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Leachates from weathered polypropylene items, but not from polylactic acid, induce ecotoxicological effects on a marine diatom

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Abstract

Plastic items released in the marine environment are subject to weathering processes which alter their surface physiochemical properties, and which can induce the leaching of associated chemicals from the polymer matrix to the aquatic media. Even though plastic leachates (i.e., an aqueous solution of released compounds) can induce negative effects on aquatic biota, the effects of plastic leachates from weathered plastics are still poorly known. The goal of our work was to assess the ecotoxicological effects of plastic leachates from pristine and weathered items on a marine diatom. To do so, we exposed the marine diatom *Phaeodactylum tricornutum* to the leachates from pristine and UV-weathered self-reinforced (SR-) polylactic acid (PLA) and polypropylene (PP) items. As a positive control, we assessed the response of the diatom growth to two associated compounds (dodecan-1-ol and 2,4-di-tert-butylphenol), components of the tested items. Weathered plastic items were obtained after being exposed to 57-day artificial ultraviolet radiation, simulating 18-months of solar exposure in central Europe. Our results indicate that neither leachates from pristine nor weathered SR-PLA items had adverse effects on *P. tricornutum* growth. This outcome was corroborated by the measured concentrations of associated compounds (i.e. dodecan-1-ol) in leachates, which were at least three orders of magnitude lower than the 72-h EC_{50} of diatom growth (dodecan-1-ol: 1.56 mg / L, 2,4,-DTBP: 1.52 mg / L). Leachates from weathered SR-PP items at full-strength concentration induced 51% greater growth inhibition than control treatments, and 43% greater inhibition than leachates from pristine SR-PP items at the same concentration. Our results suggest that leachates from the tested weathered SR-PP items inhibited the population growth of *P. tricornutum*, although at plastic concentrations above environmentally relevant levels.

Keywords Plastic leachates, Polylactic acid, Polypropylene, Self-reinforced composites, Growth inhibition, Marine diatom, Ultraviolet weathering

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Introduction

Leachates from plastic materials and litter, i.e., solutions of compounds that have migrated from the polymer matrix to the aqueous media, can induce negative effects on aquatic organisms [1–3]. Of particular concern are the effects in primary producers [4], as these occupy a key trophic level in aquatic ecosystems [5]. For example, leachates from high density polyethylene (HDPE) and polyvinyl chloride (PVC), with plastic-to-water ratios up to 25 and 5 g / L respectively, altered the growth and photosynthesis efficiency of marine photosynthetic cyanobacterium *Prochlorococcus spp* [6]. Similarly, the growth of the marine microalgae *Skeletonema costatum* can be inhibited when the organisms are exposed to plastic leachates (80 g / L, car tire rubber, polypropylene (PP) and PVC) [3]. This susceptibility of microalgae and other phytoplanktonic organisms to plastic leachates, and the consequent potential for population growth inhibition, underscores the risk of cascading trophic disruptions within aquatic ecosystems [7, 8].

The ecotoxicological effect assessment of plastic leachates are often done using pristine plastics, i.e., items prior to use and disposal stages, rather than from environmentally aged plastics [9]. However, most plastics that accumulate in the environment are subject to weathering processes that alter their surface physicochemical properties [10, 11]. In particular, the surface of stranded plastics can undergo photochemical reactions due to the absorption of ultraviolet (UV) radiation, which causes oxidation of polymers, cleavage of the carbon-chains and the formation of cracks and grooves textures on plastic surfaces [12–15]. Therefore, the leaching of associated compounds from weathered plastics may alter when plastics enter aquatic environments, and the composition of these plastic leachates may differ from those of pristine plastics [16–18]. As a result, the effects of the plastic leachates from weathered items on aquatic species may differ from pristine plastics [19, 20]. For example, when the photosynthetic marine bacteria *Prochlorococcus* was exposed to leachates from HDPE items weathered in the field for 112 days, their growth was 50% inhibited when compared to leachates from pristine HDPE [20].

To date, the potential effects of plastic leachates from weathered plastics have been studied to a lesser extent compared to pristine ones, leaving a gap in the current knowledge of the ecotoxicological effects of environmentally aged plastics [21]. Therefore, our goal was to assess the impact of leachates from plastic items on the growth of the *Phaeodactylum tricornutum* [22] (marine diatom), from both pristine and UV-weathered self-reinforced (SR-) polylactic acid (PLA) and SR-polypropylene (PP). To do so, we exposed this marine diatom to the leachates from pristine and UV-weathered items of both polymers, following the standardised ISO 10,253 protocol [23], and used two individual associated compounds as a positive control. These compounds, dodecan-1-ol and 2,4-di-tert-butylphenol, had been identified in the tested plastic items, and were used to compare the half maximum effect concentration (EC_{50}) with the concentrations assessed in the plastic leachates and values reported in literature [24].

Results

Organic volatile and semi-volatile associated chemicals to plastic items and leachates

Eight organic compounds were identified in the plastic items' extracts from pristine SR-PLA and ten in plastic items' extracts from pristine SR-PP items (Tables S1, S2 and S5). Two of them, dodecan-1-ol and 2,4-DTBP (also the positive controls for ecotoxicity test used in this work), were selected for leachates quantification. For leachates' samples, the recovery rate ($n = 3$) of dodecan-1-ol and 2,4-DTBP with internal standards was 90% and 110%, respectively. After accounting for the loss during extractions, concentrations of dodecann-1-ol and 2,4-DTBP in all leachates were below the respective limit of quantification (LOQ: Dodecan-1-ol, 100 µg / L; 2,4–DTBP, 25 µg / L; values calculated from Figure S1). In addition to dodecann-1-ol and 2,4-DTBP, 2-propenoic acid, oxybis(methyl-2,1-ethanediyl) ester and 1H-pyrrolo[2,3-b]pyridine, 3-amino-2-phenyl- were observed in pristine SR-PLA leachates, while tributyl acetylcitrate and 4-methyl-2,4-bis(4'-trimethylsilyloxyphenyl)pentene-1 were observed in UV-weathered SR-PLA leachate (Table S5). For SR-PP, hexadecane and 4H-Furo(3,2-g)(1)benzopyran-4,7,9-trione were detected in weathered leachates, and no other compounds were identified in pristine leachates (Table S5).

Ecotoxicological effects on microalgal growth

Exposure to leachates from UV-weathered SR-PP induced a decrease in the specific growth rate (d^{-1}) of *Phaeodactylum tricornutum* (Fig. 1). The mean specific growth rate in 72 h were 0.96 ± 0.06 d^{-1} in negative controls, 0.98 ± 0.11 d^{-1} in pristine SR-PP leachates, and 0.86 ± 0.25 d^{-1} in UV-weathered SR-PP leachates. When comparing the effects of pristine and UV-weathered leachates on microalgal growth, we observed that the mean specific growth rate (d^{-1}) in 100% leachates concentration of UV-weathered SR-PP (0.46 ± 0.23 d^{-1}) was 51% lower than in negative controls (0.96 ± 0.06 d^{-1}, $p < 0.001$, Dunnett's test) and 43% lower than in 100% pristine SR-PP leachates (0.81 ± 0.11 d^{-1}, $p = 0.002$, Dunnett's test). However, exposure to pristine SR-PP leachates had no effects on the growth rate ($p > 0.05$, ANOVA). With respect to SR-PLA, no inhibitory effect was observed on the growth of *P. tricornutum* by the leachates from pristine and weathered items (Fig. 1). The mean

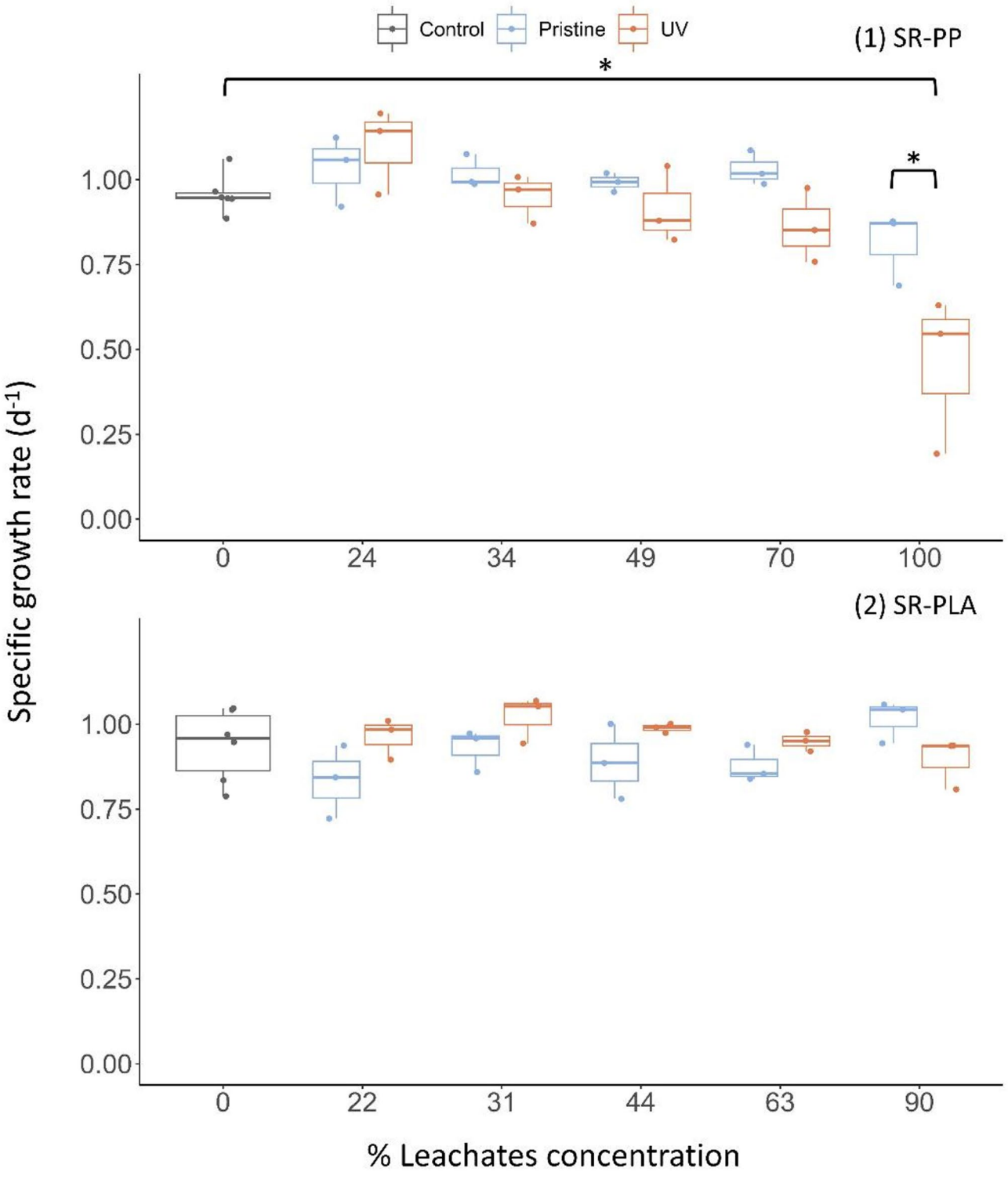

Fig. 1 Boxplot of growth rates, with the median specific growth rate (d^{-1}) according to dilutions (%) of leachates from (1) self-reinforced polypropylene (SR-PP) and (2) self-reinforced polylactic acid (SR-PLA). The specific growth rate (d^{-1}) was calculated using cell density (cells / mL) counts [Eq. 1]. The colour of boxes and dots represents controls (no leachates, $n = 6$ separate flasks, in grey), leachates from pristine plastic items ($n = 3$ separate flasks, in green) and leachates from UV-weathered plastic items ($n = 3$ separate flasks, in orange). The mean specific growth rate of controls was 0.96 ± 0.06 d^{-1} (1) and 0.94 ± 0.11 d^{-1} (2), respectively. The mean initial cell density was $3.36 \pm 0.7 \times 10^4$ cells / mL (SR-PP) and $1.81 \pm 0.5 \times 10^4$ cells / mL (SR-PLA). Asterisks (*) indicate statistically significant differences between groups ($p < 0.05$, Tukey's HSD for SR-PLA, Dunn's multiple comparison test for SR-PP)

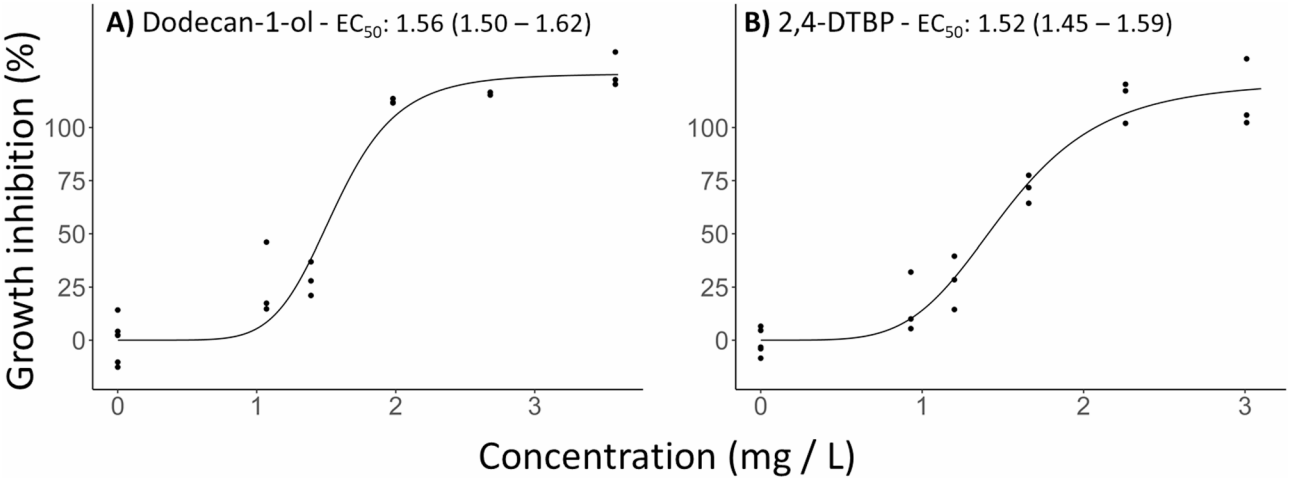

Fig. 2 Concentration-response curves of the growth inhibition (%) after a 72-h exposure according to the mean measured concentrations (mg / L) of two associated compounds: (**A**) dodecan-1-ol and (**B**) 2,4-di-tert-butylphenol (2,4-DTBP). The number of replicate flasks for each treatment and control was three and six, respectively. Doedecan-1-ol was detected in extracts from pristine self-reinforced polylactic acid (SR-PLA) items while 2,4 – DTBP was detected in pristine self-reinforced polypropylene (SR-PP) items (Tables S1 and S2). The concentration (mg / L) of these additive compounds were measured in parallel samples (Table S4). The mean initial cell density was $2.2 \pm 0.5 \times 10^4$ cells / mL (**A**) and $1.9 \pm 0.6 \times 10^4$ cells / mL (**B**). The mean specific growth rates of controls in these three panels are 1.03 ± 0.10 (**A**) and 1.13 ± 0.07 d^{-1} (**B**), respectively. The growth inhibition (%) was calculated by [Eq. 2] using specific growth rate over 72 h. Curves were fitted with four-parameter log–logistic dose–response models (bottom plateau constrained to 0). EC_{50} values (effective concentration inducing 50% growth inhibition) are reported with the 95% confidential intervals

specific growth rate in 72 h was 0.94 ± 0.11 d^{-1} in negative controls, 0.91 ± 0.09 d^{-1} in pristine SR-PLA leachates, and 0.96 ± 0.06 d^{-1} in UV-weathered SR-PLA leachates. Up to 90% leachate concentrations, either pristine or UV-weathered SR-PLA leachates, had no significant effect on the mean specific growth rate ($p > 0.05$, ANOVA).

As a positive control, *P. tricornutum* were exposed to two known additive compounds (dodecan-1-ol and 2,4-DTBP) (Fig. 2). For both dodecan-1-ol and 2,4-DTBP, the specific growth rate in solvent control (0.01% DMSO) was not significantly different from the negative controls ($p > 0.05$, Tukey's HSD). The determined 72-h EC_{50} of dodecan-1-ol and 2,4-DTBP to algal growth were 1.56 (1.50–1.62) and 1.52 (1.45–1.59) mg / L.

Discussion

In the present study, the leachates from pristine SR-PLA and pristine SR-PP items, extracted in seawater from items at concentrations up to 80 g / L, did not alter the growth of *Phaeodactylum tricornutum* (Fig. 1), suggesting that leachates from the tested items had no effect on the growth of this marine diatom. The plastic concentration (80 g plastic / L water medium) used to generate leachates was aimed at studying the ecotoxicological effects of plastic leachates [3, 25], and was four orders of magnitude higher than that of macroplastics concentrations reported in estuarine and harbour waters (0.0008 ± 0.0204 g / L in the North Sea ports of Belgium [26]). Similarly, and at a lower plastic-to water ratio (0.1 g / L), leachates from pristine PET powder did not impede the population growth of the same diatom species, *P. tricornutum*. However, and at the same plastic-to-water ratio as the present study (80 g / L), leachates from pristine commercial items such as polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and car-tire-rubber (CTR) hindered the specific growth rate of the marine diatom *Skeletonema costatum* over a 72-h exposure [3]. Indeed, the marine diatom species *P. tricornutum* and *S. costatum* can show a different sensitivity to hydrophobic pollutants [27, 28]. The fact that the same species show diverging ecotoxicological effects highlights that the observed effects on the population growth of microalgae are specific to the leachates of particular plastic items rather than species-specific [21]. In the present work, the marine diatom *P. tricornutum* was selected due to its well-established role as a model species in ecotoxicology [23], even though the effect thresholds for growth inhibition observed may differ from other microalgal taxa [3]. Moreover, although no inhibition of microalgal population growth rate was observed in the present work, plastic leachates may still induce early cellular stress such as oxidative damage and transcriptional shifts [29, 30]. For example, Gao et al. [30] found that leachates from aged PE and PVC, exposed at 10 g / L, increased the intracellular reactive oxygen species (ROS) levels by up to 52% and altered the expression of genes involved in translation and ribosomal function in *Chlorella vulgaris*, even without growth inhibition being observed. However, the present study provided a baseline for future research to build upon by incorporating multiple microalgal species and endpoints, enabling a more comprehensive assessment of species-specific sensitivities and

refining estimates of effect thresholds across diverse phytoplankton communities.

Even though leachates from pristine items in this study did not induce ecotoxicological effects, the full-strength leachates from UV-weathered SR-PP items induced growth inhibitory effects in *P. tricornutum* (Fig. 1). Similarly, leachates from UV-weathered polyethylene (PE) induced inhibition of the photosynthesis and growth of the microalgae *Scenedesmus vacuolatus*, whereas pristine PE leachates had not [31]. The inhibitory effects of leachates from weathered SR-PP in microalgal growth observed in the present study (Fig. 1) were not observed in leachates from UV-weathered SR-PLA. These results are most likely the result from the polymer degradation at the surface of the SR-PP items [32], as most plastics, including PP and PLA, can undergo a photochemical reaction under UV radiation, inducing cleavage of C–C and C–H bonds and thus formation and release of low-molecular-weight monomers and oligomers [12, 14]. In the present study, this was confirmed as we identified the presence of Hexadecane, a monomer of PP, in the leachates from UV-weathered SR-PP, but not in leachates from pristine SR-PLA (Table S5).

The non-toxicity on microalgal growth of the leachates from pristine SR-PLA and pristine SR-PP items tested in the present study is in accordance with the low observed content of organic compounds in the resulting leachates of this study. Our chemical analysis (see *Results, "Organic volatile and semi-volatile associated chemicals to plastic items and leachates"*) confirmed that the measured concentrations of associated compounds, i.e. dodecan-1-ol and 2,4-DTBP, in both SR-PLA and SR-PP leachates respectively were lower than three orders of magnitude compared to their 72-h EC_{50} on microalgal growth determined in the present study (Fig. 2), as well as values reported in reported in the REACH dossier [24]. Similarly, leachates from commercial PET containing no organic additives had no effects on the growth of *S. costatum* [3]. As the leaching protocols vary among studies [33], the low content of associated compounds observed in our plastics leachates likely result from two key methodological choices: the use of synthetic seawater at ambient temperature and the use of large, macro-sized plastic items. In this work, all plastic leachates were generated in synthetic seawater (salinity 33 PSU) at 22 °C, conditions chosen to reflect the salinity and temperature of North Sea surface waters during summer [34]. By avoiding the use of intermediate solvent and an elution step, we aimed to preserve a natural constitution of associated chemicals in the plastic leachates. Meanwhile, the plastic items used in present study are at macro-sized range (> 2.5 cm) and at least 10-fold larger than the tested plastics in literature [3, 35]. For example, the concentration of phthalate additives in the leachates from 0.1 µm PS was up to two orders of magnitude higher than in leachate from 1.0 µm PS, due to a greater-specific-surface area, and had a higher growth-inhibition of *S. costatum* [29]. Smaller plastic items, with a greater-specific-surface area, can also be more susceptible to weathering-induced degradation [36], which can in turn alter the composition of leachates (e.g. the release of degradation products of the polymer and associated chemicals) and their potential effects on microalgae. Still, the mass-wise majority of plastic litter in the environment are at macro-sized range (> 25 mm) and will remain within this size range for years until fully fragmentated into microplastics [37, 38]. As such, the leaching rate of associated compounds from the tested pristine SR-PLA and SR-PP items in the present study were either lower or slower [39, 40], mimicking the potential leaching rates of stranded plastic litter in the environment.

Despite having observed effects on leachates from UV-weathered SR-PP items, a more specific inspection as to which exact compound(s) led to the observed growth inhibition of *P. tricornutum* is challenging. For example, the hexadecane, a PP monomer detected in the weathered leachates, has been shown to impede the population growth of the freshwater microalgae *Raphidocelis subcapitata* at concentrations exceeding 100 mg / L [41]. However, by using gas chromatography–mass spectrometry (GC–MS), the chemical analysis methods used in the present work focused on volatile and semi-volatile compounds, as a systematic and high-resolution chemical profiling was not our main intention. This means that other non-volatile chain-scission products (e.g., dicarboxylic acids), which are commonly analysed using liquid chromatography–mass spectrometry (LC–MS) [42], may also have been present in the plastic leachates of UV-weathered PP. These compounds, such as dicarboxylic acids are known to have inhibit the growth of the microalgae *S. vacuolatus* [31] and could have contributed to the observed effect in the present work (Fig. 1). Although there is currently no consensus on standardised methods for non-targeted analysis of organic compounds in plastic leachates [14, 18], future studies should consider employing complementary analytical techniques, such as a combination of GC–MS and LC–MS, to achieve a more comprehensive chemical characterisation and a better understanding of the contribution of leachate components to the observed ecotoxicological effects. Moreover, the combined effects of multiple chemicals in the leachates may also play a role, as the combined effects could have either a synergistic, additive or antagonistic manner [43]. For example, the combination of two flame retardants triphenyl phosphate (TPP) and tris(1,3-dichloro-2-propyl)phosphate (TDCPP) exhibited synergistic effects on cytotoxicity [44]. This also highlighted the importance of assessing the ecotoxicological effects

of plastic leachates as a black box as per the present work and earlier studies [3]. Although the plastic leachates were considered as a whole in the present study, future research is encouraged to further investigate the underlying ecotoxicological mechanisms, including the identification of interactive effects (e.g., synergistic, additive, or antagonistic) among the various compounds present. In addition, the formation and leaching of low-molecular-weight monomers and oligomers has been confirmed from PP after exposure to UV radiation [17], due to their bond dissociation energy under UV irradiation where PP (77 kcal / mol) < polyester (88 kcal / mol) [45]. The reason why this phenomenon most likely did not take place in the tested SR-PLA item is confirmed by our previous work where the same PLA items retained their oxidation resistance after a 57-day UV exposure (same UV dose), while the same PP items lost all oxidation resistance [32, 46]. This implies that, after a longer UV-radiation exposure (equivalent to two-year solar exposure in Central Europe), once PLA items lost their oxidation resistance, the composition of plastic leachates may alter, possibly inducing adverse effects on the marine diatom population at environmentally relevant macro-plastics concentrations.

Overall, our results showed that leachates from the tested petroleum-based plastic items inhibited the population growth of the marine diatom even after an 18-month equivalent of UV-weathering in the Central Europe (Fig. 1). However, the inhibitory effect was observed at a plastic concentration of 80 g / L, which is four orders of magnitude higher than that of macroplastics concentrations reported in estuarine and harbour waters [26]. Even though the plastic concentration of 80 g / L is considerably higher than the environmentally relevant levels (e.g., 0.0008 ± 0.0204 g / L in the North Sea ports of Belgium [26]), we followed previous reports on ecotoxicological assessment of leachates under laboratory conditions, including a dose-response assessment along a decreasing gradient leachates concentration [3, 35]. This approach is intended for effect assessment, which can inform risk assessment, but may differ from desorption dynamics in the environment or environmental exposure levels [47]. Moreover, interactions between plastic leachates and other contaminants, particularly naturally occurring organic substances such as humic acids or algal exudates, may modulate their bioavailability and combined-ecotoxicological effects on microalgae [48–50]. Our results provide an overview of leachates ecotoxicological effects, which can serve as building blocks for future assessments which incorporate additional environmentally relevant co-contaminants. Therefore, our results inform that the weathering of plastic items or litter in the marine environment may alter the (potential) effects of their leachates on marine microalgae, leading to a more complex situation for assessing their environmental impact. In a broader context, the plastic leachates of weathered SR-PP in this study induced a reduction of the population growth of marine diatoms, an important component of the marine phytoplankton community [51, 52]. This potential reduction of the primary production can induce cascading effects on the trophic web, by limiting food availability to grazers [primary consumers] [53, 54]. Therefore, future efforts should consider removing the existing plastic litter in the aquatic environment, particularly in accumulation zones, to prevent possible adverse effects on the key species of marine primary producers.

Conclusion

Our results indicate that leachates from the pristine tested items of SR-PLA and SR-PP did not inhibit the growth of *P. tricornutum*, a primary producer with a high relevance in marine trophic webs, in accordance with the assessed concentrations of associated-compounds (dodecan-1-ol and 2,4-DTBP), which were at least three orders of magnitude lower than the 72-h EC_{50} on microalgal growth. However, leachates from UV-weathered SR-PP items (100% leachates concentration) induced 51% and 43% growth inhibition compared to controls and leachates from pristine items. Even though the tested plastic concentration (80 g / L) was higher than current environmentally relevant levels of plastic litter accumulation in aquatic environments, the observed growth inhibition of microalgal populations could occur in the immediate vicinity of large plastic weathered debris. Our study informs that UV-weathering of plastics can alter the toxicological effects of plastic leachates on microalgal growth, and so regulators should consider the removal of plastic litter before UV-weathering occurs to avoid adverse effects in plankton species.

Materials and methods

Test materials and their preparation

All substances and reagents used in this study were of high-performance-liquid-chromatography (HPLC) grade and deionized water was of Milli-Q quality (MilliPore®). Artificial seawater (salinity 33 PSU) was prepared according to the ISO 10,253 protocol [23] and filtered through 0.22 µm with a sterile filter (Sterivex®). Pristine self-reinforced polylactic acid (SR-PLA) and self-reinforced polypropylene (SR-PP) plates, obtained from the European BIO4SELF project [55], were cut into $2.0 \times 7.5 \times 0.13$ cm items (surface area = 32.5 cm^2) using stainless steel scissors. All plastic items were dried at 22 °C in the dark prior to use. To obtain 80 g UV-weathered plastics, plastic items were placed in pre-cleaned 25 mL quartz cuvettes in dryness and in a UV chamber (Altas Suntest CPS+). Irradiation was conducted at 60 W / m^2 with the emission range at 300–400 nm and the temperature

in the chamber was maintained at 30–35 °C and plastic items were collected after 57 days, simulating 18 months of natural solar exposure in Central Europe [17].

Pristine plastic items were subjected to a non-target screening for volatile and semi-volatile organic associated compounds after a solvent extraction of the materials and the extracts were analysed by gas chromatography mass spectrometry (GC-MS) (See *Materials and Method*, *"Leachates characterisation"*). Among all identified substances (Table S1 and S2), we selected two compounds for leachates quantification and ecotoxicological assessment: Docecan-1-ol (CAS: 112-53-8, Chem-lab NV), detected in SR-PLA items, and 2,4-di-tert-butylphenol (2,4 - DTBP, CAS: 96-76-4, Chem-lab NV), detected in SR-PP items. The selection of the two compounds was based on four aspects: firstly, dodecan-1-ol was only detected in SR-PLA and 2,4-DTBP was only detected in SR-PP; secondly, both dodecan-1-ol (a residual solvent) and 2,4-DTBP (a UV stabiliser) are known plastic-associated chemicals [56]; thirdly, both compounds are water soluble, water absorbable and stable in water [24]; fourthly, both compounds (in HPLC grade) and their internal standards are commercially available, which enables their analytical detection and quantification in leachates.

Test species

The microalgae *Phaeodactylum tricornutum* strain 1052/1A was obtained from the Culture Collection of Algae and Protozoa (Oban, United Kingdom) and cultured in the Marine Station Ostend of the Flanders Marine Institute (VLIZ, Ostend, Belgium). The microalgae growth medium was prepared in artificial seawater (see *Materials and Methods*, *"Test materials and their preparation"*) according to the ISO 10,253 protocol [23]. *Phaeodactylum tricornutum*, were cultured in 250 mL Erlenmeyer flasks (volume of culture = 100 mL) at 18 °C under a continuous white light (> 2,500 lx) and manually shaken twice a day [57]. All glassware was pre-cleaned with diluted Decon [58] and Milli-Q water and autoclaved at 132 °C for over an hour, to avoid contamination of the cultures by other organisms.

Leachates Preparation

Leachates of pristine and weathered plastic items were generated according to the method of Capolupo et al. [3] with a few modifications. Plastic items (32 g each) were added at a plastic-water ratio of 80 g / L to 400 mL pre-filtered synthetic seawater [57], in a sterilised glass 1 L bottle wrapped in aluminium foil, to avoid light degradation of photo-sensitive compounds. For pristine and UV-weathered plastic items, triplicated leachates were prepared. Then these bottles were incubated on an orbital shaker (80 rpm) at room temperature (22 °C) for 21 days. To monitor the background variance of water pH and salinity, triplicated bottles of water blanks (not containing plastics) were incubated alongside the "leachate bottles". After the incubation period, the leachates were filtered through a 0.22 µm sterile filter (Sterivex®) and mixed into a total volume of 1,200 mL, to which the algal nutrient components were added according to the ISO protocol [23]. The pH in the leachates solution from weathered SR-PLA (7.75) was lower than in seawater blanks (7.87, Table S3) and so the pH in all leachates was adjusted to 8.0 ± 0.2 prior to growth inhibition tests [23]. Triplicated subsamples (2 mL) from each leachate bottle were aliquoted and acidified (15% HCl, pH < 2) for chemical analysis (see *Materials and Methods*, *"Leachates characterisation"*), and stored at -20 °C until further processing.

Leachates characterisation

Solvent extraction of plastic items' samples

The solvent extraction of plastic items' samples was performed based on Davies et al. [59] with a few modifications. Samples (0.5 g) of plastic items were extracted with 10 mL methanol (HPLC grade, Fisher Scientific) and the sample bath sonicated for 15 min at room temperature. The plastic items' extract was then filtered (0.2 µm) through a pipette packed with Bilson cotton and a small amount of anhydrous Na_2SO_4 to remove particulates, concentrated by solvent evaporation (40 °C under a gentle flow of N_2) to approximately 2 mL.

Solid-phase extraction of leachates samples

Plastic leachates samples were subject to a wet to solid-phase extraction [60]. To do so, samples (2 mL) of aqueous leachates were spiked with surrogate internal standards (2 µg dodecanol-d25 and 2 µg 2,4-di-tert-butylphenol-d21). Samples were then passed through a solid phase column (Isolute® ENV + 100 mL / 6mL, Biotage, Sweden). The column was conditioned and equilibrated consequently with 2 mL dichloromethane, 2 mL methanol, and 2 mL acidified water. After this, analytes were eluted with 2 mL dichloromethane. Samples were spiked with 2 µg anthracene-d10 to establish the recovery rates of the procedure.

Gas chromatography mass spectrometry (GC-MS) analysis

The solid and leachates' extracts were analysed by gas chromatography mass spectrometry (GC-MS) using a non-target screening method. The GC-MS system comprised a Trace 2000 GC equipped with a Trace DSQ-Mass Spectrometer (Thermo Fisher Scientific). The carrier gas was helium (6.0), at a constant flow of 1 mL / minute. A 1 µL aliquot of sample was injected by pulsed spitless injection. The GC-column was an Agilent DB5-MS GC column (30 m, 0.25 µm film thickness, 0.25 mm

internal diameter). The GC oven was held at 40 °C (1 min), ramped by 15 °C / min to 300 °C (5 min hold). Mass spectra were recorded in the range of 40–335 m / z. Chromatograms and mass spectra were recorded using the X-Calibur software for acquisition (version 1.4) and processing data (version 4.2). After initial inspection of chromatograms, peaks with a signal-to-noise ratio ≥ 35 were deconvoluted using AMDIS software (version 2.66) and best hits from national institute of standards and technology (NIST) 2017 mass spectra library [61] were extracted. Following the methodology described by Capolupo et al. [3] and Sørensen et al. [62], compounds were identified by matching peaks (≥ 70%) to the NIST17 library [61]. For the leachates' extracts, Dodecan-1-ol and 2,4-DTBP were then quantified, and the internal standards were used to correct for losses during extraction and injection (Figure S1).

Microalgal growth inhibition tests

In this study, we exposed the marine diatom *P. tricornutum* to a dilution series of the plastic leachates in four independent batches: from pristine and weathered SR-PLA, and from pristine and weathered SR-PP. The effects of plastic leachates in *P. tricornutum* were determined as the inhibition of growth over 72 h. Considering the complexity in chemical-composition of the leachates' solutions, we used a nominal dilution series (% leachate concentration) instead of absolute concentrations (mg / L) [3, 25]. The dilution series was obtained by diluting the leachates solution 1.4 times in algal growth medium (see *Materials and Methods*, *"Test materials and their preparation"*) at each step. For all SR-PP leachates, this was 0 (no leachate), 24, 34, 49, 70 and 100% leachates concentrations. For all SR-PLA leachates, the dilutions comprised 0 (no leachate), 22, 31, 44, 63 and 90% (I.e. due to limited leachates volume) leachates concentrations. Besides the exposure to the leachates' solutions, diatoms cultures were further exposed to a dilution series of two individual associated compounds, previously detected in the tested plastic items (see *Materials and Methods*, *"Test materials and their preparation"*), as a positive control in independent batches. Dodecan-1-ol (CAS: 112-53-8) had been detected in SR-PLA items (Table S1), and 2,4-di-tert-butylphenol (2,4 - DTBP, CAS: 96-76-4) had been detected in SR-PP items (Table S2). To do so, stock solutions of dodecan-1-ol (reagent grade, Chem-lab NV) and 2,4-DTBP (reagent grade, Chem-lab NV) were prepared with 0.01% v / v dimethyl sulfoxide (DMSO) (Chem-lab NV) as a carrier solvent, at a concentration below which would induce any additional ecotoxicological effects in microalgal growth [62]. For both compounds, the six different concentrations were prepared in a dilution series. For Dodecan-1-ol, the dilution series comprise 0 (control), 1.23, 1.69, 2.25, 3, 4 mg / L. For 2,4 – DTBP, this was 0 (control), 0.92, 1.23, 1.69, 2.25, 3 mg / L. The concentrations of these two compounds in the dilution series were then measured in parallel samples following the same methods as leachates extracts (see *Materials and Methods*, *"Solid-phase extraction of leachates samples"*).

The 72-h microalgae growth inhibition test was performed according to ISO 10,253 [23]. Four days prior to the set-up, a microalgae pre-culture was incubated at 20 °C with a light regime of 10,000 lx to obtain exponentially growing *P. tricornutum*. All treatments were prepared with three independent biological replicates, and the negative control (growth medium only) included six replicates. Each flask was inoculated to an initial average cell density of $2.3 \pm 0.9 \times 10^4$ cells / mL (mean ± standard deviation). All flasks were incubated in the above-mentioned conditions for 72 h and continuously aerated with pre-filtered air to maintain the pH. The cell density (cells / mL) in all flasks were quantified every 24 h for the duration of incubation by a haemocytometer counting chamber under a light microscope (DM1000, Leica) with 10x objective lens. According to ISO 10,253 [23], the average specific growth rate μ (d^{-1}) for each flask was calculated as:

$$\mu = \frac{\ln N_e - \ln N_0}{t_e - t_0} \quad \text{(Eq. 1)}$$

where N_e and N_0 were the cell density (cells / mL) at time t_e (time of test termination) and t_0 (time of test start). The inhibition $I_{\mu i}$ (%) to specific growth rate μ_i (d^{-1}) for test flask i was expressed as:

$$I_{\mu\, i} = \frac{\bar{\mu}_c - \mu_i}{\bar{\mu}_c} \times 100\% \quad \text{(Eq. 2)}$$

where $\bar{\mu}_C$ and μ_i were the mean growth rate (d^{-1}) in control flasks and in test flask i, respectively. The specific growth rate μ (d^{-1}) and inhibition $I_{\mu i}$ (%) inferred was expressed as mean ± standard deviation (SD).

Data analysis

All data [63] was compiled and made open access according to the FAIR principles [64]. Statistical tests were performed in R v4.2.1 [65]. An analysis of variance (ANOVA) was performed to assess the difference of the specific growth rate (d^{-1}) in treatments (UV-weathering of plastic items and dilution (%) of leachates). Normality and homoscedasticity were checked using the Shapiro-Wilk and Levene tests, respectively, and statistical significance was accepted for $p \geq 0.05$. Tukey honestly significant difference (Tukey HSD) test was applied to compare the variance in specific growth rate among groups. When the assumption of normality was not met ($p < 0.05$,

Shapiro–Wilk test), a Kruskal-Wallis rank sum test was performed with the data concerning the specific growth rate obtained in the test with plastic leachates from pristine and UV-weathered SR-PP items, and statistical significance was accepted for $p < 0.05$. The Dunnett's test was applied to compare the variance in growth among groups (leachates concentration per treatment), using DescTools package [66]. Data from algal growth exposed to individual compounds was subjected to a non-linear regression analysis (growth inhibition (%) versus concentration (mg / L) of tested compound, bottom plateau constrained to 0) and 72-h EC_{50} calculation (95% confidential interval) using R 'drc' package [67]. To ensure precision and accuracy of the dose-response analysis, 72-h EC_{50} calculations were considered applicable only to data showing statistically significant differences compared to controls [1–3].

Quality assurance quality control

Quality Assurance and Quality Control (QA/QC) measures were implemented during the experimental procedures to avoid external contamination [68]. All solvents used were of analytical grade with minimal impurities. All glassware was pre-cleaned using diluted decon-90 detergent and rinsed thoroughly with Milli-Q water and acetone, and glassware was sterilized in an autoclave at 134 °C for one hour. Potential sources of microplastic contaminations were minimized by avoiding the use of any plastic equipment and by preferably using prewashed glass and metal items. All microbiological manipulations were performed in a clean flow cabinet. The validity of leachates quantification method was confirmed by the measured concentrations of dodecan-1-ol and 2,4-DTBP in individual compounds exposures (Table S4). The determined 72-h EC_{50} to microalgal growth of dodecan-1-ol and 2,4-DTBP were compared with reported EC_{50} values in the REACH dossiers database of European Chemicals Agency [24].

Abbreviations

2, 4 DTBP	2,4-Di-tert-butylphenol
ANOVA	Analysis of Variance
CTR	Car-Tire-Rubber
DMSO	Dimethyl Sulfoxide
EC50	Half-Maximal Effect Concentration
GC-MS	Gas Chromatography-Mass Spectrometry
HDPE	High-Density Polyethylene
HPLC	High-Performance Liquid Chromatography
LOQ	Limit of Quantification
NIST	National Institute of Standards and Technology
PLA	Polylactic Acid
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl Chloride
REACH	Registration, Evaluation, Authorisation, and Restriction of Chemicals
SR-	Self-Reinforced
Tukey HSD	Tukey Honestly Significant Difference
UV	Ultraviolet

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43591-025-00143-8.

Supplementary Material 1

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Author contributions

Z.N. conceived the study, conducted the investigation, developed the methodology, performed formal analysis, curated the data, generated the visualizations, and wrote the original draft. S.A.S. contributed to the investigation and formal analysis and participated in manuscript review and editing. M.C. and M.L.G contributed to methodology development and project administration and revised the manuscript. E.D. provided resources, conducted the investigation, contributed to methodology development, revised the manuscript, and acquired funding. J.A. provided resources, participated in the investigation and methodology development, revised the manuscript, and supervised the project. C.R.J. provided resources, revised the manuscript, and supervised the project. D.H. conceived the study, led project administration, acquired funding, contributed to methodology development, revised the manuscript, and supervised the project. P.D. contributed to methodology development, revised the manuscript, and acquired funding. G.E. conceived the study, developed the methodology, led project administration, revised the manuscript, supervised the project, and acquired funding. A.I.C. conceived the study, developed the methodology, led project administration and manuscript revision, acquired funding, and supervised the project. All authors read and approved the final manuscript and agreed to be held accountable for their contributions and the integrity of the work.

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Data availability

The dataset produced and presented in the work can be found in the official data repository Marine Data Archive, https://doi.org/10.14284/745.

Declarations

Competing interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors Elke Demeyer is employed by Centexbel (Ghent, BE), a private company highly active in the knowledge transfer of textile and plastic processing industry. The other authors report no conflict of interest.

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