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Research Article

# Comparative toxicity of conventional versus compostable plastic consumer products: An in-vitro assessment

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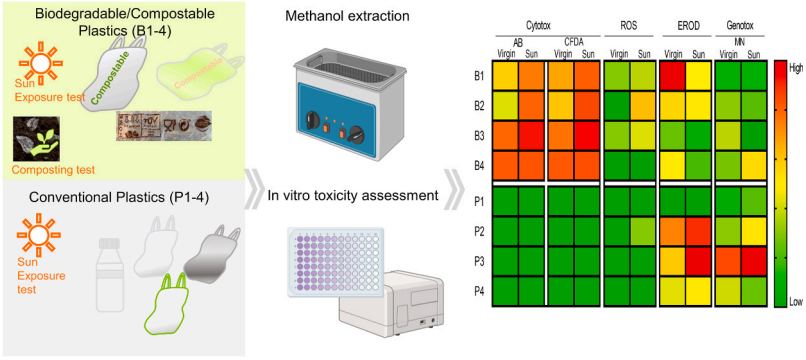
## HIGHLIGHTS

- Incomplete photodegradation and composting increases the toxicity of plastics.
- Recycled plastics contain elevated levels of CYP1A inducers and genotoxic compounds.
- Compostable plastics show higher toxicity than conventional and recycled plastics.
- Additives in bioplastic formulations require careful evaluation.

## GRAPHICAL ABSTRACT

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## ABSTRACT

This study investigates the toxicity of methanolic extracts obtained from compostable plastics (BPs) and conventional plastics (both virgin and recycled). Additionally, it explores the potential influence of plastic photodegradation and composting on toxic responses using a battery of in vitro assays conducted in PLHC-1 cells. The extracts of BPs, but not those of conventional plastics, induced a significant decrease in cell viability (<70%) in PLHC-1 cells after 24 h of exposure. Toxicity was enhanced by either photodegradation or composting of BPs. Extracts of conventional plastics, and particularly those of recycled plastics, induced 7-ethoxyresorufin-O-deethylase (EROD) activity and micronucleus formation in exposed cells, indicating the presence of significant amounts of CYP1A inducers and genotoxic compounds in the extracts, which was enhanced by photodegradation. These findings highlight the importance of investigating the effects of degradation mechanisms such as sunlight and composting on the toxicity of BPs. It is also crucial to investigate the composition of newly developed formulations for BPs, as they may be more harmful than conventional ones.

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## 1. Introduction

The annual global production of plastics has doubled in the last 20 years, from 234 million tons (Mt) in 2000–460 Mt in 2019, while plastic waste has more than doubled, from 156 Mt in 2000–353 Mt in 2019 (OECD, 2022). Although countries around the world are struggling to manage the current volume of plastic production and to reduce plastic waste, the recycling of plastics is still far from being optimized [11]. Single-use plastics (SUPs), such as plastic bags and disposable water bottles, represent a significant source of plastic pollution [14,62]. The excellent properties of SUPs (durability, lightness and stability) combined with their extensive use and inadequate recycling, and resistance to natural degradation, pose a serious environmental threat [36]. In recent years, governmental bodies worldwide have initiated measures to diminish and ban the use of fossil-based SUPs [5,8], and BPs have been presented as one of the key solutions to replace conventional plastics [26,55]. From January 2021, light and ultralight bags were banned in Spain, with the exception of biodegradable and compostable bags. Currently, the utilization of BP bags labeled as 'compostable' for bulk products (fruits, vegetables, etc.) is prevalent within the supermarkets and grocery stores and is anticipated to experience sustained growth in the coming years due to the implementation of new regulations.

Compostable plastics are distinguished by their capacity to undergo degradation without leaving noticeable residues or toxic substances that could be harmful to animals or plants [18]. Typically, they can be degraded by bacterial and fungal enzymes into carbon dioxide, methane, water and biomass/compost under controlled disposal conditions. Nevertheless, the degradation of BPs in open environments is still controversial [19,26,32,43,55]. Numerous factors, such as sunlight, heat, humidity, physical stress, and microorganisms, can substantially modify degradation rates [27,39].

The detrimental effects of plastics on the environment and living organisms are not only related to the visible pollution caused by their debris, but also to the leaching of hazardous chemicals from plastic materials [25]. A single plastic product may contain hundreds of chemicals or additives, most of which are not covalently bound to the polymer and can therefore leach out to the surrounding environment and be taken up by living organisms [63,65]. Bio-based and biodegradable plastics still require additives to improve their properties such as elasticity, color, electrostatic behavior, strength and toughness. These additives are often the same as those applied to traditional plastics, but new additives are specifically developed for improving the processing and properties of biopolymers [37]. Zimmermann et al. [65] found that 80% of bioplastics, including BPs and plant-based alternatives, contain over 1000 different chemicals. Many of these chemicals, such as phthalates, benzotriazoles, cyanoacrylates, aluminum trihydroxides, are known to be hazardous, due to their persistence, bioaccumulation, toxicity, and endocrine disrupting properties. Nevertheless, a large proportion of these additives remain unidentified [24,63].

Weathering of plastics, such as photodegradation, can cause irreversible modifications in their chemical composition, including chain scission reactions in synthetic polymers [47]. Ultraviolet (UV) irradiation plays a pivotal role in the degradation process of plastics, promoting the release of harmful substances and intensifying the fragmentation of both macroscopic and microscopic plastics [29,52]. In addition, semi-degraded plastics present in compost have also been identified as problematic, as a substantial amount of plastic fragments, particularly in bio-waste compost, can end up in farmland and ultimately in the aquatic environment [12]. Consequently, the question of whether BPs can be a promising solution to the waste disposal problem and global plastic pollution in the long run remains unresolved.

Recently, Barbale et al. [7] emphasized the importance of assessing the ecological hazard of packaging and single-use items based on the release of leachable chemicals, chemical release during degradation, and the physical hazards of debris or small size weathering particles (e.g. micro- and nanoplastics). However, when a compostable plastic bag was tested according to the EN 13432 standard with a plant growth test, it did not cause ecotoxicity. The degradation of BPs is accelerated during composting (high temperature, moisture, microorganisms), but it will be much lower in the natural environment, leading to the additional release of chemicals, but also micro- and nano-plastics [32]. Therefore, additional studies are needed to assess potential toxicity of SUPs in the environment, and particularly, in the aquatic environment, using representative organisms or bioassays that allow the sensitive detection of toxic responses and an accurate evaluation of the risks [57,6,65].

The goal of this study is to compare the toxicity of BPs and conventional plastics (virgin and recycled), and to investigate whether photodegradation and composting of plastics can affect the toxic responses in PLHC-1 cells, a fish liver cell model successfully used in toxicological research. To this end, we selected eight single-use plastic consumer products, including four BPs (light bags for foodstuff and waste) and four conventional plastics (water bottles, carrier bag, garbage bags). Among the conventional plastics, we included two garbage bags that were produced through mechanical recycling of plastics. We then investigated the in-vitro effects of methanolic extracts in terms of cytotoxicity, generation of reactive oxygen species (ROS), presence of CYP1A inducers and genotoxic compounds.

## 2. Materials and methods

### 2.1. Reagents and chemicals

Eagle's Minimum Essential Medium (MEM), fetal bovine serum (FBS), and other biochemicals were purchased from Gibco (Life Technologies Limited, Paisley, United Kingdom). 2′,7′-Dichlorodihydrofluorescein diacetate ($H_2$DCF-DA), 3-morpholinosydnonimine (SIN-1), dimethyl sulfoxide (DMSO), and methanol were purchased from Sigma-Aldrich (Schnelldorf, Germany). Alamar Blue (AB) and 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) were from Thermo Fisher Scientific (Waltham, US) and Molecular Probes (Invitrogen, Eugene, Oregon, US), respectively. 7-Ethoxyresorufin, 7-hydroxyresorufin, β-naphthoflavone (BNF), clotrimazole and bovine serum albumin (BSA) were purchased from Sigma–Aldrich (Steinheim, Germany).

### 2.2. Origin of plastic samples and characterization

Eight common plastic consumer products (biodegradable/compostable plastics B1–4; conventional plastics: P1–4) were selected from the market (Barcelona, Spain) (Table 1). Both, thermogravimetric analyses (TGA) and infrared spectroscopy (FTIR) were used to assess the gross composition of the plastic and bioplastic bags. Very briefly, thermogravimetric curves (TG) were recorded with a TA 550 (Waters- TA Instruments, New Castle, EEUU) by heating the samples (~ 5 mg) at 10 °C/

**Table 1**
Plastic consumer products analyzed including biodegradable/compostable plastics and conventional plastics.

| Plastic category | Sample ID | Product | Polymer | Color |
|---|---|---|---|---|
| Biodegradable/ compostable | B1 | Bag for foodstuff | PBAT + starch | Transparent |
| | B2 | Bag for foodstuff | PBAT + starch | Transparent |
| | B3 | Bag for foodstuff | PBAT + starch + erucamide | Transparent |
| | B4 | Garbage bag | PBAT + starch | Green |
| Conventional plastics | P1 | Water bottle single use | PET | Transparent |
| | P2 | Carrier bag | LDPE | White |
| | P3 | Garbage bag | Recycled PE | Black |
| | P4 | Garbage bag | Recycled PE | Green |

*PBAT: polybutylene adipate terephthalate; PET: Polyethylene terephthalate; LDPE: Low-Density Polyethylene; PE: Polyethylene.

min from 30 to 800 °C under a nitrogen environment. The different bags were also analyzed by FTIR in attenuated total reflectance (ATR) mode using a Thermo Nicolet Nexus (GMI, USA) equipment. The spectra were taken at 4 $cm^{-1}$ resolution in a wavelength range between 400 and 4000–1 and averaging a minimum of 32 scans. The results were processed using Origin Pro 2019 software (OriginLab Corporation, Northampton, MA, USA).

### 2.3. Photodegradation of plastics

Photodegradation experiments were performed in a Suntest CPS solar simulator (Atlas, USA), fitted with an air-cooled 1500-W xenon lamp (300–800 nm). The device allowed an effective illuminated surface of 560 $cm^2$. The irradiance was preset at 600 $W/m^2$ and controlled with a VLX-3 W radiometer (Vilber Lourmat). The intensities of UVA and UVB were 5.14 $mW/cm^2$ and 2.58 $mW/cm^2$, respectively. Plastics were cut into small pieces (1 $cm^2$), split in two parts (weight), and one part irradiated for 6 h, which corresponds to approximately 3 days of sun exposure at European average solar irradiance [23].

### 2.4. Disintegration test

A representative sample of BP (B3, different batch) was submitted to the disintegration test, using two distinct compost media, namely a normalized solid matrix and a mature compost (originated from a vegetable waste composting plant). The normalized solid matrix consisted of a mixture of sawdust, rabbit feed, mature compost, corn starch, sucrose, corn oil, urea and water (Table S2). The normalized media is the one requested by the corresponding standard for this test, while the mature compost mimics the conditions existing in composting plants. The test bioreactors were polypropylene containers measuring 30 cm × 20 cm x 10 cm (length, width and height). Each reactor contained 5 g of test material (plastic) and 1 kg of synthetic or mature compost medium. The ratio between the mass of the test materials and the mass of the wet synthetic residue must be between 0.5% and 2% according to the standard (UNE-EN ISO 20200:2016). The reactors were incubated at a constant temperature of 58 ± 2 °C for a period of 60 days (being 90 days the maximum testing time). To guarantee a good composting process, the content of the reactors was mixed and moistened according to the standard timetable (final water content of 55% in total). The degree of disintegration was determined after the composting period and the final substrate was sieved through standard 10 mm, 5 mm and 2 mm sieves (according to ISO 3310–1) to recover undecomposed residues. The reduction in the mass of the test sample was used to calculate the degree of disintegration. This percentage value ($D_i$) was calculated by the following formula:

$$Di = \frac{m_i - m_f}{m_i} \times 100\%$$

According to ISO 20200 standard, a plastic is considered disintegrated when its size is equal or less than 2 mm. Thus, at the end of the test, the visible plastic fragments (> 2 mm) were retrieved from the compost media using tweezers, in order to proceed with their extraction.

### 2.5. Extraction of plastic and compost samples

All plastic and compost samples were extracted with methanol as described in Zimmermann et al. [64], with some modifications. Briefly, compost (2 g) and plastic samples (0.2–2.6 g) cut into small pieces (~1 cm × 1 cm) were placed in a glass tube, immersed in 20 mL of methanol and extracted in an ultrasonic bath for 1 h at room temperature. After centrifugation (2000 rpm, 10 min), the supernatant was transferred to a clean glass vial and evaporated to dryness under a stream of nitrogen. A second extraction with 10 mL of methanol was performed, and the final dry extract reconstituted in DMSO and stored at − 20 °C. The final concentrations tested in the cells are detailed in Table S3.

### 2.6. Cell culture

PLHC-1 (*Poeciliopsis lucida* hepatocellular carcinoma) cell line was obtained from American Type Culture Collection (CRL-2406). Cells were cultured at 30 °C and 5% $CO_2$ in complete growth medium (CGM) that consisted of Eagle's MEM supplemented with 5% FBS, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 1.5 g/L sodium bicarbonate, 50 U/mL penicillin, 50 μg/mL streptomycin and 2 mM L-glutamine, as previously described in P é rez-Albaladejo et al. [46]. Plastic and compost extracts were applied to cell cultures by diluting the extracts in culture medium, the final concentration of DMSO in the wells was of 0.5% (v/v). Controls and solvent controls (0.5% DMSO) were included in every assay.

### 2.7. Cell viability and reactive oxygen species (ROS) generation

To assess the cytotoxicity and ability to generate ROS of plastic and compost extracts, $10^5$ PLHC-1 cells were seeded in 200 μL CGM in 96-well plates (Nunc; Roskilde, Denmark) and allowed to attach overnight. Exposure experiments were performed in serum free medium (SFM, without the addition of 5% FBS). Cytotoxicity was tested as reported in Marqueño et al. [38] with some modifications. Briefly, after 24 h exposure to the extracts in SFM, cells were washed with phosphate-buffered saline (PBS) and assessed cell viability with 5% Alamar Blue (AB: 530/590 nm) and 4 μM 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM: 485/530 nm). Fluorescence was measured in a Tecan Infinite M Plex plate reader (Männedorf, Switzerland). The generation of ROS was assessed as described by P é rez-Albaladejo et al. [46]. Cells were first loaded with 20 μM H2DCF-DA for 30 min, washed with PBS, and the fluorescence of oxidized H2DCF was measured after 15, 30, 60, and 120 min of exposure to the different extracts. Three biological replicates of different passages and six experimental replications in each plate were performed.

### 2.8. EROD activity

The presence of CYP1A inducers in plastic/compost extracts was assessed as the induction of EROD activity as described in P é rez-Albaladejo et al. [45]. PLHC-1 cells (6.5 × $10^5$ cells/well) were seeded in 48-well plates (Nunc; Roskilde, Denmark), and exposed to varying concentrations of plastic/compost extracts, positive control (1 μM β-naphthoflavone; BNF) or 0.5% DMSO (solvent control) in CGM. After 24 h, cells were washed with PBS and incubated with 2 μM 7-ethoxyresorufin for 15 min at 30 °C. Fluorescence was read at 537/583 nm in a Tecan Infinite M Plex plate reader. Quantification was performed by calibration with 7-hydroxyresorufin. After fluorescence reading, cells were washed with PBS, and total cellular protein was measured with fluorescamine (150 μg/mL) using bovine serum albumin (BSA) as a standard. EROD activity was expressed as pmol resorufin formed per minute and per mg protein (pmol/min/mg protein).

### 2.9. Micronucleus (MN) determination

The genotoxicity of plastic extracts on PLHC-1 cells was estimated as the frequency of micronuclei formation, as described in Schnell et al. [53] with some modifications. 5·$10^4$ cells were plated on a glass coverslip (Ø 12 mm; Marienfeld, Germany) placed in a 12-well plate. After attachment, cells were exposed for 48 h to the plastic extracts at non-toxic concentrations, starting with their respective 1/10 $EC_{50}$ value, 0.5 μM mitomycin C (positive control) or 0.5% DMSO (solvent control). Next, cells were fixed with 4% formaldehyde, stained with 5 μM bisbenzimide Hoechst 33342 (Sigma-Aldrich, Steinheim, Germany) and the coverslips mounted with Vectashield H-1000 (Vector Laboratories, CA, USA). Slides were kept at 4 °C in the darkness prior to observation, and

micronuclei were scored using a confocal laser scanning microscope (Zeiss LSM 880; Tokyo, Japan) equipped with a Plan-Apochromat 63 × /1.4 Oil DIC M27 objective and use of the diode laser emitting 405 nm for DAPI visualization. During scanning, a double dichroic mirror (DM 405/488) was used with an emission window set in the 415–587 nm range. Z-stack images (voxel size: 0.13 × 0.13 × 1 μm) were obtained from 5 × 5 tile scans, stitched and processed into maximum intensity projections to score micronuclei. A Python 3.8 script was created to perform automatic counting of nuclei. More specifically, the script provided automatic segmentations of nuclei, their size, as well as grid images to facilitate manual counting of micronuclei. Firstly, the images were imported using the OpenCV library version 4.7.0.68 and converted to gray scale, collapsing the three-color channels into a single dimension. A 5 × 5 pixel Gaussian kernel was then applied to remove noise that approximately follows a Gaussian distribution. Therefore, the light speckles found within the nuclei were smoothed, decreasing sharp intensity transitions within nuclei. A threshold was subsequently applied to the smoothed image so as to detect the nuclei. Otsu's binarization was chosen to perform this task, since this method automatically detects the optimal threshold for the input image. Otsu's thresholding returned a binary mask of the image, where pixels within nuclei had an intensity value of 255, and the background pixels had a value of 0. The result of this step was used to count the number of nuclei in the image, as well as their size (in pixels). Furthermore, the contours of the binary image were generated and added to the original image, to assess the goodness of the segmentation, and a 5 × 5 grid was added to the images to ease the manual counting of the micronuclei. The criteria used to score micronuclei were based on Fenech et al. [17]. Briefly, the micronuclei must be round or oval shapes, stained with the same intensity as the main nucleus, and not overlap or connect to it. For each treatment, a total of 2000 cells were scored from different coverslips and results were expressed as ‰ micronucleated cells.

### 2.10. Statistical analysis

Half maximal effective concentrations ($EC_{50}$) were calculated and dose response curves plotted using the software GraphPad Prism 9. One-way ANOVA with Tukey test was used to analyze the statistical differences (SPSS Statistics 27). Significance level was set at $p < 0.05$.

## 3. Results

### 3.1. Plastics and bioplastics properties

TG curves and FTIR spectra of the various samples are shown in Fig. 1. The FTIR spectra reveal that samples P2, P3 and P4 were made of polyethylene, with samples P3 and P4 (recycled plastic bags) additionally containing calcium carbonate. In contrast, the FTIR spectrum from sample P1 corresponds to a polyester, specifically PET (polyethylene terephthalate). The spectra obtained from the biopolymer bags corresponded to biopolyesters. Sample B2 featured an inscription indicating the commercial grade of the material, Mater-Bi®, a commercial compostable thermoplastic containing polybutylene adipate terephthalate (PBAT) and starch. The FTIR spectra demonstrated that all the

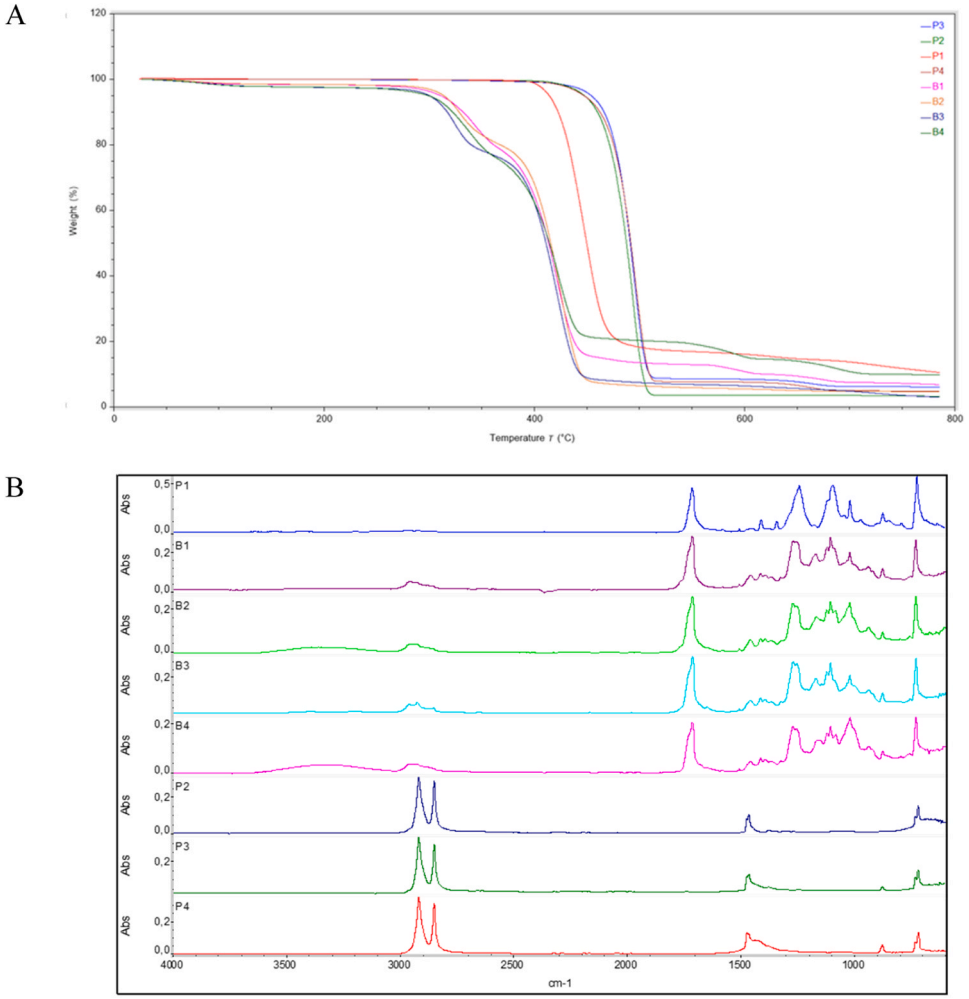


**Fig. 1.** Thermogravimetric curves (A) and FTIR spectra (B) of the various plastic (P) and bioplastic (B) samples.

biopolymer samples displayed similar vibrational bands, and two main thermal degradation peaks were observed from TGA, indicating that all the samples had essentially the same basic composition, i.e. PBAT and starch. Sample B3 also showed spectral bands corresponding to compounds related to erucamide (clearly observed in the range from 2850 to 3000 $cm^{-1}$). The TGA results also showed a variable amount of inorganic residue in the different samples. Table S1 compiles the composition of the different samples together with their ash contents.

### 3.2. Cell viability

BP extracts (B1-B4) lead to a significant decrease of cell viability on PLHC-1 cells after 24 h exposure, while extracts of conventional plastics (P1-P4) did not alter cell viability (Table S4). The dose-response curves for BPs (B1-B4) and the concentrations resulting in a 50% decrease in cell viability ($EC_{50}$) are shown in Fig. 2 and Table S4. The toxicity of BPs significantly increased after simulated photodegradation, particularly B3 extracts resulted in the highest toxicity ($EC_{50}$: 3.4 mg/mL (AB); 3.2 mg/mL (CFDA)).

B3 bags were disintegrated in compost, being their degree of disintegration, measured as percentage of weight loss, which was 97.01% in mature compost and 98.40% in synthetic compost (Table S5). After 60 days, the volatile solids content in the dry material decreased and the pH value of the mature and synthetic composts increased (Table S6). Extracts of the remaining BP fragments showed increased cytotoxicity compared to the original plastic bag, particularly those fragments that disintegrated in mature compost (Fig. 3). The extracts of compost after removal of the visible plastic debris also showed significant cytotoxicity in PLHC-1 cells, both mature ($EC_{50}$: 11.2 ± 2.3 mg/mL (AB); 4.5 ± 0.8 mg/mL (CFDA)) and synthetic ($EC_{50}$: >20 mg/mL (AB); 12.6 ± 0.9 mg/mL (CFDA)) composts (Fig. S1). However, the toxicity of compost samples did not change after 60 days of composting, indicating that the degradation of BPs in the compost did not significantly increase their toxicity (Fig. S1).

### 3.3. ROS generation

Only two out of four BP extracts (B1: 22 mg/mL; B3: 26 mg/mL) resulted in a slight increase in ROS generation, while the extracts of conventional plastics tested at maximum concentrations (20–26 mg/mL) did not induce ROS in PLHC-1 cells (Fig. S2). Photodegradation enhanced ROS production in 3 out of 4 BP extracts (B1-S: 2.5-fold; B2-S: 2.5-fold; B3-S: 1.7-fold), but had no significant effect on conventional plastic samples (P2-S: 1.2-fold) (Fig. S2).

Interestingly, extracts of plastic debris collected after composting significantly induced ROS production (1.5–1.9-fold) at all tested concentrations (0.5–4 mg/mL), while virgin BPs only generated ROS (1.3-fold) at concentrations higher than 2.0 mg/mL (Fig. 4 A). The extracts of compost (5 mg/mL) also lead to significant ROS induction: mature compost (1.4–1.7-fold) and synthetic compost (1.2–1.4-fold) (Fig. 4B). No significant increase in ROS production was detected after 60-days composting.

### 3.4. Induction of EROD activity

All plastic extracts, except those from water bottles (PET), significantly induced EROD activity, with dose-response plots shown in Fig. 5. Photodegraded BPs induced lower EROD activity than virgin BPs, while conventional plastics (P2–4) induced higher EROD activity after photodegradation. The highest EROD activity was observed after exposure to B1 (130.2 ± 11.5 pmol/min/mg protein), and two conventional plastics after photodegradation (P3-S: 117.5 ± 5.1 pmol/min/mg protein; P2S: 82.4 ± 3.3 pmol/min/mg protein).

Interestingly, the small BP fragment collected after 60-days of composting, showed an increased ability to induce EROD activity (5.3-fold increase in mature compost; 3.6-fold increase in synthetic compost) (Fig. 6A). The compost extracts, both the mature and synthetic compost, induced EROD activity in PLHC-1 cells, with the highest activity detected for mature compost (62.1 ± 3.2 pmol/min/mg protein) (Fig. 6B, C). After 60 days of composting, the ability of compost sample

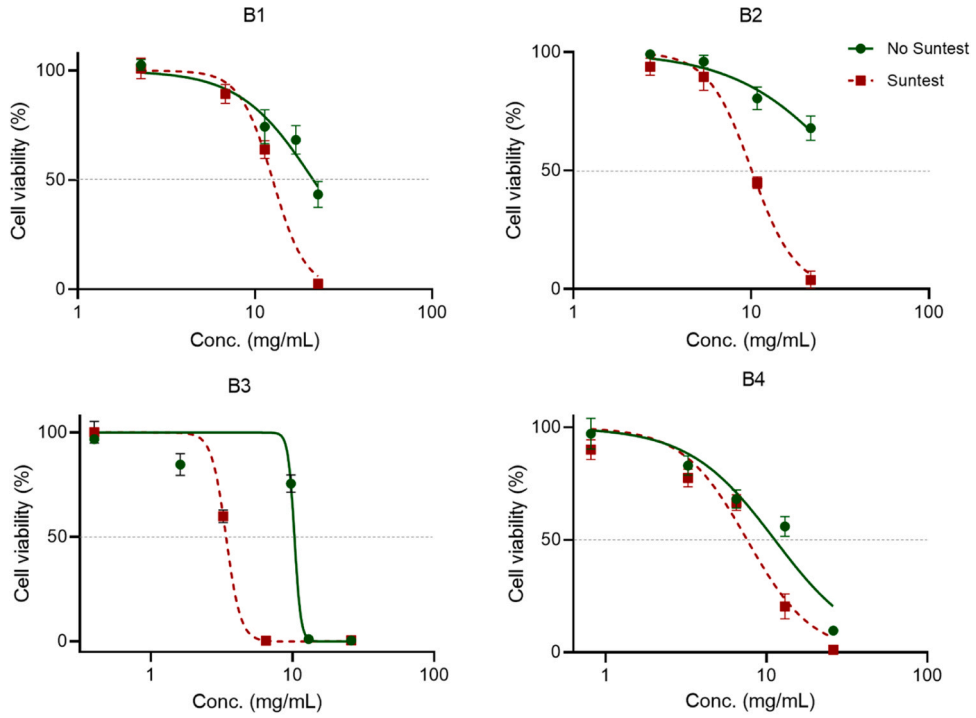


**Fig. 2.** Cell viability of PLHC-1 cells exposed to extracts of virgin or photodegraded BPs (B1-B4) for 24 h, assessed with Alamar Blue. The results for the virgin plastic extracts are shown in green, while photodegraded plastic extracts are shown in red. The dashed line represents 50% depletion of cell viability. Values are the mean ± SEM of at least three independent experiments.

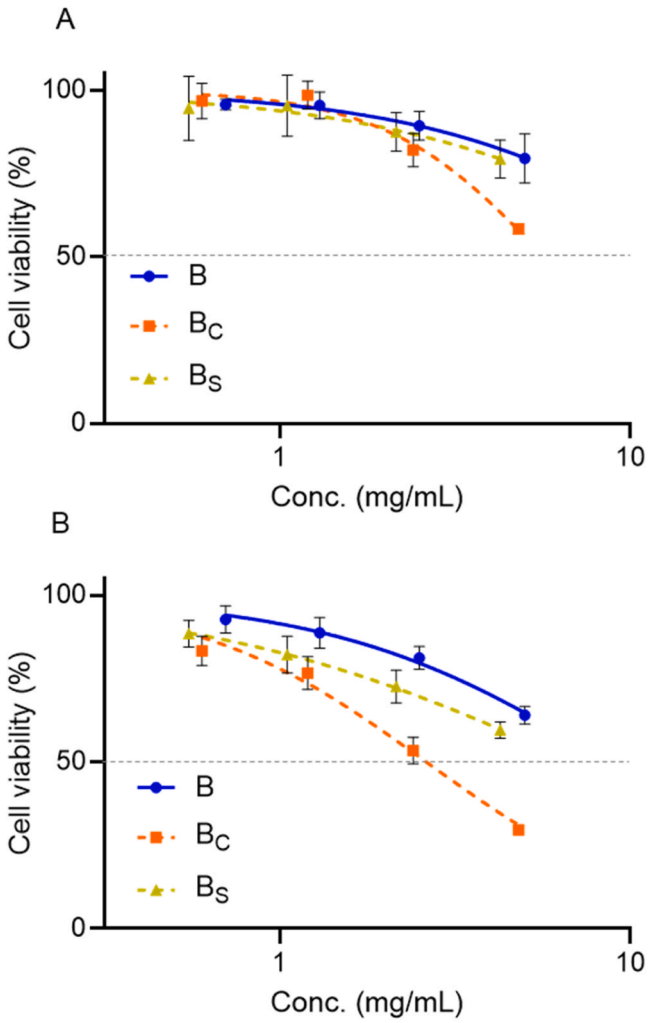


**Fig. 3.** Cell viability of PLHC-1 cells exposed to extracts of virgin or composted BPs assessed with Alamar Blue (A) and CFDA-AM (B). Blue line: virgin BP (B); orange and green: composted plastic after composting in mature compost ($B_C$) and synthetic compost ($B_S$), respectively. The dashed line indicates 50% depletion of cell viability. Values are the mean ± SEM of at least three independent experiments.

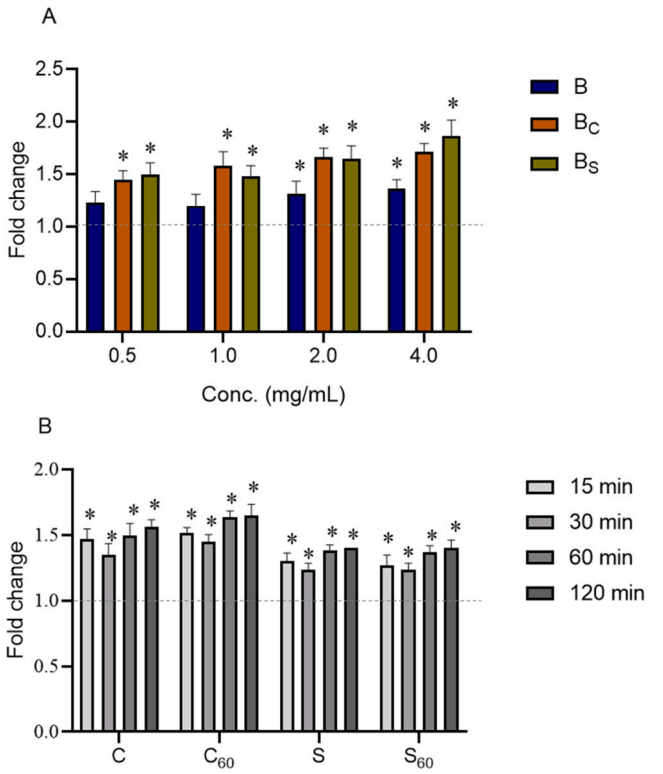


**Fig. 4.** (A) ROS production in PLHC-1 cells after 15 min exposure to extracts of biodegradable/compostable plastic (blue), and the extracts of plastic residues after composting in mature compost (orange) and synthetic compost (green) at different concentrations (0.5–4 mg/mL). (B) ROS production of PLHC-1 cells after 15, 30, 60, 120 mins exposure to extracts of compost samples (5 mg/mL). Labels C and S represent samples of mature compost and synthetic compost, respectively. The subscript '$_{60}$' represents the compost sample after 60 days of composting. The dashed line represents the level of ROS in control cells (0.5% DMSO). Values are the mean ± SEM of at least three independent experiments. *Significant differences respect to control ($p < 0.05$, one-way ANOVA).

extracts to induce EROD activity in PLHC-1 cells remained the same.

### 3.5. Genotoxicity

MN frequencies (‰) determined in PLHC-1 cells exposed to plastic extracts (0.4–26 mg/mL), positive control (0.5 μM mitomycin C), and solvent control (0.5% DMSO) are summarized in Table S4. The frequency of micronuclei in the positive control was of 272 ± 40‰, 10-fold higher than in cells exposed to 0.5% DMSO (20 ± 5‰). Generally, plastic extracts exhibited low genotoxic responses in PLHC-1 cells, with MN frequencies between 1.6- to 2.2-fold higher than the solvent control, with the exception of sample P3 that induced the highest frequency of micronuclei before (141‰) and after photodegradation (217‰) (Table S4). P3 corresponds to the extract of a black recycled-PE garbage bag. Two other extracts from photodegraded plastics also significantly increased the frequency of MN in PLHC-1 cells (B4-S: 61‰; P2-S: 54‰).

## 4. Discussion

### 4.1. Toxicity of plastic extracts

Bio-based and compostable plastics (BPs) have gained attention as environmentally friendly substitutes for conventional non-biodegradable plastics. However, BPs still require the incorporation of additives to improve their processing and enhance their characteristics. To the best of our knowledge, few studies have investigated the toxicity of chemical leachates from BPs, especially following composting or photodegradation, when BPs components are released into the environment. Given the diversity of BP-based items, this work focused solely on light or ultralight plastic bags, where BP has almost completely replaced traditional plastics. Our goal was to get a first insight into the hazards of extractable compounds in BPs compared to conventional plastics (virgin and mechanically recycled). We are aware that methanolic extracts represent the worst-case scenario, but even so, one of the most striking findings was that the chemicals extracted from BPs (B1-B4) were cytotoxic to PLHC-1 cells, while those extracted from conventional plastics (P1-P4) were not. Remarkably, among the four BP samples selected, B3 demonstrated the most pronounced toxic effects. Upon drying the methanol extract, a comparatively higher amount of white precipitate was observed, suggesting a potentially higher extraction of chemical additives for this particular sample. In terms of polymer composition, the four BPs, consisting of PBAT and starch, showed no significant differences, except for the detection of erucamide in B3. Erucamide is commonly used as a slip additive in the plastic manufacturing industry and has been reported to have non-toxic properties [28,40]. Therefore, the increased toxicity observed in B3 samples may not be directly related to the presence of erucamide, but to additional compounds released during extraction.

Likewise, none of the conventional plastic extracts generated ROS,

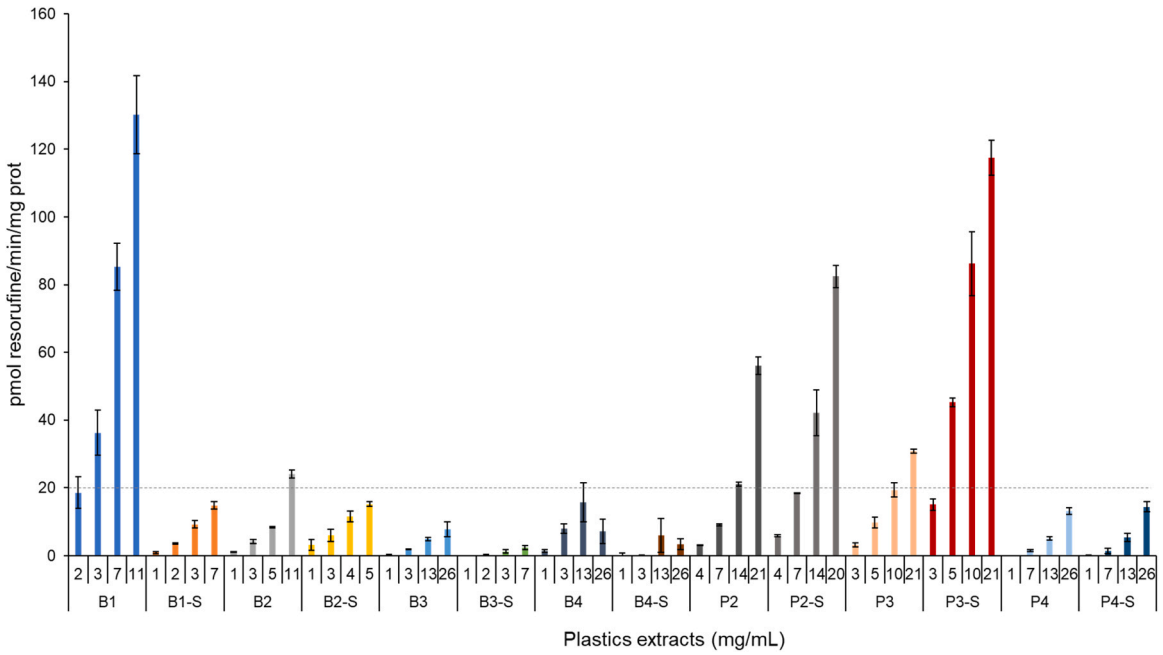


**Fig. 5.** EROD activity of PLHC-1 cells after exposure to various concentrations of the tested plastic extracts for 24 h, with the exception of the water bottle (P1), which did not show CYP1A induction and was not included in the figure. Results are expressed in pmol resorufin/min/mg protein. Label -S indicates samples that have been irradiated in a SunTest for 6 h. EROD activity induced by 1 μM β-naphthoflavone (BNF; positive control) was 60 ± 5.9 pmol resorufin/min/mg protein. The dashed line represents 1/3 activity of 1 μM BNF ($R_{BNF}$). Values are mean ± SEM of at least three independent experiments.

whereas 2 of the 4 BP samples did. The highest toxicity of bioplastics and plant-based materials compared to conventional plastics was highlighted by Zimmermann et al. [65]. Other studies have also shown the highest cytotoxicity of BPs in different bioassays [29]. This high toxicity is very likely due the addition of new plasticizers to biopolymers to improve their mechanical properties [30,59]. However, the compounds causing toxicity remain unidentified.

EROD activity is frequently used as a measure of CYP1A activity and as a biomarker to predict the impact of xenobiotics on aquatic organisms [61]. Polycyclic aromatic hydrocarbons (PAHs), dioxins and dioxin-like polychlorinated biphenyls are among the compounds that activate aryl hydrocarbon receptor (AhR)-dependent pathways, inducing *cyp1a* expression and EROD activity in fish cells [10,45,49]. Additionally, some plastic additives have also been found to induce EROD activity [1, 60]. In this study, all samples except for the single-use water bottle, showed the presence of CYP1A inducers. The highest EROD activity was detected in B1, probably due to the higher density of letters/patterns on the bag surface. Chromophores are a significant source of PAHs, which are often used as additives in plastics pigments (black carbon) and in plasticizer oils/softeners (extender oils) [21,31]. Van et al. [58] detected concentrations of PAHs in polystyrene foam one to two orders of magnitude higher than in virgin polymer particles, indicating that contamination occurs during the manufacturing process and reinforcing the idea that the presence of toxic chemicals in plastics often depends on the manufacturing process of the product. High concentrations of PAHs have also been detected in virgin LDPE plastics, but particularly in two samples of black recycled plastics (LDPE), which had higher PAH concentrations due to the addition black carbon as a heat stabilizer [44]. Accordingly, in our study, the highest CYP1A induction was detected in the extracts of a black recycled plastic bag.

Apart from containing CYP1A inducers, plastic particles (e.g. MPs and NPs) and their additives, such as bisphenol A, di(2-ethylhexyl) phthalate, and PAHs, have been shown to be genotoxic in certain organisms, including mussels, fish and human cells. They induce the formation of micronuclei, cellular apoptosis, genome instability, or cancer progression [16,20,35,4,41,56]. Plastic-induced genotoxicity can be caused by different mechanisms, including the induction of oxidative stress, inhibition of DNA replication, damage to lysosomal or mitochondrial structures, impaired replication and/or repair mechanisms, or changes in DNA methylation patterns [41,50]. In this study, the extracts of black recycled PE bags led to the highest induction of MN (7-fold), indicating the higher presence of genotoxic compounds in these extracts compared to the other tested samples. This can be attributed to the processing technology used for recycling, as previous research has shown that the concentration of PAHs in recycled HDPE can be 4–20 times higher than that in virgin material, depending on material source (e.g. content of additives or other pollutants) and the processing conditions (e.g. addition of new additives) [2]. Other studies have also demonstrated that the recycling process can concentrate or introduce new chemicals, increasing the toxicity of the plastic items [22,29]. Therefore, the quality of recycled plastics must be carefully evaluated before promoting their wide application.

### 4.2. Photodegradation and disintegration

Generally, UV irradiation enhances the toxicity of plastics by increasing the release of toxic chemicals and/or the formation of active compounds and degradation products, such as dicarboxylic acids [23, 29,51]. Accordingly, we observed increased cytotoxicity and ROS generation in BP samples after photodegradation. But, this increase was not so evident for conventional plastic samples. Also, Bejgarn et al. [9] reported that leachates from various plastics differ in toxicity and that the toxicity can be modulated by weathering. Interestingly, the toxicity of leachates from a biodegradable garbage bag (50% corn starch, 50% aliphatic polyester) significantly increased after exposure to sunlight, whereas photo-oxidized PE (96 h UV treatment) leachates did not show any toxicity [52].

On the other hand, while extracts of biodegradable plastics showed a sharp decrease in EROD activity after simulated photodegradation, a marked elevation of such activity was detected in conventional plastics after photodegradation. These results could be attributed to distinct additives and/or byproducts that were released or formed upon photodegradation, which may have acted as inducers of EROD activity [3,61]. Previous studies on the effects of UV aging on plastics have mainly

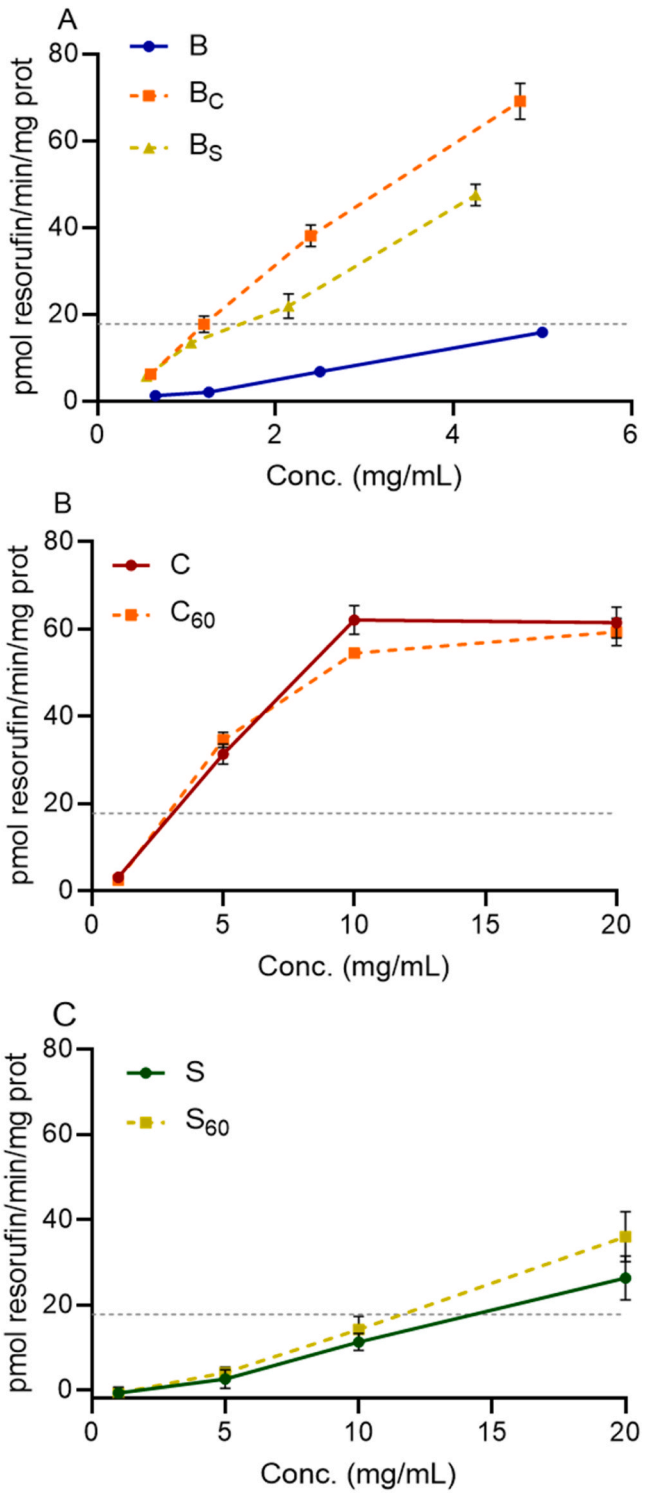


**Fig. 6.** EROD activity in PLHC-1 cells exposed to extracts of: (A) biodegradable/compostable plastics, (B) mature compost, and (C) synthetic compost. Results are expressed in pmol resorufin/min/mg protein. EROD activity induced by 1 μM β-naphthoflavone (BNF; positive control) was 53.5 ± 5.0 pmol resorufin/min/mg prot. The dashed line represents 1/3 activity of 1 μM BNF ($R_{BNF}$). Values are mean ± SEM of at least three independent experiments.

focused on the assessment of cytotoxicity, oxidative stress and endocrine activity, as well as changes in chemical composition, indicating among others, an increased release of estrogenic plasticizers [15]. These authors also found that UV-irradiated conventional plastics elicited significantly higher AhR activity than prior irradiation. Thus, long term sun-irradiation may facilitate the release of CYP1A inducers from conventional plastics and the consequent contamination of the environment.

Regarding BP disintegration, we observed that 2–3% of the plastic residue remained after 60 days of the disintegration test, indicating that decomposition was efficient (the standard requires disintegration to occur between 45 and 90 days in composting conditions). However, the extracts obtained from plastic fragments that remained after 60 days of composting showed increased cytotoxicity, ROS generation, and EROD induction, possibly due to the release of chemicals from degrading plastics [13,42,48]. At the same time, we observed an increase in pH for both mature and synthetic composts after 60 days (Table S6). Increased pH and leaching time have been reported to promote the release of chemical additives from plastics [33]. In most studies, no deleterious effects of polymer degradation products were detected, except for PLA, which exerted cytotoxic and genotoxic effects on common onion and inhibited microbial activity after degradation [26]. However, improper use and waste disposal practices lead to many plastics, including non-compostable items, being disposed of in compost [12]. These plastics can be a source of microplastics and hazardous chemicals, which may end up in agricultural fields and the aquatic environment [32,54].

In fact, extracts of mature compost, which was sourced from a vegetable waste composting plant, caused significant cytotoxicity, ROS and induced CYP1A activity in PLHC-1 cells, indicating the presence of hazardous chemicals in the compost (Fig. S1). Typically, compost toxicity is assessed through a seed germination test prior to its release into the environment [34]. However, we postulate that these tests may not be sufficient for actual risk assessment, given the high levels of in-vitro toxicity detected in mature compost and the risks for pollutant accumulation during vegetable and crop cultivation, which could have detrimental effects on human health. Therefore, a more comprehensive evaluation of compost toxicity should be conducted before it is applied to agricultural lands.

In summary, this study demonstrates that compostable and non-biodegradable plastic products contain extractable chemicals that induce cytotoxicity, CYP1A activity, and micronuclei formation in fish liver cells. Methanolic extracts from BPs were the most cytotoxic, while those from conventional plastics, particularly mechanically recycled plastics, had higher levels of CYP1A inducers and genotoxic compounds. Our results also show that plastic-induced toxicity can be enhanced by UV exposure, and that plastic residues remaining in final compost can be a significant source of pollutants to the environment.

## 5. Conclusions

This work demonstrates the elevated toxicity of recycled plastics, compostable plastics, and semi-degraded compostable plastics resulting from partial disintegration, as compared to virgin conventional plastic extracts. These findings underscore the need for additional research efforts and the implementation of regulatory measures prior to the release of mature compost into the environment. Furthermore, improvements in plastic production and recycling processes are needed in order to generate safer plastics, with particular emphasis on the development and use of safer additives, in order to mitigate the negative impact of plastic pollution on human health and the environment.

## CRediT authorship contribution statement

**Tiantian Wang:** Methodology, Investigation, Data acquisition, Writing – original draft and Reviewing. **Mahboubeh Hosseinzadeh:** Methodology, Investigation, Data analysis, Reviewing. **Alice Cuccagna:** Investigation, Data analysis. **Rakhat Alakenova:** Investigation, Data analysis. **Paula Casademunt:** Methodology, Data analysis. **Alcira Reyes Rovatti:** Methodology, Investigation, Data analysis, Reviewing. **Amparo López-Rubio:** Methodology, Investigation, Data analysis, Reviewing. **Cinta Porte:** Conceptualization, review and editing of the

manuscript, Funding acquisition.

## Environmental Implication

Compostable and recycled plastics have been advocated as environmentally friendly alternatives to conventional plastics, aiming to mitigate plastic pollution. Our research examines the toxicity posed by their leachable plastic additives to aquatic organisms and underscores the need for regulatory action regarding chemicals used in plastic formulations. The widespread usage of compostable plastics and their incomplete degradation may result in increased release of plastic additives and plastic particles to the environment with the consequent adverse implications for the environment and human health.

## 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.

## Data Availability

Data will be made available on request.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.132123.

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