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Alternative end-of-life options for disposable bioplastic products: Degradation and ecotoxicity assessment in compost and soil

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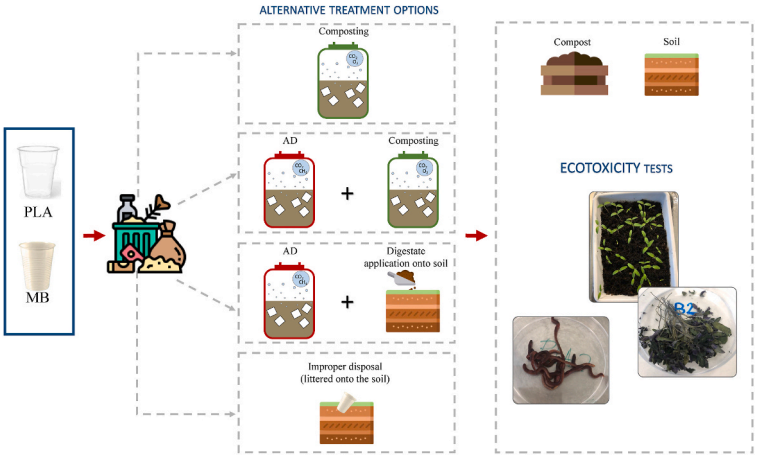
HIGHLIGHTS

- Four end-of-life options for disposable bioplastic cups were investigated.
- Biodegradation rates, polymeric matrixes and operating conditions were correlated.
- Coupling AD and composting lead to 100% degradation in over 6 months.
- Low degradation (23%) of digestate on soil warns over micro-bioplastics release.
- Phytotoxicity tests revealed a biomass gain <80% across all samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Four different end-of-life options for disposable bioplastic cups were investigated and compared based on their environmental implications. Two products with distinct polymeric composition were tested simulating the following scenarios at laboratory scale: i) industrial composting (180 days at 58 °C); ii) anaerobic digestion followed by industrial composting (45 days at 55 °C and 180 days at 58 °C); iii) anaerobic digestion followed by direct digestate use on soil for agricultural purposes (45 days at 55 °C and 180 days at 25 °C); iv) uncontrolled release into a soil environment (180 days at 25 °C). Ecotoxicity tests were run at the end of each experiment to investigate the effects of the materials on three main groups of terrestrial model organisms: plants, earthworms and nitrifying bacteria. Complete biodegradation of the cups was observed in 180 days in the scenarios involving composting environment. A low degree of biodegradation (22.9 ± 4.5%) of the digestates in soil was observed, warning for a potential micro-bioplastics discharge into the environment. No degradation was observed for the cups in soil during the same testing period. Ecotoxicity tests revealed a negative effect on plants biomass growth across all samples, which was 17–30% lower compared to the blank sample. The experimental campaign

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highlighted the need for a systematic assessment of controlled treatment of bioplastics, as well as the need for a harmonized legislative framework.

1. Introduction

In the latest years in Europe there have been targeted efforts to reduce single-use plastics (Directive (EU) 2019/904), as they account for 60% of the annual plastic waste generation (Eurostat). This has led to the spread of bioplastic products, replacing commodity plastics for some applications such as packaging and disposable tableware. Bioplastics are a wide category of materials, which can be bio-based, biodegradable or have both such characteristics. They are assumed to exert lower impacts in terms of carbon footprint and have a higher potential for recovery at their end of life (Bishop et al., 2021). In particular, the ones that are biodegradable have attracted attention, since biological treatments can be applied as end-of-life options. However, many scientific concerns are arising regarding the effective environmental sustainability of bioplastic products, which is currently under scrutiny (Dolci et al., 2023). Two of the most widely used biopolymers for single-use commercial items are currently polylactic acid (PLA) and thermoplastic starch (TPS) (Sangeetha et al., 2018). The first is an aliphatic polyester which is generally produced from lactic acid through ring opening polymerization or direct polymerization (Farah et al., 2016). It has a brittle behavior, so it is usually blended with plasticizers for easier processability. TPS is blended with other polymers and additives (Jumaidin et al., 2020; Ju et al., 2022), since it has a hydrophilic nature. One of the most successful blends available on the market is Mater-Bi® (MB), which is commercialized in various grades by Novamont S.p.A. (Italy). The biodegradability of bioplastic products can be potentially advantageous since, once discarded at their end of life, they can be collected and treated together with organic residues or biowaste using the existing waste collection and treatment infrastructure (Vardar et al., 2022). On the other hand, in the worst-case scenario where they are dispersed uncontrollably in the environment, they should degrade faster than their conventional counterparts. The strategy currently adopted by the European Union provides that they should be handled in the same way as organic waste, as indicated by the Directive 2018/851 (Directive (EU) 2018/851). Most commercially available bioplastic products are designed to be compostable in compliance with the EN, 20082 standard (EN, 20082:2008). Other standards, such as ISO 16929 or ISO 20200 (ISO 16929:2021; ISO, 2015), can be used to test the material disintegration. However, the policy framework for bioplastics certification is currently underdeveloped, with concerns arising about the standards' ability to accurately represent full-scale conditions (Folino et al., 2023), as well as their harmonization.

Scientific data on biodegradation of commercial bioplastic items are still relatively limited (Cazaudehore et al., 2022; Falzarano et al., 2023) and as yet it is hard to derive indisputable conclusions on bioplastic waste behavior. Commercial bioplastics are usually produced by blending biopolymers with plasticizers and additives, which improve their physical and mechanical properties but also affect their biodegradability and could lead to the production of some undesired final products or undegraded residues (Xu et al., 2016; Sangeetha et al., 2018). For instance, starch is a relatively easily degradable polymer, but the fact that it is typically mixed with other more recalcitrant biopolymers, such as polybutylene succinate (PBS) or polybutylene adipate terephthalate (PBAT), can affect the kinetics of its degradation (Ruggero et al., 2020). The design criteria commonly adopted for biological treatment plants may not be adequate for the optimal management of bioplastics, resulting in digestate and compost contamination (Dolci et al., 2022; Calabrò and Grosso, 2018), that could in turn be carriers of bioplastics across the environmental compartments (Le et al., 2023). In order to identify the main issues in bioplastics treatment and design technical solutions, the criticalities during composting should be carefully evaluated (Folino et al., 2020; Kale et al., 2007b).

Another pressing concern regards the risk of bioplastic residues ending up in the environment, which could represent an issue just as it happens for the conventional plastic counterpart, in particular when they are designed to be single-use. Some other bioplastic products are specifically designed to be applied and degrade in the environment, such as mulch films. In any of these scenarios, it is therefore necessary to understand the fate of bioplastics and the risks their presence may pose to the natural compartments. So far, the impact of bioplastic debris on the ecosystems is not clearly understood (Boots et al., 2019; Huerta-Lwanga et al., 2021), but there are evidences of micro-bioplastics accumulation and persistence in soil (Fojt et al., 2020), affecting its structure and the taxonomy of the biota (Chah et al., 2022; Rauscher et al., 2023). Fine particles can adsorb and transport heavy metals and toxic compounds, resulting in a potentially higher risk for living organisms (Abe et al., 2022; Khaldoon et al., 2022; Liwarska-Bizukojc, 2021).

The aim of the present study was understanding the aerobic degradation in compost and soil of commercial bioplastic products and their residues from a previous anaerobic digestion treatment. Bioplastic cups were selected for this experimental campaign, as they are among the most used disposable tableware worldwide. The material behavior was assessed by applying different testing methods addressing physical alteration, mass loss, biological degradation and ecotoxicological effects on different organisms. Lab-scale batch tests were adopted to have better control of the operating parameters and tracking of the processes' evolution. The following scenarios representing possible destinations of bioplastic waste were simulated: i) aerobic degradation of disposable bioplastics under industrial composting conditions; ii) coupling of anaerobic digestion and industrial composting for the biodegradation of the bioplastic products; iii) aerobic degradation of disposable bioplastics in soil, assuming they are dispersed in the environment in an uncontrolled manner; iv) biodegradation of digestate containing residual non-degraded bioplastics after direct application onto natural soil. The final ecotoxicity of all the runs with compost was evaluated on microorganisms, plants and earthworms.

2. Materials and methods

2.1. Feedstock materials

Two bioplastic products were selected among the types most widely used as single-use commercial materials and compliant with EN, 20082, a PLA-based cup (PLA_C) and a Mater-Bi-based cup (MB_C), with the aim of comparing how materials of different composition behave from the viewpoint of biodegradation. The products were characterized in previous studies using Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FT-IR) spectroscopy and thermogravimetric (TG) analysis, with the aim of identifying the chemical composition of the commercial blend used for the products (Bracciale et al., 2023, 2024). Analytical results showed that PLA_C had a relatively neat composition and the concentration of additives or co-polymers potentially present in the matrix was below the detection limit of the equipment used. On the other hand, MB_C consisted of two main fractions: a minor, easily degradable fraction of PLA/starch and a fraction of polybutylene succinate (PBS) as a co-polyester. In addition, some Ca-based additives and talc fillers were detected. The digestates (PLA_D and MB_D) were obtained from previous lab-scale studies (Bracciale et al., 2023, 2024) in which PLA_C and MB_C were exposed to thermophilic (55 °C) batch anaerobic digestion until the plateau was reached, which took 45 days. This choice was made to observe the final biodegradation potential of the products under the selected environmental conditions, despite the

fact that this eventually required longer biodegradation times than commonly practiced in full-scale digesters. The final digestates were stored at 4 °C until the aerobic tests. The positive control used for all the aerobic degradation experiments was cellulose powder (CreaTech TC 40). Mature vegetal compost (Hnos. Aguado, Toledo, Spain) was purchased from Leroy Merlin Spain. Soil was sampled from a wooded area in Castellon (Spain) and screened on a 5-mm sieve to remove the coarse fraction.

The experimental design adopted is reported in Table 2 and is described in detail in the following paragraphs.

The synthetic solid waste (SSW) for disintegration and mass loss tests was prepared according to ISO 20200 (ISO, 2012a:2015) by mixing 40% of sawdust, 30% rabbit feed, 10% mature compost, 10% corn starch, 5% sugar, 4% corn oil and 1% urea (wet weight basis), then water was added to set the initial moisture content at 55%. A commercial potting soil (PS) (Inferco S. L., Sagunto, Spain), prepared using a mix of peat, coconut fibers and compost, was used in the ecotoxicity tests. The main characterization parameters of the materials used in the experiments are reported in Table 1.

2.2. Aerobic biodegradation in controlled composting conditions

The evaluation of the ultimate aerobic biodegradability was carried out following an adapted methodology (Feijoo et al., 2023) based on the ISO 14855-1 method (ISO 14855–1:2013). Bioplastic cups were powdered prior to the degradation tests through mechanical grinding with a maximum output size of 0.1 cm. The biodegradation tests were performed in triplicate using 2-L airtight reactors (Fig. S1). For each test, 15 g TS of mature compost (initial moisture 26.9 wt%) were mixed with 2.5 g TS of the bioplastic sample, and water was then added to reach a 50% moisture content for the blend. The digestates (initial moisture >90 wt%), were dried at 40 °C to ensure a 50% moisture when blended with compost. The reactors were incubated at 58 °C for 180 days, in accordance with ISO 14855-1. Aerobic conditions (O_2 > 15% vol. in the headspace) were maintained throughout the biodegradation process. The headspace gas composition was routinely analyzed for its main constituents (volumetric O_2 and CO_2 concentrations) using a portable infrared gas analyzer (Dansensor® CheckPoint 3, Ametek Mocon Europe, DK). After the periodic measurements of gas composition, the reactors were flushed with air to restore the initial conditions in the reactor headspace (O_2 = 21% vol.). The reactors were opened once a week to adjust moisture and stir the material. Blank tests were conducted to evaluate the CO_2 production of the compost. Cellulose was used as a positive control to assess the validity of the experiment (see below for details).

Table 1
Main characterization parameters of the materials (TS = total solids; VS = volatile solids; TOC = total organic carbon).

Material	Type	TS [% ww]	VS [% ww]	TOC [gC/kg]	Thickness [μm]
Cellulose	–	95.9 ± 0.1	95.9 ± 0.1	436.3 ± 0.8	–
PLA_C	Cup	99.6 ± 0.0	99.6 ± 0.0	530 ± 1.4	205
MB_C	Cup	99.8 ± 0.04	69 ± 0.02	386.5 ± 1.2	0.07
PLA_D	Digestate	6.6 ± 0.6	5 ± 0.5	23.7 ± 1	–
MB_D	Digestate	8.3 ± 0.2	5.3 ± 0.1	29.5 ± 1.7	–
Mature compost	–	73.1 ± 0.8	32 ± 0.1	104.3 ± 1.1	–
SSW	–	44 ± 0.5	92 ± 0.5	402.9 ± 2.8	–
PS	–	31.4 ± 0.1	76 ± 0.3	369.1 ± 13.8	–
Soil	–	91.4 ± 0.2	5.5 ± 0.04	91.4 ± 0.1	–

The results of the tests in terms of cumulated volume of CO_2 evolved during the process were modelled using the modified Gompertz equation (Eq. 1):

$$P(t) = P_m * \exp\left\{ -\exp\left[\frac{R_m * e}{P_m}(\lambda - t) + 1\right]\right\} \quad (1)$$

where $P(t)$ is the cumulated CO_2 production at time t, P_m is the maximum CO_2 production, R_m is the maximum CO_2 production rate and λ indicates the lag phase duration. The process kinetics was evaluated through the parameter $t_{x\%}$, which represents the time required to reach a predetermined percentage (x%) of the maximum conversion yield into CO_2 (with x% = 10, 25, 50, 75 or 95%). Modelling was performed using the average CO_2 production of replicated experiments and the degree of fitting was found to be highly accurate, with values for the correlation coefficient (R^2) > 0.99.

Material biodegradation was calculated according to the following equation (Eq. 2):

$$Biodegradation\ (t) = \frac{(CO_2(t))_S - (CO_2(t))_B}{ThCO_2} \times 100 \quad (2)$$

where $(CO_2(t))_S$ is the measured cumulated CO_2 production of the sample at time t, $(CO_2(t))_B$ is the measured cumulated CO_2 production of blank at time t and $ThCO_2$ is the theoretical CO_2 production calculated stoichiometrically from the elemental analysis results.

According to the method, the test is considered to be valid if the positive control's (i.e., cellulose) biodegradation exceeds 70% within 45 days and if the percent difference of the results among the replicates of each tested sample at the end of the test is <20%. Moreover, the blank test is required to produce 50–150 g CO_2/g VS within the first 10 days of incubation.

2.3. Disintegration and mass loss

Disintegration was evaluated according to the ISO 20200 method (ISO, 2021:ISO, 2021a). The bioplastics were cut in half and buried in SSW using plastic box reactors (19.5 × 28× 13.2 cm) for three months. The bioplastics disintegration process relied on the indigenous biomass present in the compost fraction included in SSW. Two bioplastic items were placed in each box and mixed at 1:100 ratio with SSW. The reactors were incubated at 58 °C and routinely monitored to adjust moisture and stir their content. According to the method, the disintegration degree has to be evaluated after 3 months using a 2-mm sieve.

To track the mass loss over time an additional test was run in which the standard method was modified (Feijoo et al., 2023) by introducing a plastic net that allowed the identification and monitoring of the individual sample pieces. In this case, PLA_C and MB_C were manually cut into 2.5 × 2.5 cm pieces, which were weighed individually and sewn onto wire mesh screens accommodating 12 bioplastic pieces each. Two meshes were placed in each reactor and buried in SSW. Moisture adjustment and stirring were performed manually once a day for the first 15 days and then thrice a week until the end of the test. The bioplastic fragments were sampled over time, rinsed with distilled water, dried at 40 °C under vacuum and then weighed. Photographs of samples were taken and analyzed through the ImageJ software (Rasband, 2018) in order to highlight dimensional and morphological changes resulting from materials' disintegration.

2.4. Aerobic biodegradation in soil environment

Biodegradation in a soil environment was tested using 500-ml airtight reactors according to the ISO 17556 method (ISO 17556:2012). For each test, 200 g of dry soil were mixed with 2.5 g of dry sample; the soil moisture was set at 50% of its water holding capacity (WHC = 33.4%). Bioplastic samples preparation and aerobic conditions monitoring were performed as described in section 2.1. The

Table 2
Experimental design (individual test description in the following sections).

	BIODEGRADATION IN COMPOST		Disintegration (D)/mass loss (ML)		BIODEGRADATION IN SOIL		Ecotoxicity on plants (P)/earthworms (E)/ microorganisms (M)	
Test procedure	ISO 14855-1		*(D)* ISO 20200		ISO 17556		ISO 17088	
Reference standard			*(ML)* ISO 20200 modified					
Environment	Mature compost		SSW[a]		Natural soil		*(P)* Tested compost + PS[b] (50 + 50 wt%) *or* tested soil *(E)* Tested compost + PS[b] (25 + 75 wt%) *or* tested soil *(M)* refer to ISO 15686	
Duration	180 d		*(D)* 120 d *(ML)* 45 d		180 d		*(P)* 14 d *(E)* 14 d *(M)* 2–6 h	
Temperature	58 °C		58 °C		25 °C		25 °C	
Reactor characteristics	2-L glass reactor		19.5 × 28 × 13.2 cm plastic box		500-mL glass reactor		*(P)* 20 × 20 cm tray *(E)* Φ 25 cm box *(M)* 10 mL vials	
Tested material	**material**	size	**material**	size	**material**	size	**material**	size
	cellulose	(<30 μm)[c]	PLA_C	*(D)* half cup	cellulose	(<30 μm)[3]	PLA_C MB_C	1 × 1 mm
	PLA_C MB_C	(<0.1 cm)		*(ML)* 2.5 × 2.5 cm	PLA_C MB_C	(<0.1 cm)	PLA_D	1 × 1 mm
	PLA_D	(<0.1 cm)	MB_C	*(D)* half cup	PLA_D	(<0.1 cm)	MB_D	–
	MB_D	–		*(ML)* 2.5 × 2.5 cm	MB_D	–		–
		–				–		

[a] Synthetic Solid Waste (SSW).
[b] Potting Soil (PS).
[c] Average fiber length.

reactors were incubated at a controlled temperature of 25 °C for 180 days. The reactors were opened every two weeks to adjust moisture and stir the material. Blank tests were conducted to evaluate the respiration activity of soil alone. Cellulose was used as a control material (see 2.2). Data modelling and material biodegradation assessment were performed according to Eq. (1) and Eq. (2), respectively. All tests were performed in triplicate. According to the standard, the test is valid if the reference material biodegradation exceeds 60% at the end of the process and the percent difference among the replicates is <20%.

2.5. Ecotoxicity assessment in compost/soil

A series of experiments with compost were conducted to evaluate the final ecotoxicity of the material on plants, earthworms and microorganisms in conformity with the ISO 17088 standard (ISO 17088:2020). Aerobic biodegradation was carried out in plastic box reactors (19.5 × 28 × 13.2 cm). Bioplastic cups were cut into 1 × 1 mm particles and mixed with SSW (ISO, 2012) in a 10 wt% ratio. The same ratio was used for the digestates. The reactors were incubated at 58 °C, with periodic moisture adjustment and manual stirring being performed thrice a week. Similar tests were run in soil according to the ISO 17556 method (ISO 17556:2012), with the exception of the operating temperature, which was set at 25 °C, and the sample ratio, which was 1 wt%. After three months all tests were stopped, and the samples obtained were used to carry out the ecotoxicity tests.

The assessment of ecotoxic effects on higher plants was carried out on tomato plants. Squared vessels of 20 × 20 cm were filled with a mix (50 + 50 wt%) of tested compost + PS or tested soil. Each vessel was seeded with 50 tomato seeds that were placed at a depth of 1 cm and equally spaced in 10 rows. The vessels were incubated in thermostatic chambers equipped with lights simulated day and night at 25 °C. For comparison, a blank test was carried out for each set of experiments, using compost and soil that had not been exposed to bioplastics/digestate. All tests were run in triplicate. The beginning of the test was set on the day at which the blank test reached 50% germination. The test is considered valid if the seedling germination rate of the blank is at least of 70% after 14 days. At the end of the test period, plants were counted and afterwards uprooted, dried and weighted to determine their biomass. The germination and biomass ratios must be >90% with respect to the control to consider the sample non-toxic.

Acute toxicity was evaluated on the *Eisenia fetida* species using a blend (25 + 75 wt%) of tested compost + PS or tested soil. Adult earthworms were taken from the wormery, cleaned and weighed to obtain their initial biomass. Then, 10 individuals were placed in each reactor, consisting of a closed box with a lid (to minimize loss of water by evaporation and animal escape) punched with holes to allow for ventilation, and were incubated at 25 °C in the dark for 14 days. At the end of the test the number and the biomass gain/loss of the survived earthworms were evaluated. For the test to be considered valid, survival and biomass gain/loss in the compost exposed to the sample must be >90% of those for the corresponding blank.

Ecotoxicity effects on compost and soil microorganisms were evaluated through measurements of their nitrification capacity after 2 and 6 h of incubation, which was calculated as nitrification potential in μg NO2–N/g dry compost, according to the ISO 15685 (2012). The tests were performed in triplicate and the material was considered non-toxic if the nitrite formation in the compost exposed to the sample was >80% of the corresponding blank.

3. Results and discussion

3.1. Aerobic biodegradation in controlled composting environment

For both the bioplastic and the digestate samples, the CO_2 evolution over time and the process kinetics are shown below along with the blank test. On day 45 the blank test produced 91.6 mg CO_2/g VS and the degradation degree of the positive control was 85% with a percent difference among replicates of 1.08%, thus complying with the requirement of the testing method. The evolution of biodegradation, as monitored through CO_2 production during the experimental runs, is shown in Fig. 1a.

Fig. 1b shows the evolution of tx% parameter, which represents the time required to reach a predetermined percentage of the maximum conversion yield into CO_2.

3.1.1. Bioplastics biodegradation

Visual observation of the runs on PLA_C and MB_C revealed the presence of clearly identifiable bioplastic fragments still after more than one month of testing, indicating that the kinetics of physical disintegration was notably slow, likely also affecting the biodegradation

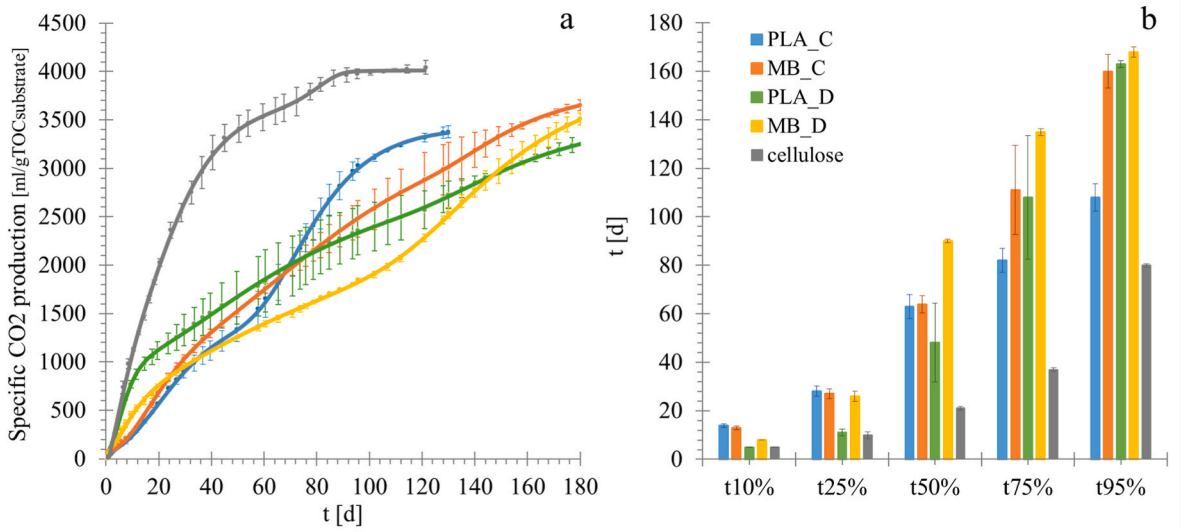

Fig. 1. Main results of the aerobic biodegradation in controlled composting environment of PLA cup (PLA_C), Mater-Bi cup (MB_C), PLA digestate (PLA_D) and Mater-Bi digestate (MB_D): a) experimental specific cumulative CO_2 production and corresponding interpolation curves (runs with compost); b) evolution of the $t_{x\%}$ parameter (time required to reach a predetermined percentage (x%) of the maximum conversion yield into CO_2) during the biodegradation process.

process. PLA_C gas evolution displayed two steps, suggesting a first degradation of the amorphous fraction of the material likely along with the hydrolysis of the more recalcitrant crystalline fraction, followed by a final mineralization stage of the hydrolysis products. MB_C displayed a single-stage degradation, which did not reflect the heterogeneous nature of the blend and could be attributed to overlapping degradation of the different components, assuming they had comparable hydrolysis kinetics. PLA_C and MB_C degradation kinetics was comparable during the initial stages of the process and diverged as biodegradation proceeded, as MB_C contained more biologically recalcitrant components. This is also evident from the calculated values of $t_{75\%}$ and $t_{95\%}$, which were 35% and 48% higher for MB_C compared to PLA_C (Fig. 1b). Full degradation of the PLA and MB cups was estimated to require 130 and 180 days, respectively (Table 3). Similar results were obtained in previous studies testing PLA in compost under thermophilic conditions. For instance, Kalita and colleagues tested two different grades of PLA film in compost (1:10 on a wet weight basis) at 58 °C and obtained 90% degradation in 140 and 95 days (Kalita et al., 2020, 2021). Other PLA specimens (20 × 20 × 0.2 cm) tested in mature compost (1:15 on a wet weight basis) at 58 °C reached complete degradation in 75 days (Narancic et al., 2018), while PLA sheets (2 × 2 cm) tested at 58 °C reached 86% biodegradation in 120 days (Leejarkpai et al., 2011). On the other hand, thermoplastic starch is usually easily degraded under composting conditions. For instance, a number of authors measured a degradation degree of 73% in 25 days (Du et al., 2008), 65% in 32 days (Del Rosario Salazar-Sánchez et al., 2019) and 100% in 75 days (Narancic et al., 2018). It should however be emphasized that commercial blends have been found to display more recalcitrant biodegradation features, due to the presence of co-polymers and additives. Gómez and Michel (2013a) tested a blend of polypropylene with corn starch at 55 °C and attained a biodegradation of 51% in 85 days. Differences in the degradation kinetics and yields can be due to the influence of several factors, such as the polymeric grade and the form of the product. The effect of material size was evaluated by Husárová et al. (2014), who tested four PLA films from different producers for 100 days and attained biodegradation degrees in the range 51–95%. However, when they tested the same products in powder form an increased biodegradation up to 66–100% was revealed. Material crystallinity can also affect biodegradation, for instance amorphous PLA films tested in compost at 55 °C reached 70% biodegradation in 28 days (Tabasi and Ajji, 2015). Additives can also play a role, for example PLA films (2 × 2 mm) tested at 58 °C for 90 days showed increased biodegradation from 62 to 78% when nanoclay was added (Stloukal et al., 2015). On the other hand, when decreasing the operating temperature from 50 °C to 25 and 37 °C no biodegradation was observed for some PLA discs tested in compost (Al Hosni et al., 2019). Different commercial products were also tested by a number of authors. PLA pots were tested at 58 °C for 60 days and obtained 13% of biodegradation (Ahn et al., 2011), while a PLA bottle was tested at 65 °C for 58 days reaching 84.2% degradation (Kale et al., 2007a) and PLA spoons achieved a disintegration of 65.1% after 22 days of composting (Bandini et al., 2022).

Table 3
Measured net CO_2 production (average value ± standard deviation), final biodegradation degree and degradation time (runs with compost).

Substrate	Net CO_2 [ml]	Theoretical CO_2 [ml]	Biodegradation [%]	Degradation time [d]
PLA_C	2630 ± 139	2500	105.1 ± 1.4	130
MB_C	2304 ± 36	2241	102. ± 0.7	180
PLA_D	1529 ± 62	1687	90.6 ± 3.6	180
MB_D	1343 ± 45	1467	91.6 ± 3.1	180
Cellulose	2019 ± 29	1944	103.8 ± 0.5	120

3.1.2. *Digestates biodegradation*

The runs on PLA_D and MB_D attained a similar final CO_2 yield and biodegradation degree (Table 3), although the biodegradation process displayed different profiles over time. It should be noted that during the tests originating these digestates, the PLA and MB bioplastic materials had attained, respectively, 92% and 45% biodegradation as well as 100% and 31% disintegration in ~ 40 days (Bracciale et al., 2023, 2024). PLA_D therefore consisted mostly of microbial biomass from the species grown during the digestion tests. On the other hand, the low degree of disintegration attained in the anaerobic digestion tests indicated that MB_D was mainly comprised of microbial biomass together with a residual undegraded fraction of the bioplastic that was likely to include the most biologically recalcitrant components of the original polymers.

The PLA_D biodegradation kinetics was comparable with cellulose during the initial stages of the process, but then slowed down as

biodegradation proceeded, suggesting the presence of a first more readily degradable fraction (including species such as hemicellulose or cellulose that likely derived from the lignocellulosic components of the original inoculum) along with more complex organic substances. On the other hand, MB_D displayed two degradation steps that appeared to proceed at a considerably lower rate compared to PLA_D. Despite the different time evolution of the biodegradation process for the two digestate samples, at the end of the test the biodegradation degree was in both cases higher than 90% (Table 3). In this case, coupling anaerobic digestion with a composting phase allowed for further stabilization of the organic matter and almost complete mineralization of the residual bioplastic fraction, possibly suggesting the need of a second aerobic stage downstream of anaerobic digestion for MB in order to achieve full degradation.

Literature data available on the combination of anaerobic and aerobic treatment topic of bioplastics is currently rather limited, however they appear to suggest that special attention must be paid to identify the appropriate combination of process conditions for both stages. The coupling of anaerobic digestion (30 days at 35 °C) and composting (15 days of active composting and 40 days of maturation) was found to be inadequate for the treatment of some starch-based shopping bags and PLA-based single-used items (Cucina et al., 2021). At the end of the test, 17.8% TS of the initial bioplastic content was retained when sieving the final compost at 2 cm. Bandini et al. (2022) tested some PLA and starch-based disposable spoons under combined anaerobic (43 days at 52 °C) and aerobic (22 days at 65 °C) conditions and observed persisting bioplastic fragments between 2 and 10 mm at the end of the process. A limited disintegration was also observed after the combined mesophilic anaerobic digestion (21 days) and composting treatment (28 days of active composting followed by 52 days of curing composting) of some cellulose acetate samples (Gadaleta et al., 2022): at the end of the process the residual amount of bioplastics accounted for 26−45% were measured.

3.2. Disintegration and mass loss

After the first disintegration test (see 2.3) no particles were retained at a 2-mm sieve. The same test with a higher concentration of bioplastics (10% w/w instead of 1% w/w) was performed by Cucina et al. (2021) on starch-based shopping bags and single-use PLA items. The test was carried out for 90 days at 58 °C and a degradation of 70% and 25% was observed for starch and PLA items, respectively, suggesting an influence of bioplastic concentration on the disintegration process. According to the same ISO 20200 method, Arrieta et al. (2014) tested the disintegration of biodegradable films synthesized in the laboratory with different PLA/PHB blends and reported weight losses higher than 90% after 28 days. Only very few studies have addressed the issue of bioplastic disintegration strictly following the ISO 20200 standard, therefore there is still lack of a consistent dataset that can be used to identify the influence of the environmental conditions on disintegration for bioplastics of different compositions. In the present study, an additional mass loss assessment was performed on 2 × 2 cm fragments of the cups with PLA_C reaching complete mass loss in 29 days and MB_C in 43 days (Fig. 2a). A similar test was performed by Sessini et al. (2019) on TPS samples (15 × 15 mm) at 58 °C and complete disintegration was observed after 56 days. The evolution of bioplastics alteration over time was monitored through eight sampling events, which evidenced different stages of physical/mechanical and biological alteration (Fig. 2b).

After the first week, the PLA pieces displayed an increased opacity, which may be linked to further crystallization of the polymer (Kalita et al., 2019) or to the onset of hydrolysis (Fortunati et al., 2012), since water absorption modifies the material's refractive index. Surface alterations, like cracks and cavities, began around day 16, leading to fragmentation by day 20 (Fig. 2b). The size range of PLA fragments did not change significantly, but the number of finer particles increased after 24 days (Fig. S2a). The cumulative area decreased with time (from 427 mm^2 on day 20–57 mm^2 on day 27), while the total number of particles reached a peak value on day 24 (695 particles) as a result of intensive fragmentation of the original material, decreasing afterwards to 263 with the progressive degradation of the material. MB_C showed an evident color change from the first extraction. The material surface showed three steps of macroscopic physical changes (Fig. 2b): i) progressive formation of cracks and cavities (day 16); ii) expansion of the cavities and appearance of darker/lighter areas together with an increased fragmentation (days 20–22); iii) further and uniform erosion (day 24–27). The measured mass loss during the sampling events on days 24 and 27 was comparable, indicating a lag time during the process. However, some differences can be observed (Figs. S2c and d) in terms of size range (0.23–296 mm^2 on day 24 and 0.02–112 mm^2 on day 27), cumulative area (596 mm^2 on day 24 and 483 mm^2 on day 27) and number of particles (11 on day 24 and 23 n day 27), highlighting the variability of the degradation and disintegration routes.

The lag phase duration for the disintegration process shown in Fig. 2a, was found to be comparable for the two cups, suggesting that the onset of biodegradation was mostly dictated by the environmental conditions of the system. The standards employed for this experiment group did not encompass the measurement of CO_2 production, therefore it was not possible to derive a direct comparison with the biodegradation tests in mature compost (see section 3.1).

3.3. Aerobic biodegradation in soil environment

At the end of the experiment in the soil environment, cellulose

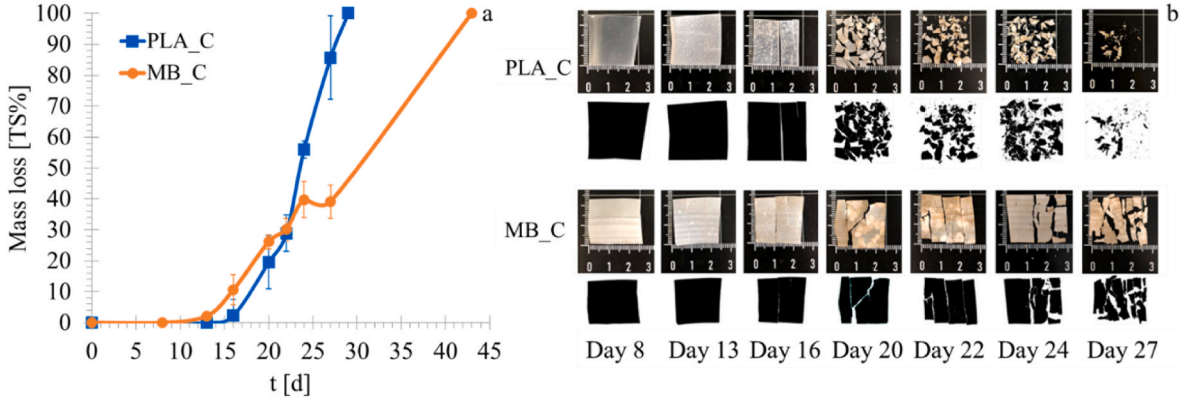

Fig. 2. a) Evolution of mass loss (TS% - total solids basis) of bioplastic particles over time and b) dimensional and physical alterations of bioplastic particles over the sampling events.

biodegradation was 62.5% and the percent difference of the replicates' biodegradation was 5.4%, thus complying with the requirement of the method. In 120 days of incubation the degradation degree for the reference material was 46.5%, which was lower than that obtained by Papa and colleagues, who reported a degradation degree of 66% (Papa et al., 2023). These differences could be attributed to the geographic location where the soil was obtained, which may influence both its biological and physicochemical characteristics. The evolution of CO_2 for PLA_C and MB_C runs (Fig. S3) was comparable to the one observed for blank (502.2 ± 54.2 ml in 180 days). It was concluded that no degradation was achieved in 180 days for the bioplastic cups (Table S1), likely due to the adverse conditions in the soil environment in terms of temperature and moisture. As already observed by other authors, native starch is more easily degradable than PLA, with an associated natural biodegradation time range of 1–2 years and 3–10 years, respectively (Cucina et al., 2021). However, commercial products are more recalcitrant than neat polymers and laboratory blends (Adhikari et al., 2016) due to their more complex composition, therefore longer biodegradation times may be anticipated. According to previous studies, TPS and TPS blended with PCL were degraded by 95% in 136 days and by 97% in 347 days, respectively (Narancic et al., 2018). On the other hand, starch-based products were found to reach 19.7%–55.1% degradation in 660 days (Gómez and Michel, 2013b), 50% in 168 days (Papa et al., 2023) and 34% in 90 days (Cucina et al., 2021). Mater-Bi was observed to have lost 34% of its weight after 400 days of testing (Alvarez et al., 2006). A PLA biodegradation degree around 20% was reported after over 500 days (Papa et al., 2023) and 5% after 90 days (Cucina et al., 2021). As far as the two digestate samples tested in our study were concerned, they attained a degree of biodegradation of 24% (PLA_D) and 20% (MB_D) (Table S1), which were likely mainly due to the contribution of endogenous respiration of the microbial biomass in the digestate samples. A previous study also concluded that mixing digestate with soil was not useful to improve bioplastics biodegradation (Papa et al., 2023). These results warn against directly applying digestate from bioplastics treatment onto soil, as it could act as a carrier of micro-bioplastics into an environment where they could potentially behave as contaminants due to their limited biodegradability.

3.4. Ecotoxicity

The results of the ecotoxicity tests with the tested compost and soil are shown in Fig. 3. The starting time of the experiments with plants was marked after 9 days, when a germination of 50% of the plant seeds was attained. After two weeks, the method requirement about the germination rate was fulfilled in both compost and soil, as all tests gave rise to germination rates higher than 90% with respect to the blank (Fig. 3a and d). Nevertheless, the achieved biomass of the plants in compost and soil, respectively, fell within the ranges 70%–83% and 83%–89% of the biomass in the blank test, suggesting a potential detrimental impact of the products of sample biodegradation in tomato plants biomass. In principle, the reduced growth of plants could also depend on an imbalanced carbon-to-nitrogen ratio in favor of carbon (Abraham et al., 2021). In such a case, microorganisms would need to extract an increased amount of mineral nitrogen from the soil for their metabolic needs, which may in turn become less available to the plants. However, since bioplastics degradation was negligible (see section 3.3) at the temperatures of the phytotoxicity tests (25 °C), the carbon in the bioplastics likely remained inaccessible to microorganisms. Furthermore, complete degradation of the bioplastics was observed during the composting tests, suggesting that the available carbon was utilized and converted into CO_2. It can thus be concluded that the toxicity effects observed in the tests probably resulted from reaction by-products associated with the presence of bioplastics (e.g. release of inorganic additives). It should however be noted that the amount of compost prescribed by the standard procedure for the phytotoxicity tests (1 g compost/g of soil, corresponding to 1000 − 1500 kg compost/m^3 soil assuming an average soil density in the range 1.0 − 1.5 t/m^3) is almost two orders of magnitude higher than that typically used in real conditions (6 − 16 kg compost/m^3 soil assuming a compost application rate in the range 20 − 50 t compost/ha on the top 30-cm layer of agricultural soil). Of course, the results of the applied tests should be interpreted in light of the fact that toxicity assessment procedures commonly do not intend to simulate real field conditions, but are often designed adopting a worst-case approach in terms of exposure assessment (exposure level, application duration, contact mode, etc.).

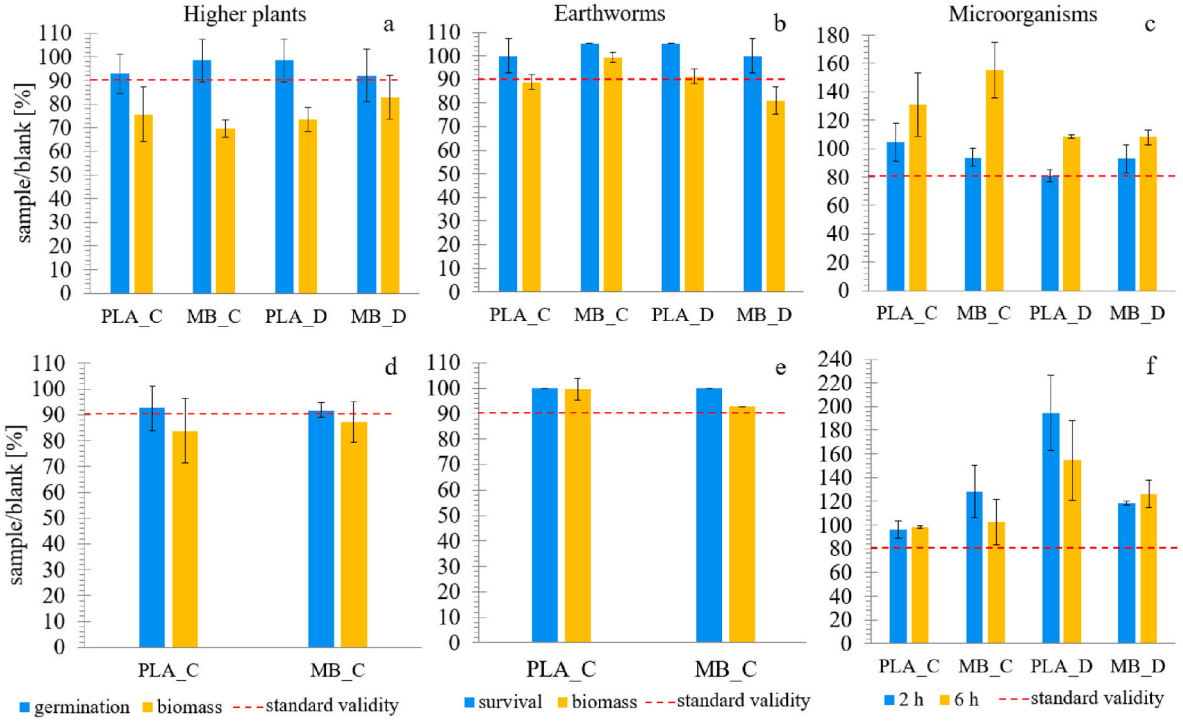

Fig. 3. Results of the ecotoxicity tests on higher plants, earthworms and microorganisms in compost (a, b and c) and in soil (d, e and f).

In the literature, a negative influence of bioplastics (both starch- and PLA-based) on plants was observed by a number of authors, which reported a decrease in the germination index for samples tested with PLA (F. Bandini et al., 2020; Boots et al., 2019). On the other hand, Huerta-Lwanga et al. (2021) did not observe any effect of PLA on plant growth. Starch-based plastics were reported to exert even severe effects on vegetative growth, and in some cases such effects were also more serious than those caused by the conventional fossil-based counterparts (Balestri et al., 2019; Qi et al., 2018). A 48-h test on *Lepidium sativum* seed germination revealed an increased phytotoxic effect of liquid digestate when it was mixed with Mater-Bi® shoppers (Pangallo et al., 2023).

Earthworms' survival was larger than 90% in all tests (Fig. 3b and c), while the biomass gain did not comply with the standard when the organisms were exposed to PLA_C (88%) and MB_D (81%). However, these values cannot be interpreted as a trend of bioplastics influence on earthworms and they are also quite close to the threshold imposed by the standard. As reported by Liwarska-Bizukojc (2021), data on bioplastics impact on soil fauna are currently scarce, but they generally indicate either no effect or a decrease in earthworms reproduction at high bioplastics concentrations (Holzinger et al., 2023). Earthworms' zero mortality was observed by a number of authors (Ferreira-Filipe et al., 2022; Sforzini et al., 2016), but the presence of ingested micro-bioplastics was also reported (Ferreira-Filipe et al., 2022; Zhang et al., 2018). On the other hand, Huerta-Lwanga et al. (2021) observed increased earthworm mortality after 16 days at PLA concentrations of 0.75% and 1% w/w, while Khaldoon et al. (2022) observed that increasing concentrations of PLA (from 10% to 80% w/w) were correlated with a lower individuals weight gain. The method requirement on the nitrification potential of the microorganisms was fulfilled for all the experimental runs, indicating that the samples were non-toxic for the nitrifying bacteria in all the tested matrixes (Fig. 3c and f). The nitrification potential of bacteria in tests with compost ranged from 1.97 to 2.54 and from 2.96 to 4.25 μgNO_2-N/g after 2 and 6h, respectively, while it ranged from 0.39 to 0.8 and from 0.65 to 1.02 μgNO_2-N/g after 2 and 6h, respectively, in soil.

4. Discussion

The set of testing procedures adopted in the present study was meant to provide different kinds of information regarding the environmental profile of bioplastics in various scenarios. Concerning the assessment of disintegration and biodegradability of the materials, it should be noted that the different conditions envisaged by the adopted standardized procedures make the results hardly relatable. In particular, the biodegradation experiments in a controlled composting environment were aimed at assessing the intrinsic (i.e. in the absence of other co-substrates) biodegradation potential of bioplastics under non-limiting conditions; these involved the use of a sufficient amount of mature compost (85% TS) to provide the required active microorganisms for the biodegradation process, as well as milling the material ($\Phi < 0.1$ cm) to guarantee a high surface area thus reducing the time for particle fragmentation. On the other hand, the disintegration tests were intended to estimate, again under controlled aerobic conditions, the degree of fragmentation of the whole original material over time but with the addition of a co-substrate (SSW) to simulate a real scenario in which bioplastic residues are treated along with biowaste. In the biodegradation tests, unaltered particles remained visible after over 30 days of degradation; on the other hand, the disintegration tests revealed complete disintegration in 29–45 days. Together, the results of the two tests suggest that the presence of a co-substrate may enhance bioplastic degradation, likely as a result of keeping higher moisture in the system, enhancing microbial activity due to the presence of readily biodegradable substances and/or promoting the chemical hydrolysis of bioplastics. The observed disintegration times may also be used for practical purposes, suggesting the minimum residence time to be adopted in full-scale waste treatment plants where co-composting of bioplastics with biowaste is performed.

Under simulated worst-case scenarios, the assessment of bioplastic ecotoxicity on plants revealed an inhibition of plants biomass growth. Altogether, the absence of degradation of bioplastics under simulated soil conditions along with the observed toxic effects onto plants warn about the real environmental behaviour of bioplastics, which should thus be further investigated and studied also in correlation with an assessment of soil exposure scenarios to bioplastics.

Finally, the results of the biodegradation tests conducted on the digestates from previous anaerobic digestion of the same bioplastic products may have practical implications regarding the potential benefits arising from coupling anaerobic digestion and composting, as this turned out to be effective in achieving complete biodegradation of the bioplastic items.

5. Conclusions

The main results and implications of the present study on the biodegradation of bioplastics and digestates can be summarized as follows.

- biodegradation of bioplastics in mature compost was strongly affected by the heterogeneity of the polymeric matrix, with Mater-Bi displaying a more recalcitrant behavior than PLA.
- the disintegration experiments, carried out with a co-substrate, displayed a faster kinetics compared to biodegradation in composting conditions. An increased moisture was identified as one of the potential accelerating factors.
- no degradation was observed for the two bioplastic products in soil after six months, likely due to the non-optimal conditions (temperature = 25 °C, moisture = 16 wt%). This casts a warning related to their potential persistence of some bioplastic products in natural soil environments when dispersed uncontrollably.
- the coupling of anaerobic digestion and composting seemed effective in achieving complete biodegradation of the bioplastic items. Nevertheless, some ecotoxicological effects on plant and earthworm biomass gain were observed (17–26% lower gain compared to the blank), suggesting the need for deeper investigation of the potential byproducts of the biodegradation process.
- when directly applying digestates on soil, the testing temperature (25 °C) was a limiting factor for their degradation. This means that the residual bioplastic fragments that may persist after the anaerobic treatment can be carried into the environment and act as potential contaminants.
- ecotoxicity tests on the final compost revealed in all cases a negative effect on higher plants growth only (17–30% lower growth compared to the blank), although conclusive remarks on the ecotoxicity profile of the investigated materials would require a more systematic and wider assessment.

Overall, the results of the present study underscore the importance of comprehensively characterizing the environmental profile of bioplastics. This entails employing a combination of testing methods capable of addressing a variety of features related to the degradation process, going beyond the sole gaseous products or mass loss analysis. Further efforts to defining appropriate testing conditions to fully capture the actual environmental profile of bioplastic products should also be made.

CRediT authorship contribution statement

M. Falzarano: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **A. Marìn:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **L. Cabedo:** Writing – review & editing,

Supervision, Methodology, Funding acquisition, Conceptualization. **A. Polettini:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **R. Pomi:** Writing – review & editing, Supervision, Conceptualization. **A. Rossi:** Writing – review & editing, Conceptualization. **T. Zonfa:** Writing – review & editing, Methodology.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Luis Cabedo reports financial support was provided by Spanish Ministry of Science and Innovation. Luis Cabedo reports financial support was provided by Government of Valencia. Luis Cabedo reports financial support was provided by FEDER Una manera de hacer Europa. Alessandra Polettini reports financial support was provided by Italian Ministry of Education and Merit. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.142648.

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