering process is commonly accelerated in the laboratory. For accelerated weathering, there exist international standard protocols developed for plastics and other polymeric materials for quality control purposes. These protocols are sometimes already incorporated in commercial weatherometers or can be adapted in custom-made laboratory chambers. The former usually offers more control over the parameters and more comparable results, but it is expensive and not readily available in environmental research laboratories. The latter offers more flexibility in terms of design and is less costly, but the results are specific to each system. Standard protocols generally recommend using specific lamp types, condensation cycles *etc*. to simulate natural conditions (ASTM., 1990). However, microplastic studies are increasingly using methods to initiate fast degradation without adequate justification which can potentially lead to unrealistic physicochemical changes and conclusions.

The majority of microplastics recovered from various environmental compartments and organisms are weathered and have been well characterized both in the macro- (bulk) and micro-scales (Garvey et al., 2020; Krause et al., 2020; McGivney et al., 2020; Rowenczyk et al., 2020). In the context of this review, environmentally relevant microplastics are defined as plastics that have properties mimicking microplastics found in the environment and those that have undergone similar processes as would be experienced by plastics in the real environment. Until recently, most laboratory studies have been carried out using pristine microplastics and nanoplastics (Waldman and Rillig, 2020); hence, the majority of the known risks associated with microplastic pollution were determined under less realistic conditions. Therefore, our understanding of the true risks associated with microplastic pollution may be limited. To advance knowledge in this field,

emerging studies now include more environmentally relevant microplastics and majority show that aged microplastics behave differently from pristine ones under same conditions. By ignoring the impact of key weathering processes, most findings in the current microplastics literature may be inconclusive. Recent reports are calling for standardization of methods across microplastics studies (Cowger et al., 2020) and quality criteria for risk assessment to lay a foundation to increase harmonization and comparability across studies (de Ruijter et al., 2020). However, there is a lack of standardized protocols for microplastic weathering.

Therefore, the purpose of this review is to: (i) highlight and discuss the typical and expected weathering pathways (especially those that might have been overlooked in water treatment processes) that microplastics will undergo before and after release into the environment during use and disposal, (ii) discuss the need to mimic weathering pathways in the water cycle where exposure is important, (iii) critically review the current methods used in weathering microplastics in laboratory effects studies to assess their appropriateness, (iv) critically review existing international standard protocols recommended for weathering bulk plastics and assess their applicability for microplastics studies, (v) propose useful weathering guidelines to address some of the identified knowledge gaps.

2.0 Key weathering conditions and pathways encountered by plastics throughout their lifecycle

2.1 UV photooxidation

Sunlight is mainly composed of infrared (wavelength λ between 700 nm to 1 mm), visible (λ = 400-700 nm) and ultraviolet light or UV (λ =100-400 nm) (Tobiska and Nusinov, 2006). The latter has higher photon energy due to its higher frequency, and is divided into three main subtypes: UVC (λ = 100-280 nm), which is completely absorbed by the ozone layer in the atmosphere, UVB (λ = 280–315 nm), mostly absorbed by the ozone layer, but

still reaching the Earth's surface, and UVA (λ = 315-400 nm), which is not affected by the ozone layer and comprises more than 95% of the UV radiation that reaches the Earth's surface (D'Orazio et al., 2013). It is believed that photodegradation initiated by UV in the presence of oxygen, or photooxidation, is the most important type of abiotic degradation pathway that plastics undergo in the environment (Gewert et al., 2015; Gijsman et al., 1999).

The three steps of photooxidation are initiation, propagation and termination. First, the photon needs to be absorbed by a chemical bond leading to chain scission and free radical creation. Cleavage of weaker C-H bonds from tertiary carbons, present in polypropylene and polystyrene for instance, is particularly favourable and forms stable radicals to continue the photooxidation (Min et al., 2020). During propagation, oxygen is quickly added to these radicals to form peroxy radicals, which in turn withdraw hydrogens from vicinal chains and form hydroperoxide groups and new free radicals. The reaction is terminated once radicals combine and form inactive/stable groups. Stabilizers commonly incorporated in plastics act to preferentially absorb UV radiation or to capture and stabilize free radicals. During photooxidation, not only chain scission but crosslinking, branching and the formation of oxidized groups in the polymer chain such as carbonyl, carboxyl and hydroxyl is expected (Gewert et al., 2015). Yellowing is a typical consequence of photooxidation, creating more chromophores and facilitating further degradation (Andrady et al., 1998). As the molecular weight of the polymer decreases, the original physical properties are lost and the materials become brittle and more prone to fragmentation (Feldman, 2002). Photooxidation increases roughness and surface area, forming flakes and grooves to a depth of approximately 100 μm, and so the fragmentation easily leads to micro- and nanoplastic release (Ter Halle et al., 2016). Mechanical abrasion after photooxidation accelerates the fragmentation process by breaking the brittle degraded surfaces of plastics such as expanded polystyrene (Song et al., 2017).

The extent of photodegradation is also determined by the intensity of the radiation (Feldman, 2002), which depends on the solar irradiance, or the total power per unit area received from the sun. Absorption and scattering in the atmosphere, reflection on Earth's surface, meteorological conditions, seasons and geographical position alter the value of

solar irradiance that reaches plastic fragments in the environment. The UV dose is a product of irradiance (I), expressed as energy per unit surface area, and time of exposure (t). A long time of exposure in a natural environment leads to a high UV dose, while artificial UV irradiation used during water treatment for pathogen inactivation has a negligible UV dose due to a very short time of exposure (few seconds) (Metcalf et al., 2014; Oram, 2014; Wolfe, 1990), even considering the high irradiance (typically 40 mJ/cm^2) used (United States Environmental Protection Agency, 1999). A UV reactor (e.g., low pressure and high intensity irradiance lamp) with a monochromatic UVC irradiance (254 nm) can provide enough energy to initiate the plastic surface photodegradation, but natural weathering over a long period of time (e.g., several weeks) contributes more significantly to plastic photooxidation. More research is needed in this area, notably for polymer degradation being driven by I only rather than by $I \times t$.

2.2 Biological weathering

Biological weathering, or biodegradation, may occur when plastics are exposed to various types of microorganisms. Biodegradation can be simplified as the hydrolysis of polymer into monomers or final mineralization products (CO_2, CH_4) by enzymatic activity. This involves extracellular depolymerases to break down polymers into molecules small enough to pass the cell membranes, and intracellular depolymerases in which those small molecules are used for cell metabolism (Shah et al., 2008). The proliferation of such microorganisms depends on environmental parameters (e.g., temperature, pH, moisture, salinity) and morphology of the microplastics that enables attachment of microorganisms and formation of biofilm (Sun et al., 2020). Biological activity can be measured by monitoring the production of final mineralization products. The degradation of specific organic molecules can be monitored, for example, by using labelled carbon to enable differentiation from the background carbon (Sander, 2019; Tian et al., 2017).

Biological weathering occurs to some extent in most environmental compartments, however, microplastics may be in contact with high concentrations of active microorganisms in soils (Sander, 2019), anoxic waters and wastewater processes (Metcalf et al., 2014). In wastewater treatment and sludge treatment streams, microplastics are contacted with a wide range of microbial ecosystems, in aerobic, anoxic or anaerobic

conditions. These processes often host specific microorganisms such as methanogenic archaea or nitrifying bacteria, with high concentrations of active biomass (e.g., 1500 to 4000 mg/L in conventional activated sludge). It is worth noting the high concentration of biological solids in sludge (60 to 1300 g/L), as most microplastics that transit wastewater treatment plants are captured in the solids stream (Carr et al., 2016). Most wastewater or sludge treatment processes provide partial biodegradation of microplastics (Rom et al., 2017). Specific species can degrade different types of plastics (Shah et al., 2008).

2.3 Chemical oxidation and disinfection

Chlorine, chloramines, ozone, potassium permanganate and hydrogen peroxide are widely used in the drinking water industry for oxidation and disinfection. While ozone has a higher oxidative potential ($E°_{red}$: 2.08 V), its concentration decreases quickly in full-scale processes and no residual concentration is expected in the distribution system (American Water Works Association, 1999). The chlorine oxidative potential of HOCl is lower ($E°_{red}$: 1.48 V) (American Water Works Association, 1999), but a residual concentration is usually maintained in the distribution system (> 0.3 mg Cl_2/L in North America). Consequently, the non-filterable plastics are exposed to chlorine for several hours. In drinking water applications, disinfection is generally performed after granular filtration which removes a fair number of microplastics (87–99 %) (Zhang et al., 2020). However, if implemented before filtration (inter-oxidation), chemical oxidation via ozonation has the potential to fragment larger microplastics into smaller plastic debris. In wastewater treatment, ozone is usually implemented at the end of the water treatment process to reduce ozone consumption caused by non-selective reactions with colloids; thus, the majority of plastics are not exposed to ozone as they are efficiently removed during settling. However, some plastic debris are persistent and remain in settled waters. In wastewater treatment, the ozone concentration is considerably higher compared to the concentration used for drinking water disinfection. This increases the risk of plastic degradation via chemical oxidation pathways. Chemical oxidation was shown to alter the polymer backbone (formation of hydroxyl and carbonyl groups), hence initiating the degradation sequence (Jia et al., 2019; Liu et al.,

2019b; Razumovskii et al., 1971; Tian et al., 2017), and to change the surface charge (reduction of the zeta potential by using 0.5–5 mg O_3/L) (Pulido-Reyes et al., 2020). The impact of ozone combined with low water flow shearing (25–80 s^{-1}) was investigated in a full-scale process and the concentration of 1–5 μm microplastics increased, although it is not clear if the increase was associated with plastic fragmentation or to a better detection due to the cleaner plastic surface after ozonation (Horton et al., 2017; Wang et al., 2020f). To date, no study has clearly explored the combination of chemical oxidation with high-shearing events on plastic degradation/fragmentation. Ozonating/fragmenting plastics into smaller pieces would reduce their settling velocity, as velocity is proportional to the diameter^2 (Johnson et al., 1996; Lapointe and Barbeau, 2016), which will affect their transport into clarifiers and aquatic ecosystems.

2.4 Thermal effects

Microplastics are exposed to thermal variation in aquatic environments and urban waters during 1) drinking and wastewater treatment, 2) sludge treatment and 3) distribution and usage of potable water. While many common drinking water and wastewater treatment processes occur between 1 and 30°C, several processes in the sludge treatment line are maintained at higher temperature (Metcalf et al., 2014). For example, anaerobic digestion occurs between 30 and 57°C, composting occurs between 50 to 70°C and incineration occurs between 650 and 820°C (Metcalf et al., 2014). As 90–99% of microplastics in wastewater facilities passes in the sludge treatment line (Nguyen et al., 2019), microplastics are likely to be exposed to a wide range of temperatures. Thermal stress encountered by microplastics in distribution and usage of drinking water occurs via hot water pipelines (50-95°C) and boiling in cooking processes (95°C). Microplastics will also undergo thermal stress at cold and freezing temperatures (e.g. freeze-thaw cycling) in cold climate regions. Stable aggregates of nanoplastics have been observed after exposure to several cycles of freeze-thaw (Alimi et al., 2020).

Several authors have characterized thermal aging of bulk plastics or microplastics using depletion of antioxidant, depth of carbonyl groups (Colin et al., 2009; Viebke and

Gedde, 1998), changes in molecular structure and crystallinity (Viebke and Gedde, 1998), appearance of fractures (Chen et al., 2020b; Colin et al., 2009; Viebke et al., 1994; Viebke and Gedde, 1998), changes in surface groups (Ding et al., 2020) and monitoring of mass loss (Rom et al., 2017). Colin *et al* observed an Arrhenius dependency of thermal aging processes of PE pipes between 20 and 105°C (Colin et al., 2009). Though fractures have been observed on the surface of plastics, studies that report release of smaller microplastics or nanoplastics following thermal degradation of bulk plastics or microplastics are sparse. Hernandez *et al.* (2019) showed that exposure of bulk plastic to 95°C for five min led to leaching of considerable micro- and nanoplastics (Hernandez et al., 2019).

Thermal aging is affected by environmental factors. First, the effect of temperature is affected by the presence of oxidizers. The presence or absence of oxygen in sludge treatment (e.g., aerobic or anaerobic conditions) favors oxidation or hydrolysis, respectively. Oxidation kinetics of commonly used oxidants in drinking water treatment (chlorine, chlorine oxide, ozone, etc.) are faster at higher temperature. Moreover, aging by thermal oxidation is affected by the presence or absence of antioxidant in bulk plastics (Viebke et al., 1994). Finally, the establishment of microbial communities that support plastic biodegradation is affected by temperature; thus, higher temperatures generally lead to increases in both thermal degradation and biodegradation (Chen et al., 2020b).

2.5 Other transformations

Plastic debris are known to be weathered via multiple pathways (e.g., (photo)oxidation, thermal degradation, biodegradation, etc.) causing alteration of the polymer backbone. However, plastic materials could experience other transformations in natural waters and water treatment processes: heteroaggregation with natural colloids, NOM adsorption, binding of salts, biofilm formation, and coagulant/flocculant adsorption. Although these may not be considered as weathering pathways affecting the polymer backbone, such transformations are nonetheless expected to affect the fate, behavior and impacts of plastics in the environment.

In sea waters and surface waters, binding of divalent ions (Ca^{2+} or Mg^{2+}), heteroaggregation with natural colloids and adsorption of natural NOM on plastics have been observed by many researchers and were reported to influence the stability of microplastics. Consequently, such pre-coating/corona on plastic surfaces could significantly impact nanoplastics and microplastics transport as some NOM fractions or colloids may act as stabilizers (limiting aggregation) while others (e.g., high molecular weight NOM fractions) promote aggregation via interparticle bridging effects (Lapointe et al., 2020; Liu et al., 2020c; Shams et al., 2020; Wu et al.). Similar results were observedby Liu et al., where nanoparticle stability and aggregation were considerably modified by organic coatings (Liu et al., 2018).

Water treatment was also reported to change plastic surface chemistry. While a large proportion of plastics is expected to be trapped in aggregates and settled sludge, a small proportion is however refractory to treatment and is *de facto* released in aquatic environments (Alimi et al., 2018; Mason et al., 2016; Talvitie et al., 2017). The coagulants (e.g., alum), flocculants (e.g., polyacrylamide) and bioflocculants (extracellular polymeric substances, EPS) present in wastewater (Sheng et al., 2010) are expected to coat the plastic surface, hence modifying its transport and fate once released in aquatic environments. It was reported that metal-based coagulants (e.g., alum; 1–6 mg Al/L) (Cai et al., 2018a; Kawamura, 2000; Lapointe et al., 2020) and organic cationic polymers (e.g., polyamines; 0.1–0.6 mg/L) (American Water Works Association, 1999; Rajala et al., 2020) interact with plastic surfaces *via* electrostatic affinities on negatively charged sites (e.g., hydroxyl and carboxyl groups) or via hydrogen bonding (Lapointe et al., 2020).Quartz crystal microbalance with dissipation (QCM-D) experiments showed that positively charged inorganic and organic coagulants deposited more on weathered plastic surfaces, as more anionic functions are available (Lapointe et al., 2020). UV exposure could also have an impact on plastics aggregation and stability. Wang et al. concluded that UV-induced weathering that degrades sulfate and amine groups of plastics reduced the electrostatic repulsion, hence promoting nanoplastic homoaggregation (NaCl solution) (Wang et al., 2020c).

2.6 Weathering processes in major environmental compartments of the water cycle

Weathering pathways encountered by microplastics in major environmental compartments are summarized in Figure 1. We present the mass flux of plastics in major compartments of the water cycle and show the important weathering processes occurring in them (Figure 1). Microplastics undergo several weathering pathways at the same time in each environmental compartment, leading to combined effects. For example, the presence of carbonyl groups on UV-degraded microplastic surfaces favors biofilm growth (Min et al., 2020). Conversely, a biofilm covers the surface of the plastic fragments and may also increase their density and make them sink in water (Fazey and Ryan, 2016). Marine snow can also transport micro- and nanoplastics to ocean sediments regardless of their density (Porter et al., 2018). This may explain the presence of buoyant plastics in sediments and a lower-than-expected presence in surface waters (Karlsson et al., 2018). Other combinations of weathering processes accelerate microplastic fragmentation: photooxidation combined with mechanical abrasion (Song et al., 2017) or thermal degradation combined with biodegradation (Shah et al., 2008).

Weathering pathways are complex even within a single compartment. For example, the impact of photooxidation on plastics depends on the plastic composition and sunlight penetration in water. Buoyant polymers such as PE (density = 0.91-0.97 g/cm^3) and PP (density 0.90-0.92 g/cm^3) are more prone to photooxidation in open bodies of water than common polymers that sink, such as polyethylene terephthalate (PET, density = 1.35-1.45 g/cm^3) and polyvinyl chloride (PVC, density = 1.1-1.45 g/cm^3). In seawater, where the water density is higher, some grades of PS and expanded PS also float and are subjected to direct solar radiation. Shape is another factor that will contribute to how a particular fragment will be exposed to radiation. Flat fragments in the water surface will tend to expose mainly one side, which will receive more radiation, while more symmetrical cubic fragments will rotate and present a more homogeneous degradation on all sides (Ter Halle et al., 2016). The impact of each weathering process is related to both the intensity and duration of exposure. Therefore, typical residence times in the water cycle must be considered when assessing microplastic weathering processes. A water droplet transits for

9 days in the atmosphere, 2 weeks in a river, 10 years in big lakes, 120 years in superficial layers of oceans, and 3000 years in deep oceans (Nazaroff and Alvarez-Cohen, 2001). The residence time of water in most drinking water and wastewater treatment processes is less than two days (Metcalf et al., 2014), however, in many cases, the sludge retention time (few days to few months) may be considered instead of the water retention time because most plastics are trapped in the sludge.

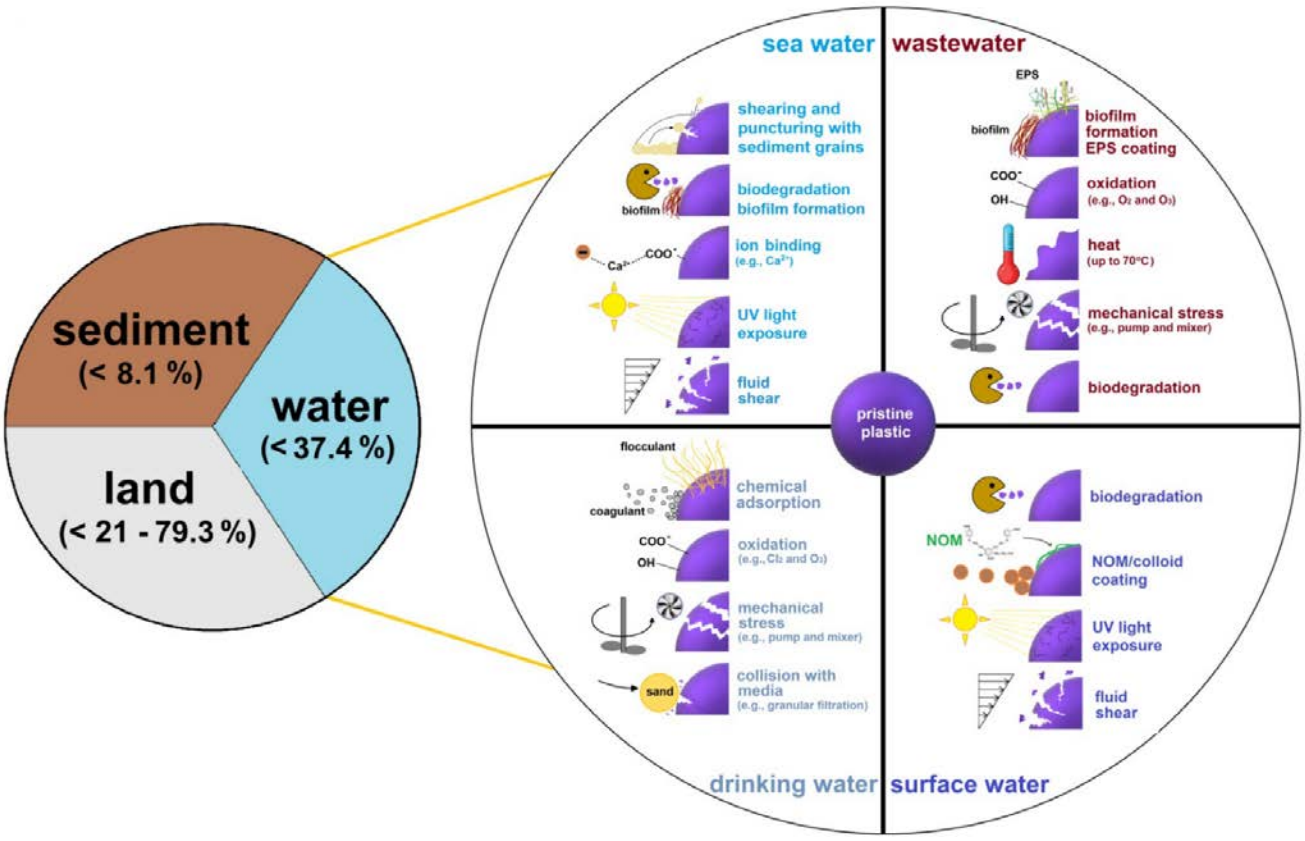


Figure 1. Major weathering pathways that plastic and its degradation products will encounter throughout its lifecycle before and after entering the environment. Percentages refer to estimated fraction of plastics released into a given compartment after manufacturing and use based on data from (Alimi et al., 2018).

3.0 Effects of weathering on microplastic fate in the environment

In the previous section, we described how weathering can change the properties of plastics. Those physicochemical changes are reported to affect plastic fate in the environment and removal during water treatment (Lapointe et al., 2020). Weathering can also affect how microplastics interact with aquatic organisms (Bråte et al., 2018). The color, size, attached biofilm and surface charge changes will determine microplastic uptake and potential effects (Chen et al., 2020a). There is a lack of understanding on how weathering affects microplastic removal during water treatment, transport and aggregation processes, hence, this section will briefly discuss these three processes.

During water treatment, weathered plastics were recently shown to interact better with coagulants and flocculant. 90-99% of weathered plastic removal was systematically measured (Hidayaturrahman and Lee, 2019; Lapointe et al., 2020; Perren et al., 2018; Rajala et al., 2020) while lower removals were observed with pristine plastics: ~ 80% (Lapointe et al., 2020) and <30% (Ma et al., 2019). Similarly, on-site measurements systematically reported removals higher than 95% for naturally weathered plastics (Bilgin et al., 2020; Rajala et al., 2020; Sun et al., 2019). Such higher interaction of coagulant, flocculant and bioflocculant (EPS) is attributable to a more heterogeneous plastic surface obtained after weathering (e.g., (photo)oxidation) and/or after other surface modifications (e.g., NOM coating), hence offering new anchoring points for coagulants, while pristine plastic surfaces are relatively homogeneous and less reactive (Lapointe et al., 2020). Consequently, studies designed with pristine plastic materials might underestimate plastic aggregation and removal in full-scale water treatment plants. Considering that pristine plastics are likely inexistent in natural environments, these studies reveal the importance of designing research protocols with realistic weathering conditions. To overcome systematic plastics release, water treatment plants could be designed considering the surface chemistry of weathered and refractory plastics e.g., by adjusting the aggregation conditions such as coagulant types and pH.

Few studies have shown the effect of weathering on the transport of nanoplastics in model groundwaters and their stability in surface waters. One study highlighted the

importance of considering weathering conditions in cold climates. Exposure of PS nanoplastics to repeated freeze-thaw cycles led to significant aggregation even in the presence of NOM, resulting in lowered mobility of the particles in saturated quartz sand compared to nanoplastics at constant cold temperature (Alimi et al., 2020). A different study showed that UV and ozone weathering increased the mobility of nanoplastics and facilitated the transport of contaminants in a loamy sand. The enhanced mobility of the weathered nanoplastics was attributed to the increase in surface oxidation and reduced hydrophobicity (Liu et al., 2019a). UV weathering also impacts the stability of nanoplastics in simulated natural waters (Liu et al., 2019c). A combination of new carboxyl functional groups and decreased particle size (from 120 to 80 nm) of the UVA-aged nanoplastics compared to pristine ones enhanced the aggregation of the nanoplastics in calcium chloride solution (ascribed to bridging via oxygen-containing functional groups) but promoted stability in sodium chloride solution (Liu et al., 2019c). Our understanding of the effects of weathering on other environmental fate processes besides toxicity and sorption is still very limited, hence, more studies are needed for realistic risk assessment.

4.0 Current knowledge about weathering protocols used in microplastics effect studies

A comprehensive literature search was conducted using the Scopus and Google Scholar citation databases (as of May 25, 2021). The search was carried out to identify laboratory-based effect studies that (i) compared weathered/aged plastics with pristine ones in the same study, (ii) used leachate from weathered plastics and, (iii) used pristine microplastic only (detailed criteria in Table S1). An effect study in this context is defined as a study that investigates the effect of weathered microplastics or leachate on transport, aggregation, or toxicity of particles, sorption of contaminants, etc. Leachates commonly contain organic/inorganic additives and monomers that are released during the weathering process (Gunaalan et al., 2020), and can also contain nano- or microplastics (Xu et al., 2020). Studies investigating the fragmentation or biodegradability of microplastics without evaluating the effects of the aged microplastics were excluded from the search.

4.1 Weathering protocols used in microplastic effect studies.

Most weathered microplastic effect studies use pristine commercial primary microplastics or nanoplastics and weather them via UV, chemical, thermal or biological degradation. A few studies use microplastics obtained from the environment (mostly beaches) for laboratory studies. A subset of these studies uses degradation products leached from bulk plastic for toxicity studies. Figure 2 shows the distribution of the various types of weathering treatments applied to microplastics or their leachates. It is evident that there are fewer studies using leachate compared to the weathered microplastics. As mentioned above, there are more studies investigating the effect of weathering on microplastic sorption/desorption capacity (Figure 2, layer 2). Few studies have used microplastics weathered via natural UV radiation (sunlight) with only two of them reporting irradiance (Liu et al., 2019b; Luo et al., 2019).

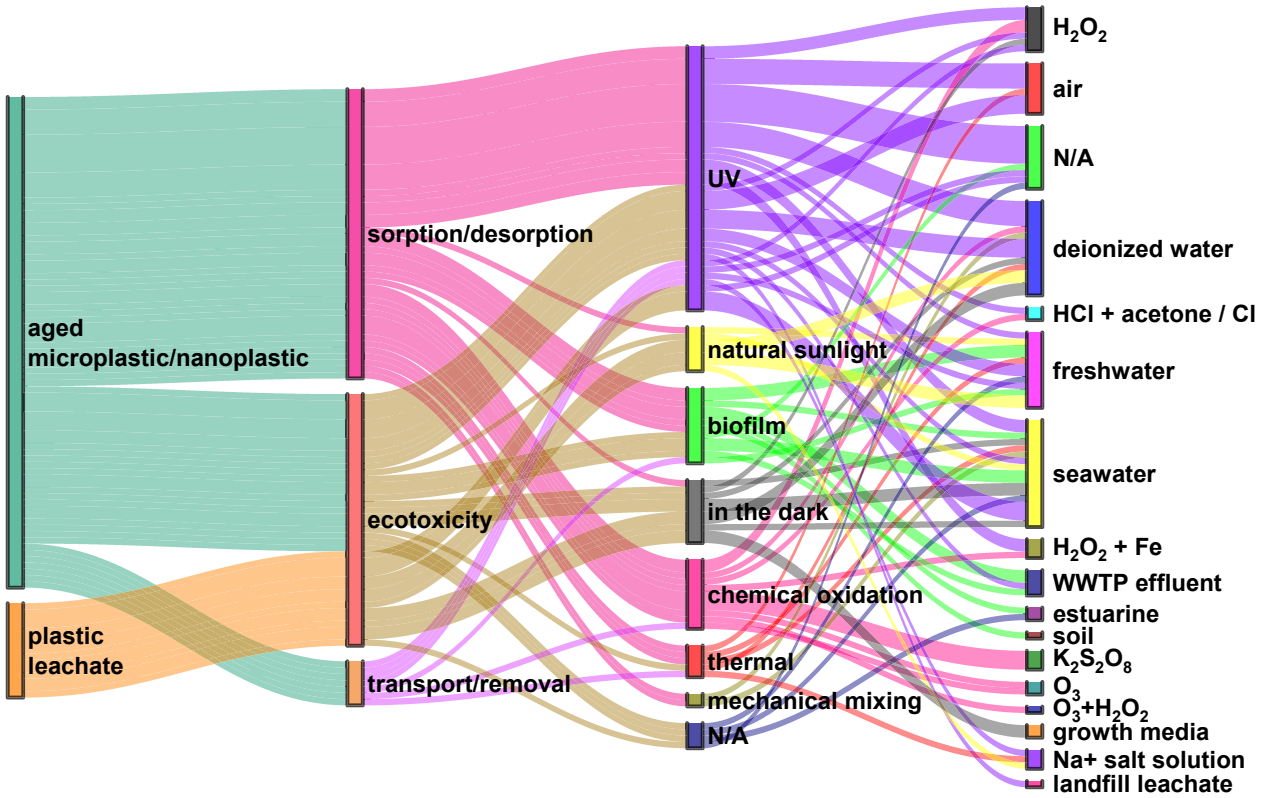


Figure 2. Distribution of the various types of weathering treatments applied to microplastics and plastic leachates among controlled laboratory effects studies. First layer = plastic state, second layer = study type/effect studied, third layer = weathering pathway/choice, fourth layer = weathering medium. WWTP – Wastewater treatment plant, N/A – Not available. Total of 93 studies identified from 63 articles. Articles reporting more than one weathering media are treated as separate studies. Data references provided in Table S2.

For studies using UV radiation, we compared the irradiance versus duration of exposure for microplastics weathered naturally or artificially as well as the type of plastic used (Figure 3). The radiation time varies from 24 to 7920 hours in these studies. There is no generalized method of exposure as the irradiance and duration of exposure vary significantly across these studies with PS having the most variation. It is worth noting that 49% of studies (31/63 articles) report the temperature in the weathering setup. The cumulative distribution (Figure S1) shows that 70% of these studies use temperatures <35°C with only two investigating effect of weathering at cold (Vroom et al., 2017) and freezing temperature (Alimi et al., 2020). The plastics weathered via UV radiation are typically suspended in media that range from deionised water to natural water and chemical oxidants (Figure 2, layer 4). Two studies used a combination of UVC light and H_2O_2 to weather microplastics for 96 hours (Hüffer et al., 2018; Mao et al., 2020). While UVC light is not most representative of the natural environment, it is sometimes used in water treatment disinfection. UVC light exposure in water treatment is usually done at short contact times (~ 5 sec), hence studies using this approach should mimic the short residence time accordingly. Other studies have used chemical oxidation approaches including Fenton reagent, hydrogen chloride, ozone, potassium permanganate and hydrogen peroxide (Liu et al., 2020b; Wu et al., 2020b) while some combined Fe^{2+} with UV light (photo-Fenton) (Liu et al., 2020a; Liu et al., 2020b) or high temperature (Wu et al., 2020b). While these chemicals are sometimes used in water and wastewater treatment, hence relevant, there is variability in the working concentrations used across studies (20–200 mM Fe^{2+}, 2 g and 10 mM $K_2S_2O_8$), making comparability and environmental appropriateness difficult to assess. Environmental appropriateness is sometimes questionable as there is a need to justify the choice of high chemical dose and weathering pathway being mimicked. One study used natural sunlight to weather PS and PE, and compared the results to microplastics weathered via Fenton reaction and heat-activated $K_2S_2O_8$ (Liu et al., 2019b). Microplastics were suspended in water samples from Yangtze River and Taihu Lake, China and placed on a building rooftop for 11 months. It was argued that the degradation products formed after initiating natural UV radiation yields same products as the advanced oxidation process i.e., free radicals. The oxygen/carbon ratio of the aged microplastics was also quantified and it was shown that it could be used as an alternative parameter to carbonyl index typically

used to measure extent of oxidation. These oxidation processes are promising approaches that could shorten the aging time of microplastics for laboratory effect studies but may require further validation.

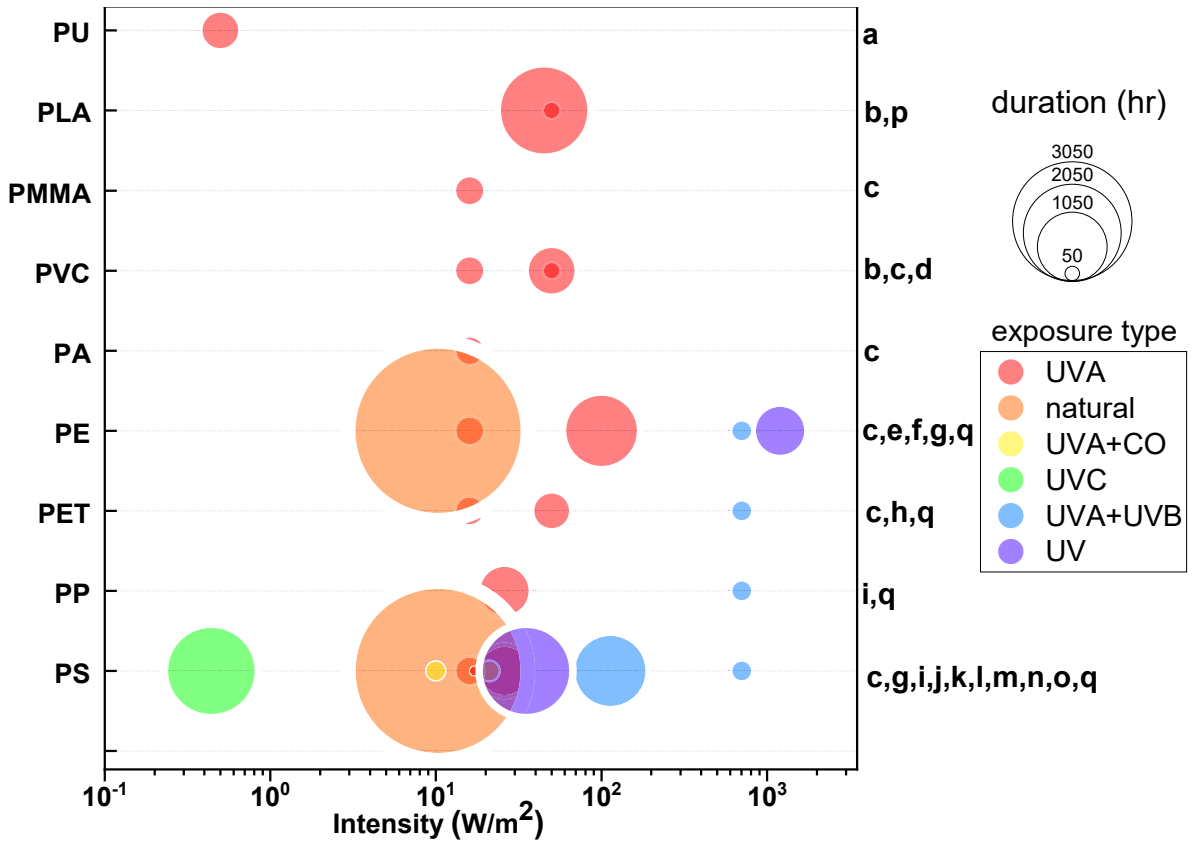


Figure 3. General trend in irradiance versus duration (hr) and plastic type across different laboratory effect studies reporting these parameters. Here, we see that the type of UV treatment and plastic type varies across studies. CO - chemical oxidation, PS - polystyrene, PE - polyethylene, PP – polypropylene, PVC – polyvinyl chloride, PET – polyethylene terephthalate, PA – polyamide, PC – polycarbonate, PMMA – polymethyl methacrylate, PLA – polylactic acid. References: a - (Černá et al., 2021), b - (Fan et al., 2021), c - (Yang et al., 2019), d - (Wang et al., 2020a), e - (Lapointe et al., 2020), f - (Luo et al., 2020), g - (Liu et al., 2019b), h - (Wang et al., 2020b), i - (Müller et al., 2018), j - (Liu et al., 2020a), k - (Liu et al., 2019c), l - (Wu et al., 2020a), m -(Wang et al., 2020d), n - (Zou et al., 2020), o - (Liu et al., 2021), p - (Zhang et al., 2021), q - (Rummel et al., 2019).

Microplastics can be weathered with the aim of growing biofilms on them (Kalčíková et al., 2020; Kaposi et al., 2014; Vroom et al., 2017; Wang et al., 2020e). Wang et al. (2020e) mimicked weathering in wastewater treatment plants by placing PE microplastics in sewage outlets in Shanghai for 20 days. This resulted in a pore size reduction (from 10 to 3 nm) and an increase in specific surface area (from 0.24-0.78 m^2/g)

of the plastic. When mimicking biofouling in a riverine, estuarine and marine system in Australia, Johansen *et al.* (2019) observed that patchy biofilm enriched with Si, Al and O developed on the plastic surface. PS microbeads placed in filtered seawater for 3 weeks in the dark showed that aging enhanced plastics ingestion by zooplankton (Vroom et al., 2017). However, no characterisation was done to confirm the presence of biofilm on the plastic surface. Even though some studies are designed to produce biofilm-aged microplastics, characterising its presence after weathering is helpful. Schur *et al.(2021)* showed this in a recent study where dissolved organic matter rather than the presence of biofilm was suggested as the driving mechanism for the multigenerational effect of wastewater-incubated PS on *Daphnia*. A recent study revealed that microplastics exposed to freshwater from an artificial pond and seawater from a marine aquarium led to the coating of biomolecules forming an eco-corona, which facilitated their uptake in mouse cells (Ramsperger et al., 2020). These non-UV weathering pathways particularly highlight the importance of exploring other weathering processes microplastics will encounter in the environment. For example, while it was shown that UV-aged PA microplastics had limited toxicity to zebrafish larvae (Zou et al., 2020), another study reported tissue alterations in mussels exposed to PE microplastics incubated in seawater (Bråte et al., 2018).

Thermal weathering pathways have also been used to obtain environmentally relevant plastics. One study exposed PS nanoplastics to temperatures typically encountered in cold climate regions (Alimi et al., 2020) for transport experiments in saturated quartz sand. The nanoplastics were suspended in monovalent salt solution (in the presence and absence of natural organic matter) and subjected to several controlled freeze-thaw cycles (from 10°C to -10°C). These temperature ranges closely mimic those encountered during the shoulder periods in southern Quebec, Canada. Another study used a higher temperature of 70°C to weather PS microplastics suspended in sea water and freshwater for sorption experiments (Ding et al., 2020). However, it is unclear which environmental compartment was being mimicked or where plastic would normally encounter such high temperatures. Since such high temperatures will not be typically encountered in freshwater and seawater, there is a need to better describe the rationale behind such choices.

Another approach used in obtaining environmentally relevant microplastics is by using leachates obtained during the weathering of bulk plastics. The particles contained in leachates could be more representative of the types of nano- and microplastics found in the environment, therefore, we included some studies using leachates in this review. It is however important to note that some of these studies do not use corresponding reference or control pristine particles for comparison. Nevertheless, we can gain some insights from the weathering methods used. Leachates were obtained either by weathering bulk plastics in the dark or exposure to natural sunlight (Luo et al., 2020; Luo et al., 2019; Xu et al., 2020). The reported leachate studies use background medium ranging from deionised water, tap water and natural/artificial seawater.

Overall, we noted considerable variability in the methods, duration, and medium used for weathering microplastics. While few of the identified laboratory-based studies follow existing international standards, a larger percentage used custom-designed weathering protocols, and some do not justify the rationale behind the choice of weathering process. There is no notable difference in the protocols used for nanoplastics versus microplastics across studies. In general, the biofilm/biodegradation related effect studies seem to use the most realistic protocols having direct environmental relevance. Some studies have weathered plastics naturally by placing them outdoors but fail to report the irradiation values, making comparison difficult. Effect studies mimicking mechanical abrasion that might occur in sandy beaches or deep bed sediments are sparse. Weathering processes occurring in biosolids streams are also overlooked.

4.2 Proportion of microplastic effect studies that use weathered plastics

Figure 4a shows the proportion of effect studies carried out with weathered plastics. Only few microplastics effect studies (~10%) used weathered microplastics, of which a considerable proportion found weathering to have a significant effect (~90%). By focusing on only pristine plastics, current models may be underestimating (or overestimating) the risks associated with microplastic pollution. Across all effect studies, the most frequently weathered plastic type was polystyrene > polyethylene > polypropylene > polyvinyl chloride > others (Fig. 4b). Comparing the type of plastics detected in various environmental compartments globally as well as the current global plastic demand, there

seems to be a mismatch (Fig. 4c). Indeed, majority of weathering studies use polystyrene whereas it is not the most commonly occurring plastic in environmental samples. Polypropylene which ranks second in most environmental studies (Alimi et al., 2021; Geyer et al., 2017; Koelmans et al., 2019), is the third most weathered plastic. Polyethylene appears to be the most commonly occurring plastic, hence should be used in more weathering research to understand its effects.

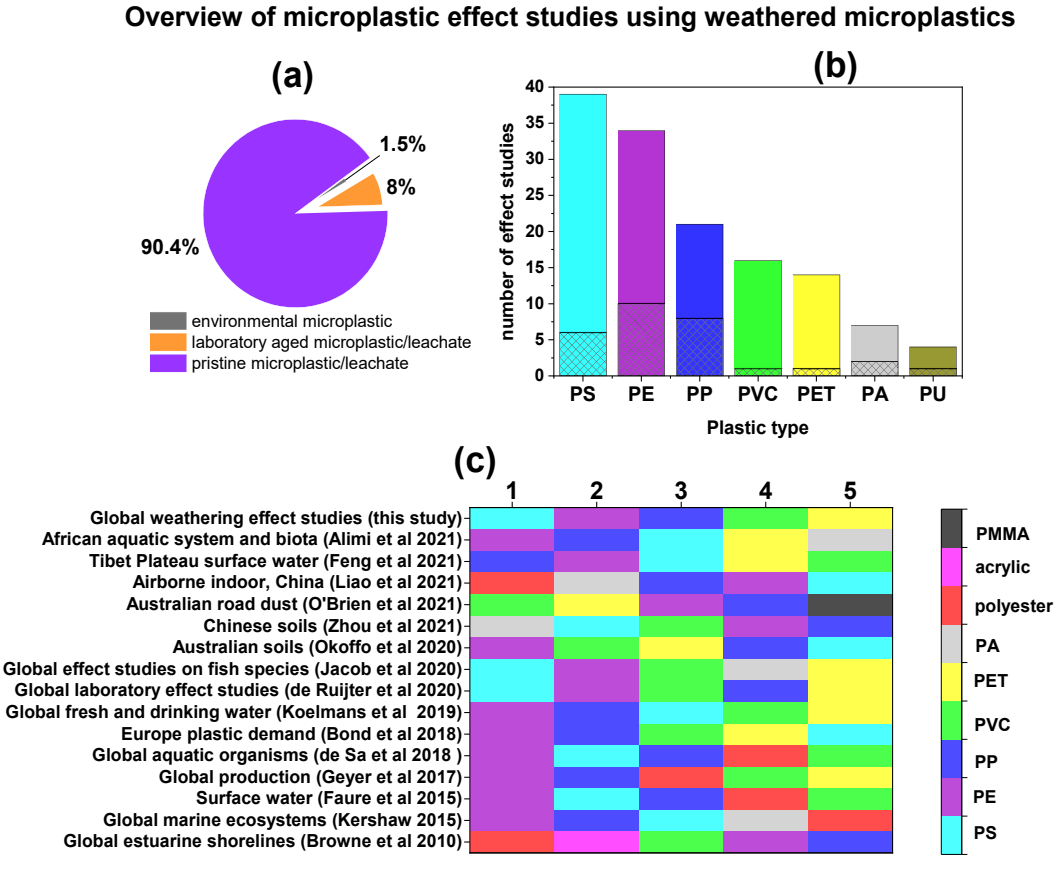


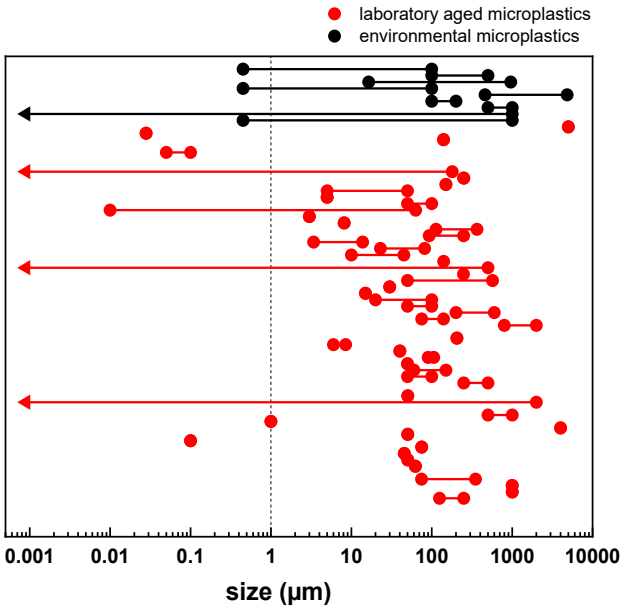
Figure 4. (a) The distribution of microplastic types used in effect studies highlighting the small proportion using weathered plastics in comparison to pristine ones; (b) The number of effects studies reporting types of polymer weathered in those studies. Dot pattern are polymer types reported in effects studies using microplastics sampled from the environment. Studies reporting both PE and HDPE/LDPE were counted as one PE; (c) A ranking of top 5 plastic types used in weathering effects studies in the present review versus those detected in the environment, produced or used in laboratory studies globally. Plastics rank 1 (most common) – 5 (least common) from left to right. PS - polystyrene, PE - polyethylene, PP – polypropylene, PVC – polyvinyl chloride, PET – polyethylene terephthalate, PA – polyamide, PC – polycarbonate, PMMA – polymethyl methacrylate, PU - polyurethane.

548 **4.3 Microplastics effect studies using environmental samples: comparison with**
549 **laboratory weathered microplastics.**
550

551 Few effect studies (<2%) have used microplastics collected in the natural environment
552 (Figure 4a, Table S3). Again, majority of these studies focus on sorption/desorption and
553 the most frequently used plastics follow PE>PP≈PS. Although this approach yields
554 microplastics that are of significant environmental relevance, it makes study
555 reproducibility quite challenging. Zhang *et al.* collected beached microplastics from North
556 China and compared their contaminant sorption capacity to virgin PS foams with similar
557 sizes (Zhang et al., 2018). The beached microplastics adsorbed contaminants two times as
558 much as the pristine ones (Freundlich isotherm constant = 425 and 894 mg/kg. $(l/mg)^{1/n}$
559 respectively). This was attributed to the higher specific surface area of the aged
560 microplastic. Using PE pellets collected from beaches in South West England, researchers
561 have shown that higher amount of trace metals adsorb on the beached plastics compared to
562 virgin ones (Holmes et al., 2012). Waldschlager *et al.* recently used microplastics
563 recovered from a fluvial environment to determine their fate (Waldschläger et al., 2020).
564 They showed that the environmentally weathered microplastics had much slower settling
565 and rising velocities compared to pristine plastics used in their previous study
566 (Waldschläger and Schüttrumpf, 2019). Some of these studies show that the aged
567 microplastics collected in the environment behaved differently than pristine microplastics
568 of the same or similar material while others do not compare with pristine ones. Generally,
569 this approach should be embraced by the microplastic community as it can provide more
570 realistic insights on the effects of microplastic pollution in the environment.

571 In Figure 5 and Table 1, we compared the characteristics of microplastics weathered
572 in the laboratory versus those collected from the environment. Interestingly, we observed
573 that only 12 laboratory effect studies have used aged nanoplastics. Additionally, only few
574 studies report the size of plastic retrieved from the environment which prevents an
575 extensive meta-analysis (some report < 5 mm without an actual value or range). The few
576 environmental microplastics with size ranges up to 0.45 µm, were obtained by grinding
577 milli-sized samples (Missawi et al., 2021; Zhang et al., 2018; Zitouni et al., 2021). The lack

578 of environmental samples using nanoplastics might be associated with the methodological
579 difficulties associated with separating the nanoplastics from the complex background
580 matrix. The shapes of plastics used are also very different as fragments dominate
581 environmental microplastics whereas aged beads/spheres are more commonly used in the
582 laboratory studies. While most environmental microplastics were collected from
583 agricultural soils and beaches, only one laboratory effect study used landfill and soil as
584 weathering media. Clearly, there exists several gaps between these two types of
585 microplastics used in effect studies.



586

587 **Figure 5.** Size ranges of weathered microplastics used in effect studies. Detailed references provided in Tables
588 S2 and S3. Arrows indicate that no lower limit was provided for that study.

589

590

Table 1. Comparison of characteristics and weathering conditions of effect studies using microplastics aged in the laboratory or collected in the environment. Detailed references in Tables S2 and S3

Characteristics	Laboratory weathered microplastics	Microplastics collected from environment
polymer types	PS > PE > PP > PVC > PET > PA > PU > PMMA, PTFE, PLA, PC	PE > PP > PS > PA > PVC, PET
shapes	beads > fragments, films, fibers	fragments > beads, films, fibers
weathering medium/sampling environment	air, deionized water, artificial and natural surface waters, chemical oxidants, landfill/soil, wastewater effluent	beach sediment, farmland soil
physical characterisation	size, density, morphology, specific surface area, crystallinity, color, contact angle, glass transition temperature, melting point	size, density, color, morphology
chemical characterisation	polymer type, surface chemistry (zeta potential, carbonyl index)	polymer type, surface chemistry (zeta potential, functional groups, carbonyl index, point of zero charge)
other conditions reported	plastic source, irradiation, wavelength, temperature, humidity, duration	location

5.0 Standardized international weathering protocols in different applications

Long before the onset of microplastics research (Figure 6a), standard weathering protocols were developed to assess whether a new plastic product will maintain acceptable properties during its lifecycle. Nonetheless, there has been an increase in the number of publications on microplastics or nanoplastics that mention these protocols (Figure 6b). In this section, we review selected active standards from ASTM International and the International Organization for Standardization (ISO) and discuss whether they are appropriate and adaptable for the study of plastic fragmentation into micro- and nanoplastics in the environment. These standards are classified below according to the type of exposure they intend to reproduce: outdoor exposure, marine exposure and solid waste conditions. A list of all standards used in this section, including ASTM/ISO equivalencies is available in Table S4.

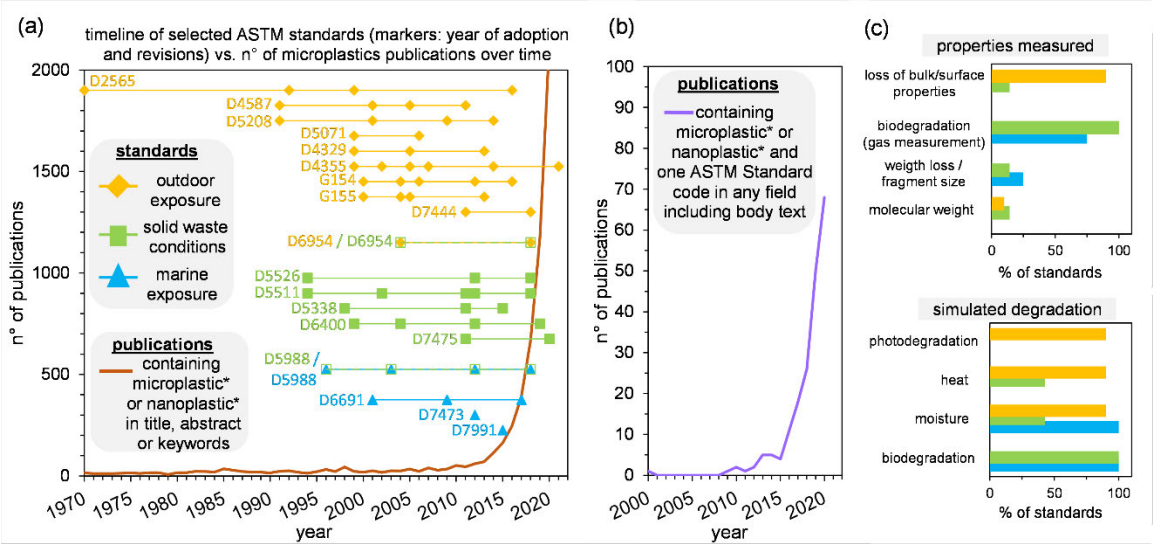


Figure 6. Selected ASTM standards for simulated weathering, classified as outdoor exposure, marine exposure or solid waste conditions: (a) timeline including the creation and revision of each standard, compared with the surge in scientific publications including microplastic* or nanoplastic* in title, abstract or keywords (Scopus, May 4, 2021); (b) publications including microplastic* or nanoplastic* and at least one code (e.g., D6400) of the selected ASTM standards in any field, including the body text (Google Scholar, May 4, 2021); (c) simulated degradation pathway and the outcome properties measured within each type of exposure. Standards of natural exposure were not included in this selection.

5.1 Outdoor exposure

The standards aimed for natural outdoor exposure, such as ASTM D1435/ISO 877.2, ISO 15314, ISO 877, and ASTM D 5272, recommend that specimens should be exposed in several locations, and state that an average result in a given location can only be achieved after several years of repeated exposure.

The standard protocols that simulate outdoor exposure using accelerated weathering are carried out in a chamber in which plastic degradation is induced by light (photodegradation), heat and moisture (Figure 6c). These standards do not intend to simulate other degradation pathways such as mechanical abrasion, biodegradation or advanced oxidation processes. To produce photooxidation, most protocols recommend the use of either a fluorescence UV lamp or a xenon arc lamp. ASTM D4329/ISO 4892-3 describe the practice for exposing plastics to UVA lamps, which match the solar irradiance in the UV region to produce the most damaging type of radiation that can occur in the environment. But even though the higher energies of UV-range radiation are more

deleterious to plastics, in the environment they are exposed to a wider range of radiation of different energies. Experiments using a narrow frequency band may overlook synergistic effects or overexpose plastics to their UV wavelengths of maximum sensitivity, which are not so abundant in the environment (Andrady, 1997; Feldman, 2002). Xenon arc lamps simulate the sunlight spectrum including UV, visible and infrared light, and therefore are generally preferred if a product is intended for outdoor use, as described by ASTM D2565 (similar to ISO 4892-2).

Weathering chambers allow for irradiance, temperature and humidity control to improve the simulation of natural phenomena. While the majority of the standards specify a temperature suitable for each exposure, some of these temperatures are higher than those encountered in typical natural waters/environments. Condensation cycles can be reproduced in UV chambers by an increase of chamber temperature and relative humidity followed by a temperature decrease. Alternatively, chambers equipped with xenon arc lamps use a water spray cycle to simulate rain and fast temperature changes. The presence of water on the surface of plastics can accelerate the reactions involved in the degradation process, while fast temperature changes cause contraction and expansion of the specimens. Different cycles with small variations in these parameters are also proposed, but in all cases, the cycle is repeated every few hours with the same parameters.

The test time depends on the materials and can be defined by the stakeholders involved, but it is recommended that the minimum test time should produce a substantial performance difference between the specimen and the control. Some standards recommend the use of two controls: one with known superior durability and another with known inferior durability. Appearance and mechanical properties of the bulk plastic parts are common properties evaluated by the users.

5.2 Marine exposure

In the marine environment, plastic specimens are in constant contact with water and microorganisms, and exposed to different levels of UV radiation depending on their buoyancy. ISO 15314 is one of the few standards aimed at natural exposure of plastics in marine environments. It provides three exposure scenarios: plastic floating on the surface,

partial immersion of plastic and complete immersion to assess the persistence of marine litter. ISO 15314 is suitable for different types of specimens commonly found in consumer products such as plastic films, sheets, fibers and ropes. This standard recommends exposure at different locations to account for variability in radiation, temperature, microorganism populations, *etc*. The accelerated weathering standards that simulate marine exposure account for the fact that material degradation in natural waters is mainly dependent on the presence of microorganisms (Viera et al., 2021), giving emphasis to biodegradation and often omitting other processes such as photodegradation and temperature variations (Figure 6c). ASTM 7473 simulates marine exposure in open system aquarium incubations with natural flowing seawater, but without sunlight as the test is aimed for non-buoyant plastics. The protocol uses marine sediments, which contain several orders of magnitude more bacteria than seawater, to guarantee the presence of microorganisms. The standard recommends evaluating the specimen visually and measuring the weight loss over time to obtain some insight on the fragmentation rate. ASTM D6691 and D7991 describe methods to assess the aerobic biodegradation of plastics in controlled laboratory conditions, in which the amount of CO_2 produced by the biodegradation of the specimen is measured over time. In ASTM D6691, a well-defined population of microorganisms present in the marine environment is used, while the method described in ASTM D7991 reproduces the tidal environment with specimens buried in natural sandy marine sediment. But just as the protocols aimed for outdoor exposure, the ones that mimic the marine environment are only concerned with the degradation of the bulk plastic specimens, which are easier to separate and recover for analysis.

5.3 Solid waste conditions

In the standards that simulate weathering in solid waste conditions, biodegradation is also the main degradation pathway, in combination with heat and moisture (Figure 6c), in different types of media. ASTM D5988 (equivalent to ISO 17556) aims to simulate biodegradation of plastics when disposed in aerobic soil environment. A biometer flask is used and the specimen is buried in equal parts of soil, sand and manure. The CO_2 produced by the system is trapped in the flask and measured periodically. Control flasks with no

specimens are important since the soil will naturally produce CO_2. The standard does not specify the type of polymer to be tested and recommends that the results should not be used to classify the material as biodegradable or not. Depending on the type of plastic, signs of biodegradation that can be captured by this method can take a long time to appear. Natural polymers more susceptible to biodegradation produce CO_2 faster than polypropylene, for instance, and are better suited for the method (Sadi et al., 2013). The aerobic biodegradation of plastics is also evaluated in controlled composting conditions at thermophilic temperatures (ASTM D5338, equivalent to ISO 14835). ASTM D5338 is often used together with ASTM D6400 (equivalent to ISO 17088), which determines the requirements needed to label a given plastic as compostable in aerobic municipal or industrial composting facilities. Based on this standard, a compostable plastic will have 90% or more of its fragments passing a 2 mm sieve after 12 weeks in composting conditions. At 180 days, 90% of the carbon present in the plastic must be converted to CO_2. ASTM D6400 mentions that the rate of degradation in the specified timeframe is thickness dependent, and each material that aims to be labeled as compostable must specify the maximum thickness at which the requirements above are met.

Anaerobic biodegradation can be simulated by the ASTM D5511 (equivalent to ISO 15985) in high-solids anaerobic-digestion conditions (wastewater sludge) or by ASTM D5526 in accelerated landfill conditions, both using sealed vessels to measure the gas residues (CO_2 + methane) over time. Both standards recommend the use of methanogenic inoculum derived from anaerobic digester as the medium to reproduce anaerobic conditions. ASTM D5526 is also designed to produce a mixture of household and plastic waste in different stages of degradation that can be used for ecotoxicological assessment.

The protocol described by ASTM D7475 combines both aerobic and anaerobic biodegradation in simulated biologically active landfills. In the aerobic phase, plastic specimens are mixed with pretreated household waste and changes in mass, molecular weight and selected physical properties should be measured before and after as indications of biodegradation. The anaerobic phase of the protocol is analogous to ASTM D5526.

5.4 Appropriateness of standard protocols for micro/nanoplastics research

Standard protocols for accelerated weathering explicitly state the claims that can be made with the measured results and highlight that the proposed exposure conditions of each protocol cannot be used to predict/extrapolate the absolute degradation rates of plastics. Their main advantage is to produce faster degradation and reproducible conditions compared to natural degradation (Gewert et al., 2018), which is sought after in micro/nanoplastics research while the drawback is creating conditions that do not match natural weathering.

The standards for outdoor exposure focus on accelerated photodegradation (Figure 5b) and are used to monitor the loss of bulk or surface properties after weathering (Figure 5c). These standards are not concerned with the generation of small fragments or leachates produced by the degradation. If a strip of textile made of plastic fibers maintains an acceptable color variation and mechanical properties after a standardized weathering test, for example, the product is approved even though it may produce microplastics during its common use. Furthermore, condensation and water spray cycles inside the weathering chambers can wash away these by-products. In microplastic research, the most mentioned outdoor exposure standards are the guides on how to operate either a fluorescent UV lamp or a Xenon arc lamp and water apparatus (ASTM G154 and G155) (González-López et al., 2020), which can be used in non-commercial weathering chambers commonly built in research laboratories. The most typical adaptation that is made in microplastic research is the use of a water-filled container containing the plastic to be weathered, to retain micro/nanoplastics and leachates during the process. This type of sample exposure is not covered by the international standard protocols, which were designed to expose plastic parts attached to a panel to produce homogeneous exposure. In some commercial weathering chambers that comply with the standards, the samples are exposed at an angle and even vertically, which makes it difficult to adapt for the exposure of open water-filled containers containing plastic to be weathered. This gap could be bridged with new standards on how to expose this type of sample aimed for leachate/micro and nanoplastic retention.

The standards for marine exposure and solid waste conditions are mainly used to evaluate biodegradable, compostable or oxo-biodegradable plastics by the biodegradation gases produced under different weathering conditions (Figure 5c). These types of plastic

759 are often presented as a sustainable alternative to conventional plastics, but the standards
760 used to evaluate biodegradability or compostability are also not concerned with the
761 generation of micro- and nanoplastics in soil or compost. A plastic classified as
762 biodegradable according to ASTM D6691, for instance, may not completely biodegrade in
763 the natural marine environment, since the test conditions described by the standard may
764 overestimate the natural biodegradation rate (Viera et al., 2021) while ASTM D6400
765 allows the presence of microplastics (fragments ≤ 2 mm) in the final compost after
766 fragmentation for a plastic to be labelled as compostable (Brodhagen et al., 2017). This
767 apparent contradiction has made ASTM D6400 one of the most cited international standard
768 protocols in microplastics research. Adapting these standards as a weathering method to
769 study plastic fragmentation is challenging due to the complexity of the remaining medium,
770 often a mixture of waste/soil/sediment and plastic fragments. As different methods to
771 separate micro- and nanoplastics from complex samples are being developed (Nguyen et
772 al., 2019), new international protocols designed specifically for the separation and analysis
773 of micro- and nanoplastics could be created and used in conjunction with existing
774 weathering standards.

775 A combination of protocols is also a potential future direction to create conditions
776 that are closer to natural weathering. ASTM D6954 is a guide that combines different
777 degradation pathways: thermal or photooxidation (outdoor exposure standards) followed
778 by biodegradation in soil or solid waste. This guide also recommends the assessment of the
779 ecological impact of degradation by-products. Each weathering step is analyzed separately
780 and consecutively. More characterization data to compare artificially and naturally
781 weathered samples (as described in Table 1) is needed to verify if this approach can
782 produce realistic samples, since natural weathering pathways often occur concurrently.

783 The size, thickness and shape of the specimens is rarely specified in most
784 weathering standards. The recommendation is that they should fit inside the sample holders
785 and be appropriate for the before/after properties measurements. But the rate of
786 fragmentation into micro- and nanoplastics is highly dependent on these characteristics. As
787 mentioned earlier, degradation pathways start on the surface, so samples with high surface
788 area are more susceptible to faster deterioration and fragmentation. This partly explains the
789 ubiquity of microfibers in the environment (Henry et al., 2019).

6.0 Overview of the current state of research on environmentally relevant microplastics and proposed weathering guidelines for future research.

This review outlined several important aspects related to protocols for obtaining environmentally relevant microplastics and nanoplastics:

- Most of the studies reviewed show that weathering largely has an effect on the behavior of microplastics in the environment, however, many studies (~90%) are still using pristine plastics.
- There is a lack of effect studies using aged nanoplastics from accelerated laboratory weathering or environmental samples.
- Environmental microplastics are dominated by fragments while those aged in the laboratory are mostly beads/spherical.
- Reported weathering studies are focused on polystyrene > polyethylene > polypropylene > polyvinyl chloride, while the most produced/detected plastics include polyethylene > polypropylene. Polystyrene has been overrepresented in microplastics research and more efforts should be dedicated to other plastic types, especially microfibers.
- Current plastic standard weathering protocols, developed before the increased concern about plastic pollution, may not be fully suited for microplastic studies as they aim to monitor durability and understand bulk plastic behavior, with little concern about fragments or leachates produced during degradation. Combining different protocols and creating new sampling protocols for micro- and nanoplastics could increase the use of international standards and improve reproducibility in microplastics research. To achieve this objective, more characterization data comparing naturally and artificially weathered samples is needed.
- Important weathering pathways are not well represented in microplastics research. Many microplastics will undergo biodegradation or biological coating under various temperature ranges, due to their predominance in biosolids streams or in land. Chemical oxidation encountered in the water treatment cycle is also overlooked.

- The combined impacts of several weathering pathways on polymer backbone alteration (e.g., mechanical stress combined with (photo)oxidation) and other surface modifications (e.g., NOM coating) are currently neglected, although such combinations are likely to drastically change interactions with surfaces and to synergistically contribute to plastic fragmentation.
- The characterization of leached plastics has particularly been overlooked. While we focus on the weathering of microplastics, we may miss an essential component: are smaller microplastics or nanoplastics being leached from primary microplastics and/or bulk plastics (Gigault et al., 2021)?

As the microplastic scientific community is now moving towards plastics and microplastics of greater environmental significance, it is important that protocols used for weathering effect studies be standardized for the sake of harmonization. Without documenting the actual conditions used and appropriate metrics, comparison across studies becomes challenging. Overall, there is a lack of justification of the choice for some weathering pathways. A selected method or protocol should attempt to mimic a weathering pathway encountered in the environment. As a way of harmonizing methods, we recommend that future weathering effect studies follow some of the guidelines presented in Table 2. In this table, important parameters related to materials and protocols are listed. Currently, only few microplastics research studies describe all these materials and parameters. Notably, too much focus has been given to the primary materials without considering the initial microplastics, leached chemicals and leached plastics as a whole.

840 **Table 2.** Proposed reporting guidelines for studies on effects of weathered microplastics

	Parameter/property	Guidance to improve comparability and reproducibility
Materials	Polymer type	Characterise polymer type before and after weathering
	Polymer source	Specify source: purchased or collected in the environment
	Physical and mechanical characterisation	Indicate the properties of the plastics before and after weathering. *e.g.,* color, size, shape, morphology, roughness, melting point, tensile strength, hardness, etc.
	Chemical characterisation	Report chemical changes before and after weathering. e.g., surface functionalization, crystallinity, surface charge, molecular weight
	Leached chemicals	For plastic leachates, report organic and inorganic products generated during weathering
	Leached plastics	Monitor the formation of secondary microplastics and nanoplastics during weathering
Methods/Protocols	Irradiance	For samples exposed to UV, report the total irradiance measured in the sample compartment and wavelength of light
	Weathering exposure time	Report the duration of each weathering exposure
	Weathering pathway	Justify the weathering pathway being mimicked in the environment
	Medium	Describe the background medium in which plastic is weathered. *e.g.,* air, activated sludge, seawater, saline solution, presence of organic matter, river water
	Oxidation	Report the dosage of oxidants (type, concentration, contact time)
	Temperature	Indicate the temperature in the weathering setup
	Humidity	Report the relative humidity in the weathering setup, especially for samples exposed to air
	Location	In the case of microplastics collected from the field, the location and environmental compartment as well as extraction procedure should be outlined
	Control	Control of same microplastic type and/or procedural blanks should be used to elucidate the effect of weathering
	Replicates	Characterise variability by replication

841

Acknowledgements

The authors acknowledge the support of the Canada Research Chairs program, the Natural Sciences and Engineering Research Council of Canada, the Killam Research Fellowship program, the Petroleum Technology Development Fund of Nigeria for an award to OSA, an NSERC Banting Fellowship to DCM, an NSERC postdoctoral fellowship to RSK, and Mitacs Canada and Kemira for a postdoctoral fellowship to ML. This project was supported partially by a financial contribution from Fisheries and Oceans Canada. We also thank Laura Rowenczyk for her helpful comments.

CRediT authorship contribution statement

Olubukola S. Alimi: Conceptualization, Writing – original draft, Data Curation, Formal Analysis, Visualization, Methodology. **Dominique Claveau-Mallet:** Conceptualization, Writing – original draft, Visualization, Supervision. **Rafael Kusuru:** Writing – original draft, Visualization, Data Curation. **Mathieu Lapointe:** Writing – original draft, Visualization. **Stéphane Bayen:** Writing – review & editing, Supervision. **Nathalie Tufenkji:** Writing – review & editing, Supervision.

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