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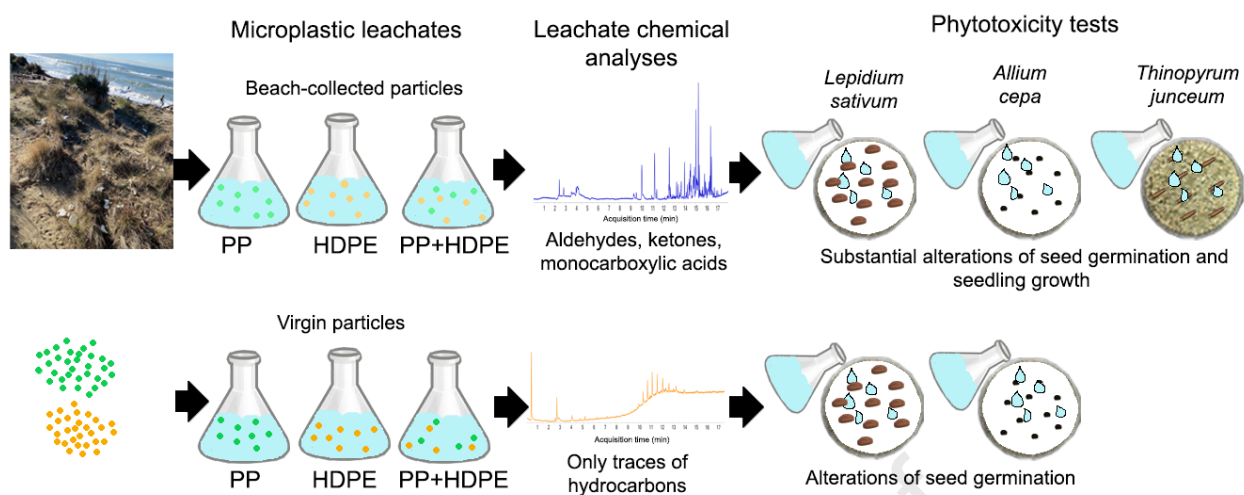
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Virginia Menicagli: Conceptualization; Methodology; Formal analysis; Investigation; Visualization; Writing-Original Draft; Writing - Review & Editing. **Elena Balestri:** Conceptualization; Methodology; Formal analysis; Investigation; Supervision; Writing-Original Draft; Writing - Review & Editing. **Greta Biale:** Formal analysis, Investigation. **Andrea Corti:** Conceptualization; Investigation; Resources; Writing - Review & Editing. **Jacopo La Nasa:** Conceptualization; Methodology; Formal analysis; Investigation; Writing - Review & Editing. **Francesca Modugno:** Supervision; Writing - Review & Editing. **Valter Castelvetro:** Conceptualization; Supervision; Project administration; Funding acquisition; Writing - Review & Editing. **Claudio Lardicci:** Conceptualization; Methodology; Supervision; Funding acquisition; Writing - Review & Editing.



Leached degradation products from beached microplastics: a potential threat to coastal dune plants

Virginia Menicagli^{a,b}, Elena Balestri^{*a}, Greta Biale^c, Andrea Corti^c, Jacopo La Nasa^c, Francesca Modugno^{b,c}, Valter Castelvetro^{b,c}, Claudio Lardicci^{b,d,e}

^a Department of Biology, University of Pisa, via Derna 1, Pisa, Italy

^b Center for the Integration of Scientific Instruments of the University of Pisa (CISUP), via S. Maria 53, Pisa, Italy

^c Department of Chemistry and Industrial Chemistry, University of Pisa, Via Giuseppe Moruzzi 13, Pisa, Italy

^d Department of Earth Sciences, University of Pisa, via S. Maria 53, Pisa, Italy

^e Center for Climate Change Impact, University of Pisa, Via Del Borghetto 80, Pisa, Italy

^{*}Corresponding author

elena.balestri@unipi.it

University of Pisa, via Derna 1, 56126 Pisa, Italy

Abstract

Plants play a fundamental role in maintaining coastal dunes but also accumulate littered microplastics (MPs). Migration tests suggest that naturally weathered MPs can leach out a broader range of potentially phytotoxic chemicals than virgin MPs. Thus, assessing MPs effects on plants using beached-collected particles rather than virgin ones is critically important. Here, the effects on plants of leachates from two pools of beach-collected and virgin MPs, high-density polyethylene (HDPE) and polypropylene (PP), and their mixture, were explored combining toxicity tests and chemical analyses. Phytotoxicity of MP leachates at different dilutions was evaluated under standard laboratory conditions using test species and under environmentally realistic conditions using the dune species *Thinopyrum junceum*. Leachates from beached PP and HDPE adversely affected all species, and the extent of these effects varied according to polymer type, concentration, and species. Virgin MPs had weaker effects than beached ones. Several potentially phytotoxic oxidized compounds were detected in water by GC/MS analysis, and their amount estimated. Results indicate that the molecular species leaching from beached MPs - at ppm concentration levels for the individual chemical species - can inhibit plant growth, and the effects of leachates from mixtures of degraded MPs can differ from those from individual polymers, highlighting the need for further investigation of MPs consequences for coastal ecosystems.

Keywords: microplastics, plastic leachates, phytotoxicity, polyethylene, polypropylene, solid phase extraction

Highlights

1. Leachates from beached PP and HDPE microplastics (MPs) were tested on plants.
2. MPs released many potentially phytotoxic oxidized degradation compounds.
3. Their amounts were determined by thermal desorption-GC-MS after SPE.
4. Leachates from beached MPs reduced seed germination and early plant growth.
5. Dune plants exposure to beached MPs is a potential threat to coastal ecosystems.

1. Introduction

The presence of microplastics (MPs, i.e., plastic particles of size equal or less than 5 mm; Frias et al., 2019) in marine habitats is a cause of major environmental concern due to the ubiquity and potential adverse effects of these pollutants on marine ecosystems. There is an increasing number of reports about adverse effects of MPs on growth, behaviour, survival rate, and reproduction of a wide range of organisms, such as zooplankton species, sea cucumbers, and fishes (Chae and An, 2017; Galloway et al., 2017). MPs might also act as carriers of absorbed/adsorbed chemicals, microorganisms and heavy metals into the marine ecosystem (Mato et al., 2001; Teuten et al., 2009). However, evaluating the actual impacts of MPs on living organisms is difficult due to the lack of systematic and accurate measurements and available measurement techniques for the wide variety of different marine environmental matrices. Concentrations of MPs in surface seawater can vary largely among geographical areas, ranging on average from 0.131 $\mu\text{g/L}$ to 6.9 $\mu\text{g/L}$ and from 0.013 to 100 particles/L (Beiras and Schöнемann, 2020; Cao et al., 2022), although the actual concentrations are most likely greater due to the missing detection of the smaller sized ones. Further to that, numerical models suggest that the abundance of MPs in ocean could increase approximately fourfold by 2060, in particular along the accumulation zones (Isobe et al., 2019). Most MPs detected in natural habitats result from the progressive fragmentation and degradation of plastic items, such so-called secondary MPs being mainly made of polyolefins such as polyethylene (PE), polypropylene (PP), and polystyrene (PS) (Andrady, 2011; Liu et al., 2020). However, a fraction of MPs is of primary origin, being introduced in the environment as microbeads contained for example in cosmetic products (Boucher and Friot, 2017). Sandy beaches and dunes are considered as a major final sink for floating plastics. Macro-plastics have recently been found embedded in sand dune volume and thus the degradation into MPs may be relevant in such environments (Turner et al., 2021; Andriolo and Gonçalves, 2022). Indeed, high MP concentrations (more than 1000 particles, or as much as 30 ppm and above, per kg dry sediment) have been detected in some coastal areas (Lots et al., 2017; Ceccarini et al., 2018), mostly consisting of the lower density hydrocarbon polymers PE, PP, and PS

(Castelvetto et al., 2021; Uddin et al., 2021). Studies have shown that plants colonizing these habitats can facilitate the accumulation of plastic debris through their above- and belowground organs (Poeta et al., 2017; Šilc et al., 2018; Andriolo et al., 2021; Gallitelli et al., 2021), and thus they could be particularly vulnerable to plastic pollution (Menicagli et al., 2019a,b; Menicagli et al., 2020). Currently, no information is available on the impact of stranded MPs on dune plants. This issue is of growing concern as vegetation plays a fundamental role in forming and stabilizing dune systems, while being globally threatened by a variety of anthropogenic factors (Feagin et al., 2005; Malavasi et al., 2016).

Studies performed on terrestrial plants have shown that MPs can influence their growth by changing the chemical and biological characteristics of ground, such as soil structure, nutrient immobilization, and soil microorganism community (Boots et al., 2019; de Souza Machado et al., 2019; Rillig et al., 2019; Larue et al., 2021). Other possible mechanisms explaining effects on plant growth involve the adsorption of MPs on seeds, leaves and roots, and their uptake by roots and subsequent internalization (Qi et al., 2018; Boots et al., 2019; Bosker et al., 2019; Ge et al., 2021; Mateos-Cárdenas et al., 2021). Most current information about the effects of MPs on plants is based on tests typically carried out using primary (virgin) polymer particles; as a result, the influence of MPs weathered in natural environments is still poorly understood. This is a relevant knowledge gap since virgin/primary MPs may substantially differ from weathered ones, in terms of shape, surface chemical composition and reactivity, additive content, and bio-accessibility (Phuong et al., 2016; Liu et al., 2020; Prata et al., 2021; Yin et al., 2021). Some studies addressing the effects of weathering under laboratory conditions, in which evidence of a decreasing toxicity of MPs with increasing aging time have been presented (Pflugmacher et al., 2020; Pflugmacher et al., 2021), cannot be considered as representative of the most common environmental cases. In that study, relatively large (3 mm) particles of polycarbonate, a polymer type much less widespread than the abovementioned hydrocarbon polymers, simply submitted to thermal treatment were used as simulants of weathered MPs (Phuong et al., 2016). In fact, natural weathering of plastics is typically activated by photo-

oxidation and then enhanced by other (mechanical, thermal, hydrolytic) polymer degradation processes that can cause fragmentation and release not only of MPs but also of molecular species in the form of volatile organic compounds (VOCs; Lomonaco et al., 2020; La Nasa et al., 2021) and leachates (Biale et al., 2021). In addition, plastic additives (i.e., substances intentionally added during manufacturing and not covalently bound to the polymer matrix; Teuten et al., 2009; Hermabessiere et al., 2017; Lithner et al., 2009; Paluselli et al., 2019), contaminants previously adsorbed onto the plastic from polluted environments (e.g., metals and persistent organic pollutants; Teuten et al., 2009; Rochman et al., 2014; Leòn et al., 2019; Khalid et al., 2021), and organic compounds derived from organisms colonizing plastic surfaces (Rummel et al., 2017) may also be released by MPs upon ageing, further contributing to soil and water pollution. Some of these molecular species (e.g., bisphenol A, BPA) are known to adversely affect a variety of living organisms, including plants (Domene et al., 2009; Staples et al., 2010; Gunaalan et al., 2020). Surprisingly, the impact of MPs via leaching, as well as the effects on terrestrial plants of different types of MPs accumulated in soils, has received little attention (Pignattelli et al., 2020; Pflugmacher et al., 2020). Recent migration assays carried out on virgin and beach-collected MPs have shown the presence of a variety of chemical compounds (Teuten et al., 2009; Cormier et al., 2021; Rendell-Bhatti et al., 2021) in their leachates. This finding suggests that the leaching of MPs in sandy areas with dune plants could expose them to complex mixtures of compounds, and thus understanding their effects on plant growth is critically important.

Here, we explored the impact on dune plants of leachates from two pools of beach-collected MPs, identified as high-density polyethylene (HDPE) and PP by La Nasa et al. (2021). For this purpose, we have adopted a multidisciplinary experimental approach in which toxicity tests were combined with leachate chemical analysis. Specifically, we assessed the potential phytotoxicity of four concentrations of each individual pool (HDPE-MPs and PP-MPs), and of their mixture (HDPE/PP-MPs), on two species, *Allium cepa* L. (onion) and *Lepidium sativum* L. (garden cress), used in standard germination tests (UNICHIM, 2003; OECD, 2006). The impacts of leachates obtained from

equivalent concentrations of virgin reference MPs (virgin HDPE and PP) were also evaluated to differentiate the effects related to the properties of polymers from those due to weathering. In addition, a seedling emergence test, using as a model *Thinopyrum junceum* (L.) Á. Löve, a typical dune building species, was performed to evaluate the effects of leachates from beach-collected MPs under a realistic dune scenario. The leachates from beached and virgin MPs were analysed to relate the plant responses to the presence of specific chemical compounds. An innovative analytical approach based on thermal desorption-GC-MS analysis after solid phase microextraction (SPME) allowed us to obtain the molecular profile of the leached compounds. Quantitative data were estimated using representative standard compounds to validate the method, evaluate the recovery of the procedure, and build calibration curves. The experiments tested the hypotheses that (i) beached MPs affect plants due to the leaching of chemical compounds, (ii) different MPs elicit different plant responses, and (iii) the impact of single MPs differ from that of their mixture due to the cumulative effects of the released compounds. The findings are expected to provide novel insights into the potential ecological risks posed by MPs on coastal systems and useful information for implementing beach/dune cleaning strategies.

2. Materials and methods

2.1 Materials

Small fragments originated from different types of degraded plastic items were collected in March 2016 from the accumulation zone (winter berm) of a sandy beach in northern Tuscany (Italy). Fragments were transported to the laboratory where they were carefully rinsed with fresh water to remove salts from their surface that might interfere with seeds. They were individually analyzed by attenuated total reflectance spectroscopy (ATR) using a Spectrum One FT-IR (Perkin Elmer Inc, USA) equipped with an ATR Platinum Diamond single-reflection module (penetration depth of 1.66 μm) to determine the polymer type. Only beached plastic fragments made of HDPE and PP with original size in the range of 2-5 mm were collected from the sand samples and used in this study. The

extent of their degradation was evaluated by calculating the carbonyl index (CI), an indicator of polyolefin oxidation, as the ratio of the carbonyl stretching peak area (1840-1600 cm^{-1}) vs. the methylene deformation peak area (1515-1420 cm^{-1}); the average CI values recorded from the surface of each individual fragment were in the 0.29-0.59 range for HDPE and in the 0.43-0.53 range for PP (Figure S1 in the Supporting Information). These fragments were then combined (HDPE and PP separately), ground (MF10 microfine grinder drive, IKA GmbH, Staufen, Germany) and sieved at 40 mesh with a stainless steel sieve, collecting the two batches of HDPE and PP micropowders with particle size $<420 \mu\text{m}$. Virgin HDPE (density of 0.952 g/cm^3 , average particle size $622 \mu\text{m}$) and PP (density of 0.900 g/cm^3 , average particle size $857 \mu\text{m}$) supplied as micronized powders produced by cryogenic quenching from the melt were kindly provided by Poliplast S.p.A. (Casnigo, Italy).

2.2 Leachate preparation

To prepare stock leachate solutions from single MPs, 200 mg HDPE or PP (either beached or virgin) were placed in flasks containing 200 mL of deionized water (LiChrosolv LC-MS grade, Merck). Stock leachate solutions from microplastic mixture were prepared using 100 mg of HDPE plus 100 mg of PP (either beached or virgin). Flasks were placed in a culture chamber in darkness on an orbital shaker (95 rotations per minute) for 72 h (Bejgarn et al., 2015) at $24 \pm 1 \text{ }^\circ\text{C}$ (mean \pm SE). A negative control (blank) comprising deionized water without plastics was also prepared. At the end of this treatment, each solution was vacuum filtered through $1.2 \mu\text{m}$ pore size glass fiber filters (Axiva SicheM Ltd., Haryana, India) and the pH and salinity of samples (30 mL) of these solutions were measured by a multiparameter meter (HI98194, Hanna Instruments). The pH of leachates from beached MPs varied from 5.66 to 5.98 while that of leachates from virgin MPs varied from 6.41 to 6.54. All leachates had a salinity equal to zero. Four leachate diluted concentrations (D1=1:10; D2=1:20, D3=1:40; D4=1:80) were then prepared by diluting the appropriate amount of leachate stock solution with deionized water, and immediately used for the phytotoxicity tests. Solutions of BPA (Sigma-Aldrich Inc., USA), a plastic additive used in phytotoxicity tests (Domene et al., 2009;

Staples et al., 2010; Pflugmacher et al., 2020), with concentrations of 100 mg/L, 50 mg/L, and 10 mg/L, respectively, were also prepared and used as positive controls in the experiments with seeds.

2.3 Chemical analysis of leachate solutions

The characterization of the leachates from MPs was performed using a Chemisorber® solid phase extraction device (Frontier Laboratories Ltd., Japan) consisting in a polydimethylsiloxane sorbent system. The leaching aqueous solution was extracted by soaking the Chemisorber for 48 h in the water-polymer dispersion (1 g/L). The extracted species were directly desorbed during 5 min at 280 °C in the micro-furnace of an EGA/PY-3030D pyrolyzer (Frontier Laboratories Ltd., Japan) coupled through a Py-GC interface set at 280 °C with a 8890 gas chromatograph combined to a Mass Selective Detector 5977B (Agilent Technologies, USA), and equipped with a MicroJet Cryo-Trap (Frontier Laboratories Ltd.). The Cryo-trap nitrogen jet at the head of the column was active for the whole desorption process. The GC injector was operated in split mode at 280 °C and with a 10:1 ratio, and the desorbed products were separated with an HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm, Agilent Technologies, USA) using the following temperature program: 5 min at 40 °C, 10 °C/min to 310 °C for 20 min. The identification of the chemical species was performed using a mass spectra library (NIST 20).

Semi-quantitative analyses were performed by selecting one reference chemical species to be used as a standard for evaluating the recovery and the response for each chemical class of those detected in the leachate solutions, namely: 1-hexene for the alkanes, 1-pentanol for the alcohols, hexanal for the aldehydes, 3-heptanone for the linear ketones, 2,2-dimethylbutanone for the branched ketones, and heptanoic acid for the monocarboxylic acids.

A standard stock solution in acetone with all the reference chemical species was prepared, containing 1-hexene (32 mg/L), 1-pentanol (56 mg/L), hexanal (37 mg/L), 3-heptanone (47 mg/L), 2,2-dimethyl-3-butanone (33 mg/L), and heptanoic acid (77 mg/L). Calibration curves in the 1-20 mg/L range were obtained by dilutions of the acetone stock solution with water (LC-MS grade,

Fluka). The calibration curves obtained for all the analytes, together with the method features, are reported in Table 1.

Since the differences between the calibration curves obtained for the branched and linear ketones, respectively, were negligible, the subsequent semi-quantitative analyses on the leachate solution were performed using the curve obtained for the linear ketone.

The limits of detection and quantitation of the method were evaluated on the blanks of LC-MS grade water. LOD values in the 0.03-0.05 mg/L range and LOQ values in the 0.09-0.18 mg/L range were obtained. Recoveries in the 95-100 % range were evaluated by using a standard solution (Table X). The intra- and inter-day coefficients of variation (CV%) were lower than 6% and 9%, respectively.

Table 1 Calibration curves, limit of detection (LOD) and quantitation (LOQ), intra- and recoveries obtained for the solid phase extraction method

	Slope	Intercept	R ²	LOD (mg/L)	LOQ (mg/L)	Recovery	
1-hexene	23181	11667	0.9945	0.04	0.13	99	
1-pentanol	7540	3046	0.9996	0.04	0.13	96	
hexanal	39481	5227	0.9907	0.03	0.09	96	
3-heptanone	4678	-2181	0.9909	0.05	0.16	95	
heptanoic acid	1728	6109	0.9982	0.05	0.18	100	

2.4 Toxicity assessment of leachates from microplastics: Standard phytotoxicity tests

Commercially available seeds of the monocotyledon *A. cepa* and the dicotyledon *L. sativum* (Figure S2; Italsementi s.n.c, Italy) were purchased from local retailers. Before starting the experiments, the seeds were examined under a stereomicroscope (Wild M3C, Leica) to remove small or deformed ones. They were also subjected to a pressure test (Borza et al., 2007) and those that did not collapse under pressure were selected as viable seeds. On average, seeds of *A. cepa* were 0.312 ± 0.003 mm long and 0.208 ± 0.002 mm wide ($n = 40$), and seeds of *L. sativum* were 0.295 ± 0.001 mm long and 0.150 ± 0.002 mm wide ($n = 40$). To evaluate the effects of leachates on germination, seeds

of each of the two species were placed in glass Petri dishes (9 cm diameter, 10 seeds of *A. cepa* or *L. sativum* per dish) containing a layer of cellulose filter paper (Whatman N°1) moistened with 1.5 mL of one dilution of leachates from beached or virgin MPs, and with deionized water as negative control and the BPA solutions as positive control. There were five replicate dishes for each treatment and species. All Petri dishes were sealed with parafilm to prevent water evaporation and placed in a culture chamber for 72 h at 24 ± 1 °C in darkness.

At the end of the tests, for each of the two species the number of germinated seeds in each dish was counted to determine the percentage of germination. For *L. sativum*, the length of the radicle of germinated seeds was measured, and its germination index (GI) was also calculated as

$$GI = [(n_i l_i) / (n_c l_c)] \times 100$$

where n_i is the number of seeds germinated in the sample, l_i is the mean root length in the sample, n_c is the number of seeds germinated in negative control, and l_c the mean root length in negative control. The GI index combines seed germination and radicle elongation, revealing the overall phytotoxicity of tested materials (Zucconi et al., 1981). A GI lower than 80% indicates phytotoxicity (Tiquia et al., 1996). In addition, visible detrimental effects on different parts of the seedlings were recorded. The latter include abnormalities in appearance of the emerged seedlings, stunted growth, chlorosis, discoloration, and effects on plant development relative to a normal seedling (Chandler, 2008).

2.5 Toxicity assessment of leachates from microplastics: Dune plant emergence test

Seeds of *T. junceum* (Figure S2) were harvested from plants inhabiting a dune system of the Northwestern Italy in July 2020 and stored outdoor in darkness in clean glass jars until their use in germination test. Seeds of *T. junceum* were on average 0.840 ± 0.014 long and 0.186 ± 0.004 wide ($n = 40$). To assess the effects of beached MPs on seedling emergence and growth, seeds were sown in mesocosms consisting of glass Petri dishes (9 cm diameter) containing 35 g of natural silica sand (0.5–1 mm, density approximately 1.6 g/mL, and <0.01% organic matter content). Due to the relatively large size of seeds, only 8 seeds per dish were used. The sand was carefully washed with

deionized water, placed in glass containers, baked in a laboratory oven at 180 °C for at least two hours and then sterilized in autoclave at 121 °C for 20 minutes before performing the test. In each mesocosm, 3 mL of one of the four dilutions of leachates obtained from beached MPs, as well as negative and positive control solution, respectively, was added. Such a volume of leachate was found to be the minimum amount necessary to achieve adequate wetting of the sand and allow seed germination. Each mesocosm was then sealed with parafilm to prevent desiccation and randomly placed in a culture chamber in darkness at 15 ± 1 °C to mimic natural optimal germination conditions for the given species (Sykes and Wilson, 1989; Thanos et al., 1989). Five replicate mesocosms were prepared for each treatment. Seeds were observed daily by using low green light intensity to check for newly germinated seeds (i.e., radicle length equal or greater than 2 mm). Germinated seeds were removed from dishes five days after their germination and stored in 70% ethanol for morphological measurements. The germination test was considered finished when no additional seeds germinated for at least three consecutive weeks.

Final germination was calculated and expressed as percentage of the number of seeds placed in each mesocosm that had germinated at the end of the test. The mean germination time, i.e., the time in days elapsed between seed sowing and germination, was also calculated as

$$t = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i}$$

where t_i was the time elapsed from the start of the experiment to the i^{th} day of observation; n_i was the number of germinated seeds in the day i , in each mesocosm and k was the day in which the last germination event was observed (Ranal and De Santana, 2006). The length of radicle and coleoptile was measured in a sample ($n = 15$) of seedlings and the radicle to coleoptile length ratio was also calculated.

2.6 Statistical analyses

Data were analyzed using the statistical software “R” (version 3.5.2; R Core Team, 2018). Prior to the analyses, data were tested for normal distribution and homoscedasticity using Shapiro–Wilk tests and Cochran tests, and data were transformed when necessary to meet assumptions of analysis of variance (ANOVA). As no transformation was effective in removing the non-normal error distribution of seed germination data in standard tests, percentage variables (seed germination and GI) were analyzed by using one-way univariate permutational analyses of variance (PERMANOVA; function *adonis* in “vegan” package; Oksanen et al., 2019) based on the Euclidean distance with seven levels of the factor MP type (no microplastic or negative control, beached PP, beached HDPE, beached mixture HDPE/PP, virgin PP, virgin HDPE, and virgin mixture HDPE/PP). These analyses were carried out separately for each species (*A. cepa* and *L. sativum*) and for each concentration of leachates. ANOVA analyses (*gad* function in “GAD” package; Sandrini-Neto and Camargo, 2014) with the same design were conducted on the mean radicle length of *L. sativum* seedlings. For each species, a one-way PERMANOVA with four levels of the factor BPA (no BPA or negative control, 10 mg/L BPA, 50 mg/L BPA, 100 mg/L BPA) was performed on seed germination and GI, and one-way ANOVA with the same design was conducted on radicle length. To evaluate the effects of beached MPs leachates on *T. junceum*, a one-way PERMANOVA (four levels of MP type: negative control, beached PP, beached HDPE, beached mixture HDPE/PP) was carried out on seed germination percentage, and a one-way ANOVA with the same design was performed on mean germination time, mean radicle length, mean coleoptile length, and radicle to coleoptile length ratio of seedlings, separately for each concentration of leachates. Permutational analyses of multivariate dispersion (PERMDISP; *permutest* function in “vegan” package, Oksanen et al., 2019) were performed on statistically significant terms of each PERMANOVAs to check for differences in multivariate group dispersion. Post-hoc SNK tests and Pair-wise tests were conducted when significant differences ($\alpha = 0.05$) among treatments were observed in ANOVAs and PERMANOVAs, respectively.

3. Results

3.1 Chemical analysis of leachates from MPs

The total ion chromatograms obtained for the leachates from PP are reported, as representative examples, in Figure 1, while the list of all the species is reported in Table 2.

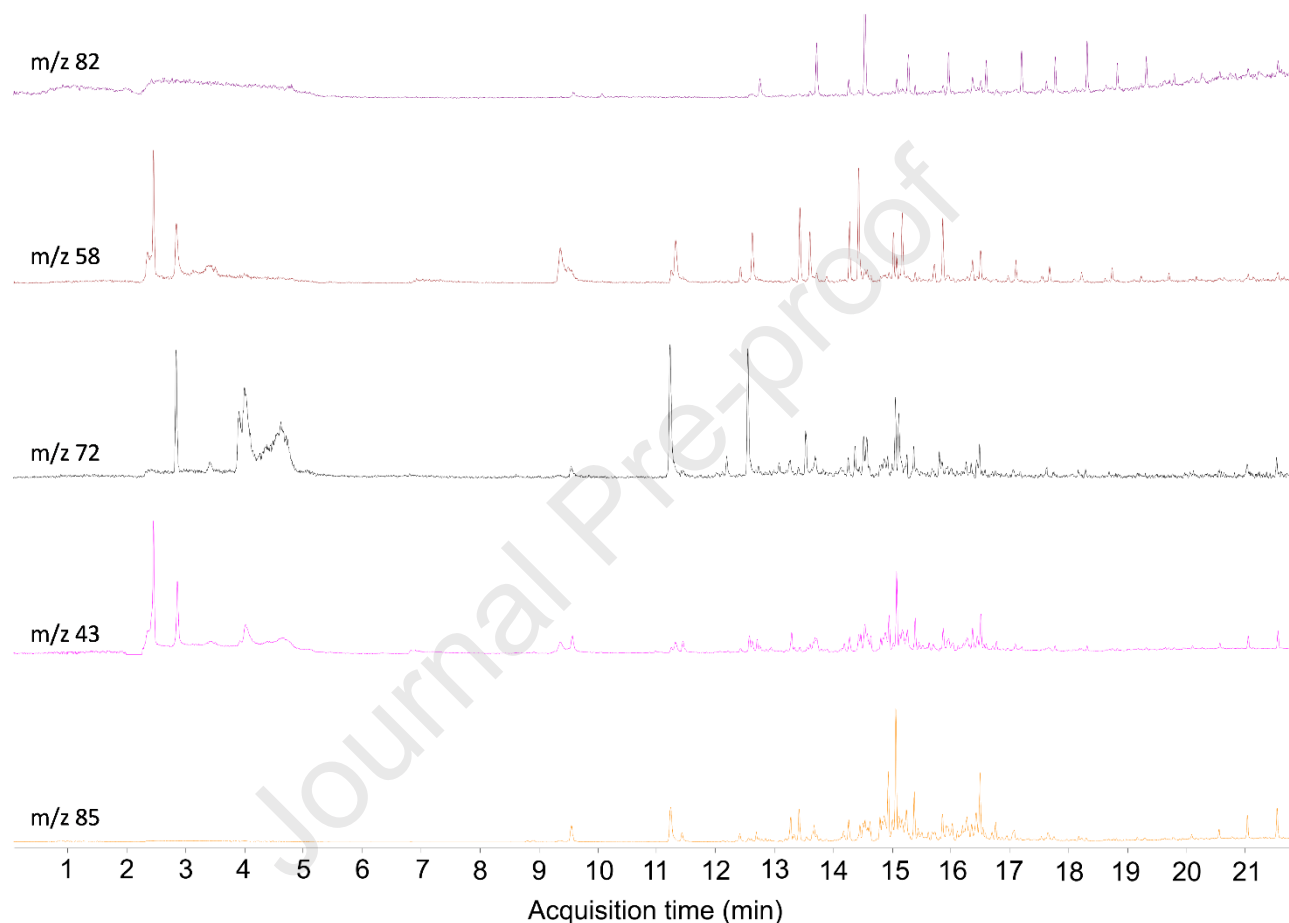


Figure 1 Extracted ion chromatograms of the ions m/z 43 (aldehydes), 58 (ketones), 72 (branched and short chain ketones), 82 (long chain aldehydes), and 85 (alkanes) obtained for the leachates of beached PP.

The leachates from beached PP comprise aldehydes, ketones, aliphatic hydrocarbons, monocarboxylic acids, and alcohols. The most abundant detected ketones, all of which with chain lengths in the C2-C18 range, were acetone, 2-butanone, 3-heptanone, 5-methyl-3-hexanone, and 3-nonanone. Traces of unsaturated ketones within the same chain length range were also detected,

although their abundances were too low to allow the accurate assignment of their chemical structure from the mass spectra. The different features of the mass spectra of the detected ketones with respect to those from the library allowed us to differentiate these compounds in two classes, linear and branched. In detail, while the detected linear ketones had ≤ 5 carbon atoms, the branched ones were characterized by ≥ 5 carbon atoms. A typical example is the set of species with molecular weight MW=128 g/mol that could include 3,5-dimethylhexan-2-one and 6-methylheptan-3-one, both characteristic oxidation products of PP. Similarly to the ketones, the leached aldehydes featured chain lengths in the C2-C18 range, the lower MW acetaldehyde, propanal, and 3-butanal being the most abundant ones. Again, the mass spectra allowed to detect and identify both linear and branched aldehydes, along with traces of unsaturated aldehydes with relative abundances that could not be unambiguously identified from their MS spectra. Linear monocarboxylic acids and alcohols with chain lengths up to 6 carbon atoms were also detected only in traces. Finally, linear alkanes in the C3-C20 range were detected and identified; among them, the lower end species (C₃-C₁₀) were the most abundant ones, while those in the higher end were only detected in traces.

The concentrations estimated for the different chemical species are reported in Table 2. From a quantitative point of view the concentrations of the single analytes were lower than 3 mg/L, while the total concentration of all the analytes could be estimated at 39.9 mg/L. In particular, saturated ketones was the most abundant class of compounds, with a total concentration of 19.9 mg/L, followed by aldehydes (7.99 mg/L), monocarboxylic acids (6.81 mg/L), alcohols (2.87 mg/L), and alkanes (2.36 mg/L).

The general composition of the soluble fraction from beached HDPE was qualitatively similar to that from beached PP, with the presence of ketones, aldehydes, monocarboxylic acids, alcohols, and alkanes, the main distinctive feature being the nearly total absence of branched degradation products, as opposed to the case of PP. Moreover, the compounds with chain length in the C5-C10 range were only present in traces, possibly due to their sparing solubility in water. From a quantitative point of view, the total amount of organic compounds leached from HDPE was 26.2 mg/L, lower than the

total amount for PP (Table 2). The most abundant compounds in the case of HDPE were ketones (14.5 mg/L), followed by monocarboxylic acids (5.23 mg/L), alkanes (3.92 mg/L), alcohols (1.52 mg/L), and aldehydes (1.03 mg/L); only small traces (below the LOQ) of hydrocarbons with chain lengths up to 10 carbon atoms could be detected in the leachates from the virgin polymers.

Table 2 Concentrations of the chemical species detected in the stock solution of MPs leachates.

The concentrations of the analytes were estimated by using the calibration curves of a specific analyte for each class of compounds; in detail: 1-hexene, for alkanes, 1-pentanol, for alcohols, hexanal for branched and linear aldehydes, 3-heptanone for branched and linear ketones, heptanoic acid for monocarboxylic acids.

Organic compound (type)	Carbon atoms (nr.)	MW (Da)	Conc. (mg/L)	
			PP	HDPE
Aldehydes				
	2	44	2.26	<LOQ
	3	58	2.04	<LOQ
	4	72	0.79	0.10
	5	86	0.29	<LOQ
	6	100	0.29	0.23
	7	114	0.41	<LOQ
	8	128	0.24	<LOQ
	9	142	0.17	0.31
	10	156	<LOQ	<LOQ
	11	170	0.63	<LOQ
	12	184	0.43	<LOQ
	13	198	0.32	<LOQ
	14	212	<LOQ	<LOQ
	15	226	<LOQ	0.11
	16	240	<LOQ	0.12
	17	254	<LOQ	0.09
	18	268	0.13	0.09
<u>Aldehydes total amount</u>			7.99	1.03
Saturated ketones				
	3	58	2.8	0.68
	4	72	2.8	0.69
	5	86	2.4	1.37
	6	100	1.2	1.11
	7	114	0.9	0.71
	8	128	1.0	0.81
	9	142	0.8	0.81
	10	156	0.7	0.92
	11	170	0.7	1.37
	12	184	0.9	0.95
	13	198	1.2	0.95
	14	212	1.0	0.74
	15	226	0.7	0.84
	16	240	0.9	0.88

	17	254	1.0	0.81
	18	268	1.1	0.86
	<i>Saturated ketones total amount</i>		19.9	14.5
<i>Monocarboxylic acids</i>				
	2	60	<LOQ	0.64
	3	74	1.64	1.91
	4	88	1.84	1.18
	5	102	2.33	0.62
	6	116	1.00	0.87
	<i>Monocarboxylic acid total amount</i>		6.81	5.23
<i>Alcohols</i>				
	2	46	0.34	0.62
	3	60	0.72	0.27
	4	74	0.33	0.33
	5	88	1.49	<LOQ
	6	102	<LOQ	0.29
	<i>Alcohols total amount</i>		2.87	1.52
<i>Alkanes</i>				
	3	44	<LOQ	0.16
	4	58	0.30	<LOQ
	5	72	0.43	0.44
	6	86	<LOQ	<LOQ
	7	100	<LOQ	0.13
	8	114	<LOQ	0.25
	9	128	<LOQ	0.25
	10	142	0.29	0.45
	11	156	<LOQ	0.74
	12	170	0.37	0.55
	13	184	<LOQ	<LOQ
	14	198	0.18	<LOQ
	15	212	<LOQ	<LOQ
	16	226	<LOQ	0.15
	17	240	0.27	0.27
	18	254	0.17	<LOQ
	19	268	0.14	<LOQ
	20	282	0.20	0.53
	<i>Alkanes total amount</i>		2.36	3.92

394

395 3.2 Toxicity assessment of leachates from microplastics: Standard phytotoxicity tests

396 Final germination percentages of *A. cepa* and *L. sativum* seeds of negative control groups were
397 always higher than 80%, confirming the validity of the tests (Figures 2,3). The germination
398 percentage of *A. cepa* seeds exposed to positive controls (BPA) was lower, regardless of the
399 concentration, compared to that of seeds treated by negative control ($p < 0.05$; Table S1; Figure S3).
400 Leachates from beached PP at all dilutions and from beached HDPE at all dilutions except the D1
401 significantly reduced the seed germination percentage in *A. cepa* compared to the negative control
402 ($p < 0.05$; Table S2; Figure 2). Leachates from the mixture of beached HDPE and PP at D1, D3, and

403 D4 also reduced seed germination compared to the negative control ($p<0.05$). Conversely, the
404 germination percentage of seeds treated by leachates from virgin MPs did not differ from that of the
405 negative control ($p>0.05$; Table S2; Figure 2).

406 No effect of BPA was detected on the seed germination percentage of *L. sativum* ($p>0.05$; Table
407 S1; Figure S3), but the radicle length in seedlings treated with the highest concentration of BPA (100
408 mg/L) was approximately 30% lower than that of negative controls ($p<0.05$; Table S1; Figure S3).
409 The GI of seedlings exposed to 50 mg/L and 100 mg/L BPA was of 23% and 36% lower, respectively,
410 than that of seedlings treated by the negative control ($p<0.05$; Table S1; Figure S3). Leachates from
411 virgin HDPE and beached PP at D3 reduced the *L. sativum* seed germination percentage compared to
412 the negative control ($p<0.05$; Table S2; Figure 3). Moreover, seedlings exposed to leachates from
413 beached HDPE at D3 and beached PP at D1 had a shorter radicle compared to that of negative control
414 seedlings ($p<0.05$; Table S2; Figure S4). Seedlings treated by leachates from both virgin and beached
415 HDPE at D3 and by leachates from virgin HDPE, virgin mixture, and beached PP at D1 had a lower
416 GI compared to that of the negative control ($p<0.05$; Table S2; Figure 3). All control seedlings were
417 morphologically normal while a low fraction of seedlings treated by all leachates, except D3 of virgin
418 HDPE (Figure S5) showed abnormalities, such as stubby and short radicles and discolored
419 cotyledons.

420

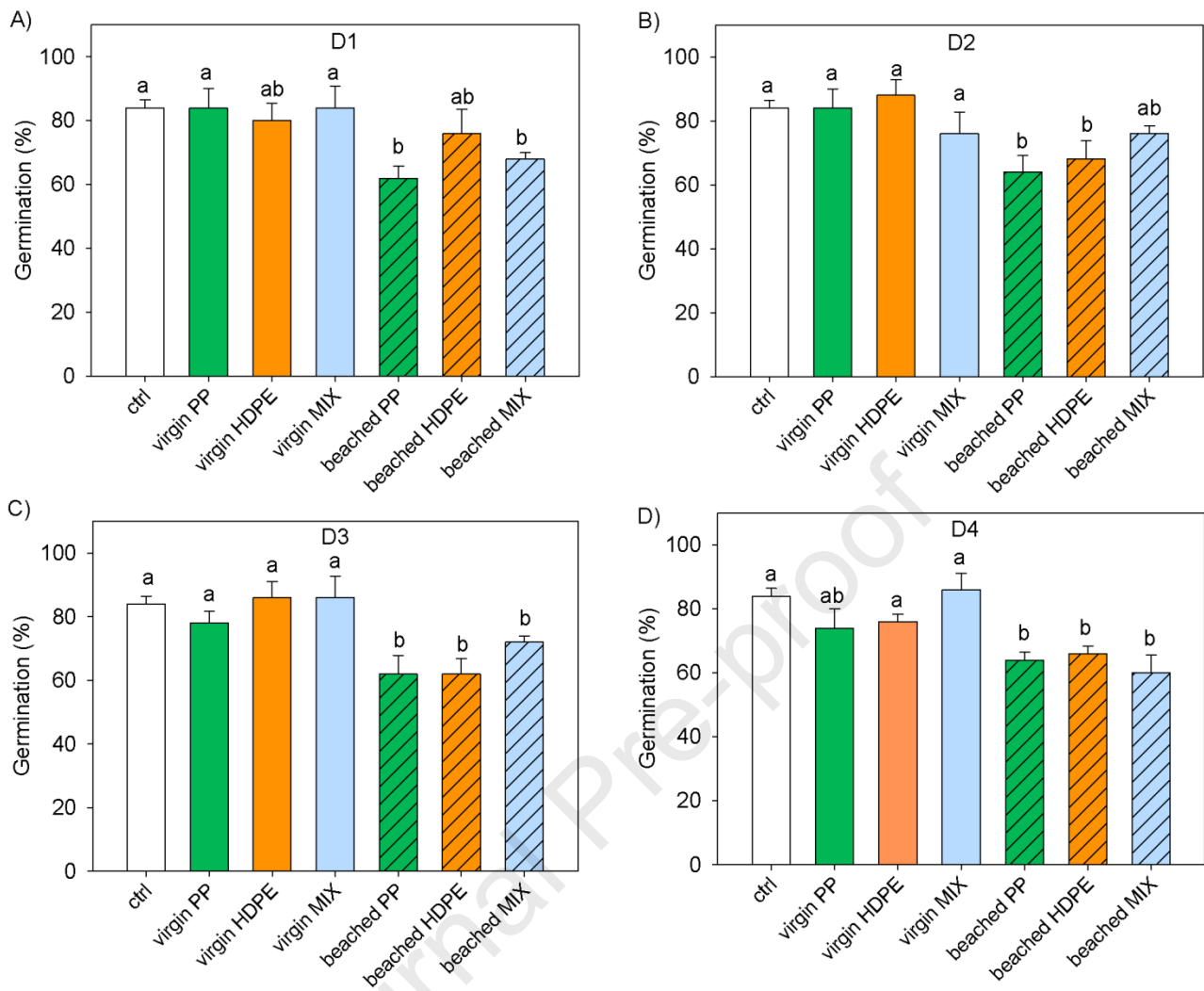


Figure 2 Germination percentage of *Allium cepa* seeds exposed to negative control water (ctrl) and leachates from virgin and beached MPs made of polypropylene (PP), high-density polyethylene (HDPE) and their mixture (MIX) at different dilutions (A) D1 (1:10), (B) D2 (1:20), (C) D3 (1:40), and (D) D4 (1:80). Data are means \pm SE (n = 5). Different letters above bars indicate statistically significant differences (p < 0.05) among treatments.

2-column fitting image; color only in online version

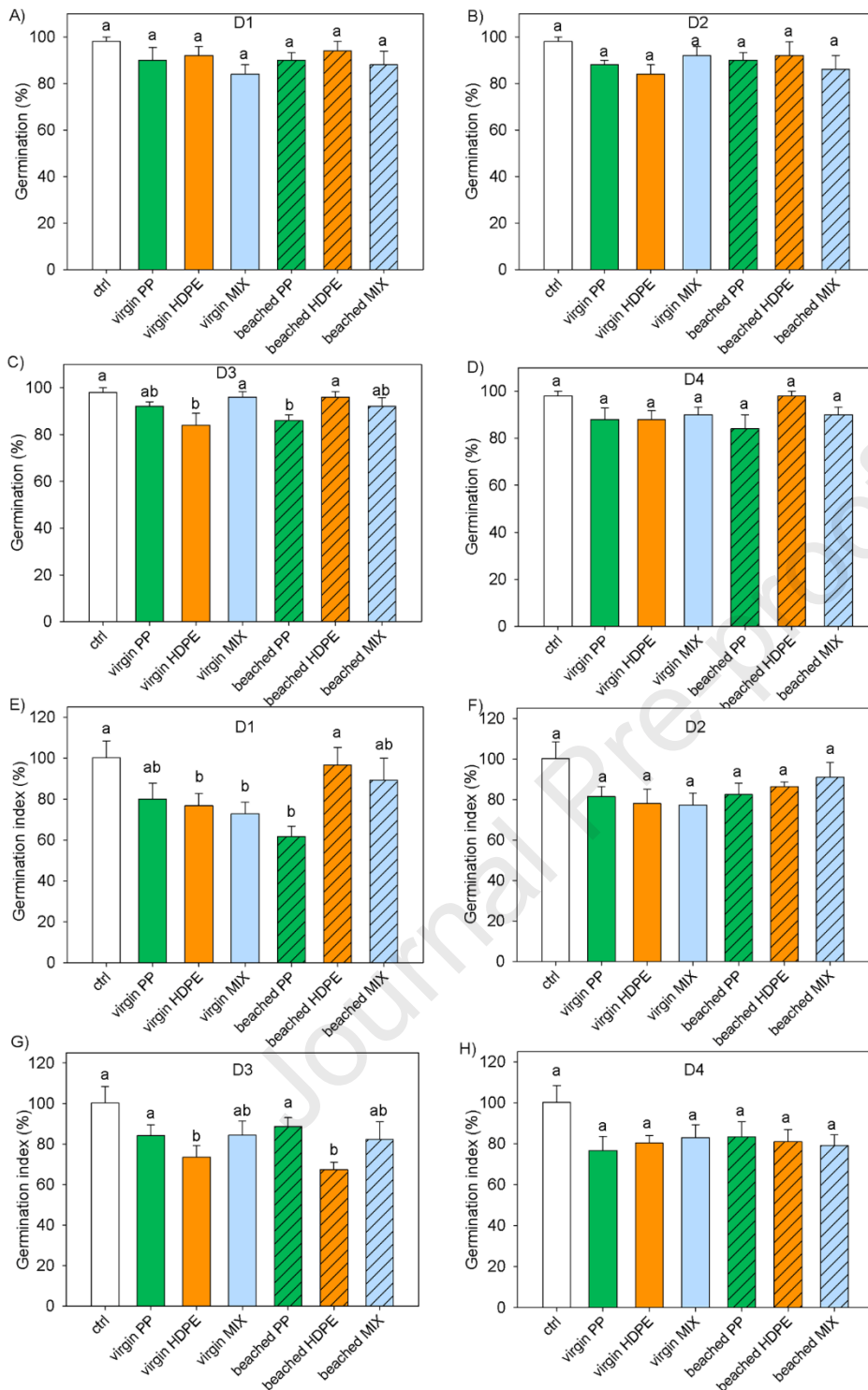


Figure 3 Effects on seedlings of *Lepidium sativum* seeds exposed to negative control water (ctrl), and leachates from virgin and beached MPs (PP, HDPE, MIX) at different dilutions (D1=1:10, D2=1:20, D3=1:40, D4=1:80). Graphs A-D: germination percentage. Graphs E-H: germination index (GI). Data are means \pm SE (n = 5). Different letters above bars indicate statistically significant differences (p<0.05) among treatments.

2-column fitting image; color only in online version

3.3 Toxicity assessment of leachates from microplastics: Dune plant emergence test

Leachates from beached MPs did not influence germination percentage and mean germination time of *T. junceum* seeds ($p > 0.05$; Table S3; Figure 4), and no abnormal seedling was observed. However, the radicle of seedlings treated by leachates from beached PP at D1 was shorter than that of control seedlings of approximately 26% ($p < 0.05$; Table S3; Figure 4). The radicle to coleoptile length ratio of seedlings treated by leachates from beached HDPE at D3 was greater than that of control seedlings due to a reduction of coleoptile length ($p < 0.05$; Table S3; Figure 4). A significant effect of the treatments was also observed on coleoptile length for leachates at D2 ($p < 0.05$), but no alternative to null hypothesis was detected by post-hoc test (Table S3; Figure 4).

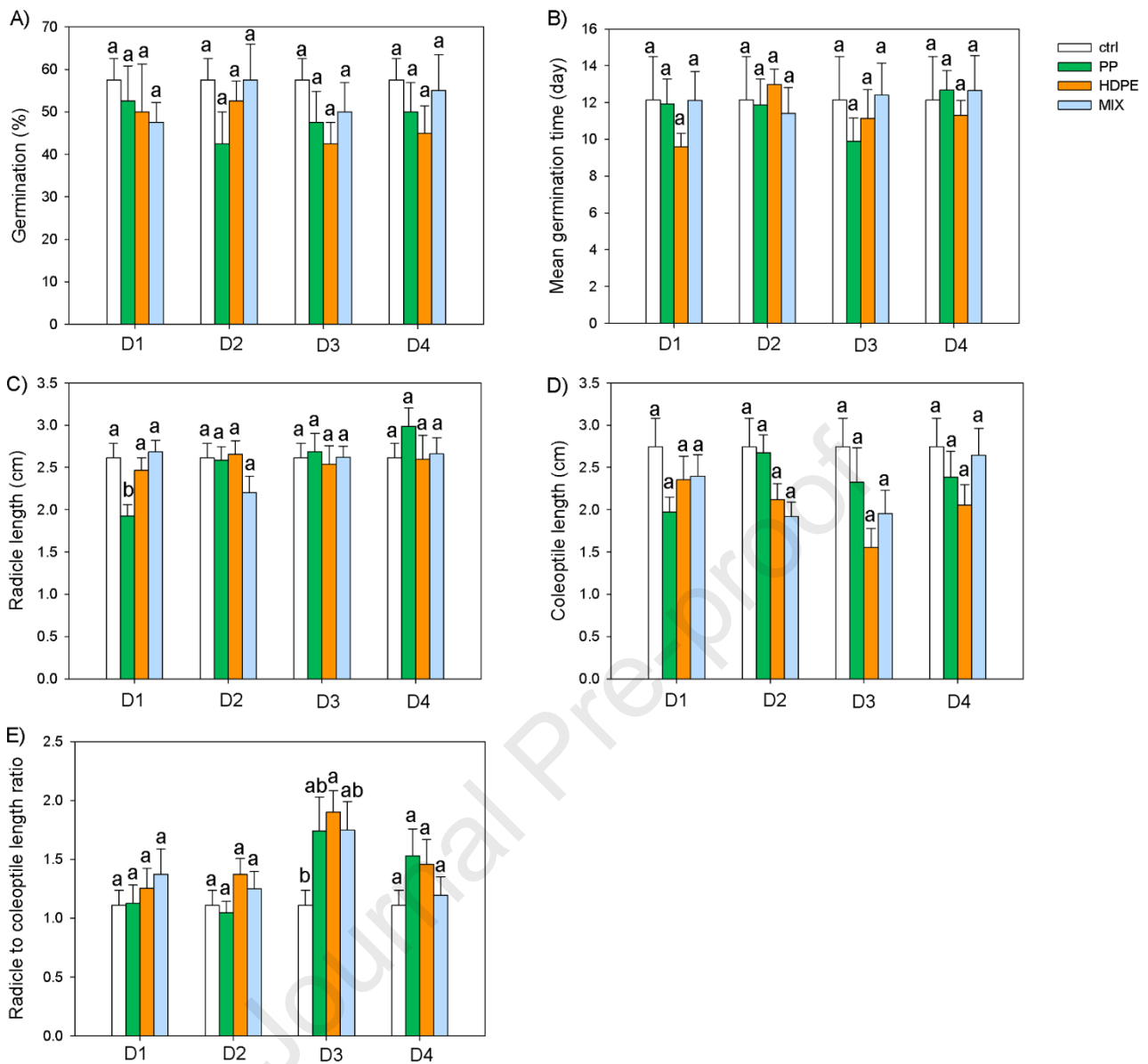


Figure 4 (A) Seed germination percentage, (B) seed mean germination time, (C) radicle length, (D) coleoptile length, and (E) radicle to coleoptile length ratio of *Thinopyrum junceum* seedlings treated with negative control water (ctrl) and different dilutions (D1-1:10; D2-1:20, D3-1:40, D4-1:80) of leachates from beached MPs made of polypropylene (PP), high-density polyethylene (HDPE) and their mixture (MIX). Data are means \pm SE (n = 5 in panels A, B, and n = 15 in panels C, D, E). Different letters above bars indicate significant differences (p < 0.05) among treatments within each concentration.

2-column fitting image; color only in online version

4. Discussion

The presence of MPs in natural environments as well as their impacts on biota have been largely documented, but no information is currently available on the effects of MPs as sources of molecular species that, in addition to being released as volatile organic compounds (VOCs), may also leach into the aqueous medium supporting the life of plants inhabiting coastal environments and their associated ecosystems. In this study, by combining germination tests with detailed chemical analyses of the profile of molecular species leached by plastic debris collected from the natural environment, we demonstrated the release of a variety of chemicals representing a potential threat to coastal dune ecosystems. Indeed, even relatively low concentrations of leached species (down to about 0.01 mg/L for the most abundant species in the more diluted solutions) were found to affect early plant development.

The results of phytotoxicity tests supported our initial hypotheses that leachates from beached MPs may negatively affect plant health. As expected, the extent of the observed effects was found to depend on the plant species, the polymer type, and the leachate concentration (in this study, the different levels of dilution of the original leaching solutions). Indeed, most of the tested dilutions of the leachates from beached MPs led to a lethal toxic effect on *A. cepa*, substantially reducing seed germination. In contrast, only beached PP at the lowest dilution (i.e., D1) and HDPE at high-intermediate dilution (i.e., D3) resulted in a toxic effect on *L. sativum*, and this was mainly evidenced as a reduction of root elongation. Some seedlings treated with MP leachates also showed abnormal development. These findings suggest a different sensitivity threshold to plastic leachates in different plant species, as also supported by results of the tests performed using BPA as positive control in this study. The leachates from beached MPs also affected the growth of the dune plant *T. junceum*, partially confirming the results observed for *A. cepa* and *L. sativum*. Indeed, no lethal leachate effect was detected for this dune plant, but the leachates from beached PP at D1 and beached HDPE at D3 elicited sub-lethal effects, such as decreased seedling radicle growth and a change in resource allocation to below- aboveground plant organs. These effects could have relevant consequences for

dune plant populations as they can lead to a greater seedling mortality. Indeed, shorter radicles could reduce plant ability to acquire water and nutrient from sediments, while shorter coleoptiles could make the seedlings more vulnerable to sand burial (Maun, 2009). A comparison between the two types of MPs revealed that at the lowest dilution the leachate from PP was more phytotoxic than that from HDPE, while at the high-intermediate dilution HDPE had a greater phytotoxicity than PP. This latter effect is consistent with the greater toxicity, based on biometrical trait alterations and oxidative stress, of PE than PP as reported in other studies (Pignatelli et al., 2020; Li et al., 2021; Yin et al., 2021). The inhibitory effects of MP leachates observed here also agree with the results of a previous study, in which a reduction of seed germination and seedling development due to leachates from MPs and macro-plastics has been reported (Balestri et al., 2019; Pflugmacher et al., 2020). The results of chemical analyses performed here showed the presence of a broad range of potentially phytotoxic chemical species such as low molecular weight aldehydes, ketones, and some alkenes, along with alkanes and carboxylic acids. These compounds shall be considered almost exclusively as degradation products of the polymer the MP particles are made of, since neither traces of the most common polymer additives nor of persistent pollutants possibly adsorbed onto the MP particles from the environment could be detected. Indeed, the detected polymer degradation products are likely the result of extended exposure to intensive photo-oxidizing conditions such as those typically found in the accumulation zone of a beach environment. This is indirectly confirmed by the presence of only traces of low molecular weight hydrocarbons and undetectable oxidized products in the leachates of the reference virgin, unaged polymers used in blank experiments. Recent studies focused on the low molecular weight organic compounds released by PP and HDPE MPs upon artificial aging have highlighted the formation, among other oxidation products, of the compounds detected in this work as leachates (Kang, et al., 2020; Biale et al., 2021). In particular, a series of isobaric species (structural isomers not individually identified due to the lack of standards in the MS libraries), including very low molecular weight water soluble oxygenated compounds such as e.g., acetone and acetic acid, can be generated by the various mechanisms involving chain scission of the PP backbone (Rouillon et al.,

2016; Kang, et al., 2020). Such degradation products of polyolefins, including biologically active aldehydes and monocarboxylic acids, have been highlighted as potentially harmful compounds to plants, inhibiting seed germination, shoot and root growth (Skinner, 1918; Gussin and Lynch, 1982; Chotsaeng et al., 2018). Further support to the direct correlation between the observed phytotoxicity on *A. cepa* and *L. sativum* and the presence of degradation products of MPs from beached PP and HDPE came from the observed negligible effect of analogous leachates from virgin MPs, in which the only molecular species that could be detected were small traces of hydrocarbons (alkanes, alkenes, carboxylic acids).

It should be emphasized that the cumulative concentration of each class of molecular species (e.g., carboxylic acids, ketones, etc.) in the leached stock solutions was estimated to be in the 1-20-ppm range (the total organic compounds estimated at about 40 ppm for the beached PP and 26 ppm for the beached HDPE), but the stock solution was then diluted from 10 to 80 times for the subsequent phytotoxicity tests. On the other hand, the concentration range resulting from simple leaching followed by dilution of the leachate cannot be compared straightforwardly with the exposure in a natural sandy beach environment since the latter is dynamic, with variable availability of leaching water and typically lack of confinement of the same, likely resulting in cyclical exposure to peak concentrations of potentially toxic molecular species within a generally highly diluted pattern.

The results of the GC/MS analysis revealed a similar qualitative chemical composition of the leachates from beached PP and HDPE, consistent with the findings of a previous study on degradation products released in water by PP and LDPE exposed to ultraviolet light (Gewert et al., 2018). Nevertheless, in our study a noteworthy difference between the beached PP and HDPE concerned the carbon skeleton of the leached compounds, which was almost exclusively linear for HDPE as opposed to the presence of several branched degradation products for PP. We postulate that these differences be responsible for the different concentrations at which beached PP and HDPE affected plant germination and growth. On the other hand, the observation of plant growth alterations with HDPE

mainly at the high-intermediate dilution suggests a non-linear relationship between MPs contamination level (and thus the exposure dose of the leached species) and effects on plant health.

Our hypothesis that the effects of leachates from a MPs mixture (i.e., different polymers) on plants would differ from those of leachates from MPs of a single polymer was partially supported by the experimental results. Indeed, in the case of *A. cepa* the leachates from the beached HDPE/PP mixture and those from the individual polymers showed a similar impact on plant growth. However, the absence of effects on *L. sativum* and *T. junceum* seedlings treated by leachates from beached MP mixture at D3 and D1 suggests a lower impact of mixture than individual polymers. Specifically, we found that at the high-intermediate dilution PP mitigated the individual adverse effect of HDPE while at the lowest dilution HDPE mitigated the individual adverse effect of PP. This latter result agrees with findings of a previous study on *L. sativum*, showing a lower effect of PE+PVC mixture added in the substrate than that of PE or PVC individually (Pignattelli et al., 2020). Differences between effects of MPs mixture and individual polymers could be related to potential interactions/reactivity among compounds released by MPs in water.

Further research will be necessary to assess the specific effects of different chemical classes and of their respective concentrations as well the potential synergistic or antagonistic effects of chemicals in the leachate from complex MP mixtures to expand our understanding of the actual hazardousness of polluting MPs for the fragile coastal environments. Moreover, future works should also investigate the MPs impact on a wider range of dune plants worldwide using same framework applied here.

5. Conclusions

Our study revealed that beached MPs made of PP and HDPE (the polymers most commonly found as polluting MPs in the accumulation zone of sandy beaches where the colonizing plants grow) can leach a broad range of low molecular weight degradation products with potential phytotoxicity at concentration below the ppm for the single chemical species. Such phytotoxicity includes inhibition of seed germination and alteration of plant development in the investigated species. The effect of

leachates from individual polymers on plants seems not to be simply additive; in particular, leachates from mixtures of HDPE and PP may result in effects that differ from those expected based on the effects produced by leachates from the individual polymers. Overall, the findings provide novel insights into how the leaching of beached MPs may affect plants including coastal dune habitat forming plant species, an issue that has been largely neglected so far. Importantly, they highlight the need of investigating the effect of other environmentally relevant MPs, in terms of polymer types, mixture composition (e.g., individual or multiple polymers), and aging status, to predict the impact of these pollutants in natural environments such as coastal dunes. They also emphasize the importance of implementing systematic actions aimed at collecting exhaustively waste macro-plastic items and debris from beach-dune environment to prevent their further degradation into polluting MPs resulting in sustained release of hazardous chemical species to plants and associated ecosystems.

CRedit author statement

Virginia Menicagli: Conceptualization; Methodology; Formal analysis; Investigation; Visualization; Writing-Original Draft; Writing - Review & Editing. **Elena Balestri:** Conceptualization; Methodology; Formal analysis; Investigation; Supervision; Writing-Original Draft; Writing - Review & Editing. **Greta Biale:** Formal analysis, Investigation. **Andrea Corti:** Conceptualization; Investigation; Resources; Writing - Review & Editing. **Jacopo La Nasa:** Conceptualization; Methodology; Formal analysis; Investigation; Writing - Review & Editing. **Francesca Modugno:** Supervision; Writing - Review & Editing. **Valter Castelvetro:** Conceptualization; Supervision; Project administration; Funding acquisition; Writing - Review & Editing. **Claudio Lardicci:** Conceptualization; Methodology; Supervision; Funding acquisition; Writing - Review & Editing.

Declaration of competing interest

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

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Declaration of interests

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

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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