Journal of Hazardous Materials 510 (2026) 142043

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

“Beached and commercial plastics’ leachates: Assessment of chemical composition and ecotoxicity to marine biota”

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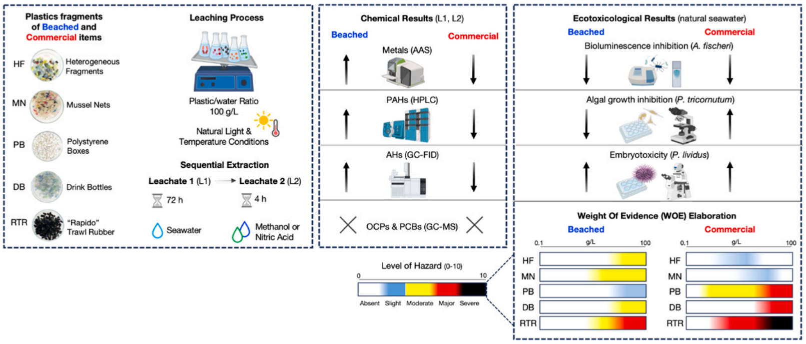
HIGHLIGHTS

- Beached plastics generally leached more chemical compounds than commercial ones.
- Al and Fe were the most abundant metals in leachate from beached mussel nets.
- Beached and commercial PS-boxes leachate showed the highest PAHs and AHs levels.
- Trawl-derived commercial rubber leachate affected all tested biological endpoints.
- WOE identified commercial PS-boxes and rubber leachates as the most hazardous.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Marine plastic litter
Plastic Leachates
Chemicals
Bioassays
Ecotoxicity
Weight of Evidence

ABSTRACT

Five plastic categories representative of beach litter from the Central Adriatic Sea were selected to assess the hazard of leachates to marine biota: Heterogeneous Fragments (HF), Mussel Nets (MN), Polystyrene Box fragments (PB), Drink Bottles (DB), and “Rapido” Trawl Rubber (RTR). Leachates (L1) were prepared by agitating beached and commercial plastics in seawater for 72 h; a sequential extraction (L2) with nitric acid or methanol quantified residual chemicals (metals or organics, respectively). Trace metals, polycyclic aromatic hydrocarbons (PAHs), and aliphatic hydrocarbons (AHs) effectively desorbed in L1, with beached plastics generally releasing higher amounts, reflecting pollutants enrichment and weathering-enhanced leaching. Specifically, Al and Fe were the most abundant metals in MN (35.78 ± 12.86 and 79.16 ± 6.59 μg/g), while PAHs and AHs reached 1070.60 ± 362.74 ng/g and 381.88 ± 57.87 μg/g in PB. L2 further mobilized some chemicals, notably Al and Fe in beached PB and Zn and PAHs in commercial RTR (up to 10-fold increase). L1 ecotoxicity showed only commercial RTR inhibited *Aliivibrio fischeri* bioluminescence and *Phaeodactylum tricornutum* growth, whereas *Paracentrotus lividus* embryos were sensitive to most leachates. A Weight of Evidence model identified commercial RTR as most hazardous, emphasizing environmental concerns over its widespread use in trawling.

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https://doi.org/10.1016/j.jhazmat.2026.142043
Received 27 November 2025; Received in revised form 6 March 2026; Accepted 10 April 2026
Available online 12 April 2026

1. Introduction

Since its invention, plastic has driven an industrial, economic and social revolution. However, its excessive use, incorrect waste management practices and low degradability have led to a global environmental crisis, especially concerning the marine environment [1]. Plastic debris accounts for a large proportion of marine litter, originating mainly from land-based sources and maritime activities [2]. Notably, plastic fishing gears are often lost or discarded at sea, persisting for decades and contributing to the 'Abandoned, Lost or otherwise Discarded Fishing Gear' (ALDFG) phenomenon [3]. The abundance and accumulation rates of plastic litter vary depending on proximity to urban, industrial, and recreational areas, maritime activities and hydrodynamics [4]. In the Mediterranean Sea, the Adriatic Basin has been identified as a preferential area of plastics accumulation, due to its semi-enclosed nature, several riverine inputs, intense coastal development and expansion of marine shellfish and finfish farms, which further contributes to the predominance of ALDFG and aquaculture-related debris [5-7].

Once in the ocean, plastic debris can act as a substrate for environmental contaminants, including heavy metals and hydrophobic organic compounds, such as persistent organic pollutants (POPs) [8], while also carrying intrinsic compounds, including monomers, manufacturing by-products and additives, which are often not chemically bound to the polymer matrix [9]. Simultaneously, plastics are exposed to physical, chemical and biological stressors [10] that drive the so called "aging" or "weathering" process, altering their structure, modifying surface properties and promoting fragmentation with micro- and nanoplastics formation [11,12]. Together, these processes can result in the release of a complex mixture of contaminants referred to as "Plastic Leachate" [13,14], which may exert ecotoxicological effects on marine organisms in addition to the physical impact of plastic itself [13-16]. While it is known that the ecotoxicity of plastic leachates can vary with multiple factors, this topic remains underexplored within plastic pollution studies, leading to significant knowledge gaps [17,18]. Among these, a major limitation concerns methodological inconsistencies and the absence of standardized protocols for generating plastic leachates under laboratory conditions. Variability in leachates' preparation arises from plastic fragment size and concentration, leaching time and experimental settings [19]. Such heterogeneity hampers cross-study comparability and ultimately constrains the development of robust, reproducible frameworks for assessing plastic leachate toxicity. Most studies investigate only few polymers, primarily focusing on thermoplastics (e.g. polyethylene, polypropylene, polystyrene), whereas elastomers, commonly defined as 'rubbers', are less frequently studied, despite evidence suggesting that they may exhibit higher toxicity, related to a higher chemical complexity of their leachates [20]. Concerning the chemical characterization of leachates, studies are largely focused on intentionally added substances, while comparatively less attention has been paid to environmentally derived contaminants. Discrepancies in the ecotoxicological hazard assessment of plastic leachates are frequently reported, with toxicity varying according to species-specific characteristics [14]. These effects are further influenced by particle size and concentration, with responses often differing depending on the specific endpoint measured. Most research has focused on micro- and millimeter-sized plastics, leaving leachates from centimeter-sized particles underexplored, despite evidence that they may exhibit higher toxicity than smaller fragments [21]. Moreover, many studies rely on single-species and/or single-endpoint approaches, limiting the robustness of hazard characterization. A comprehensive ecotoxicological evaluation of plastic leachates therefore requires a battery of bioassays encompassing multiple species and endpoints, ideally spanning different trophic levels and covering both acute and chronic effects. Furthermore, 76% of the studies reported in the literature used pristine materials for the preparation of plastic leachates, while environmentally aged plastics or laboratory-weathered plastics are less commonly employed [19]. This limits the assessment of weathering effect and of the potential role of plastics as environmental contaminants' carrier. Therefore, the use of environmentally weathered or in-field-collected plastics in laboratory studies to obtain plastic leachates is crucial for mimicking a more realistic situation. In this context, comparing the effects of leachates obtained from weathered and pristine plastics is also of primary importance [22,23].

The present study aimed to comparatively evaluate the leaching potential of weathered (beached) and unweathered (commercial) plastics in seawater by characterizing the chemical profile of their leachates and assessing the resulting ecotoxicological impact. Five categories, collected from Adriatic beaches were selected: Heterogeneous Fragments (HF); Mussel Nets (MN); fragments of Polystyrene Boxes (PB), Drink Bottles (DB); "Rapido" Trawl Rubber (RTR). Chemical analyses focused on trace metals and organic compounds, including polycyclic aromatic hydrocarbons (PAHs), aliphatic hydrocarbons (AHs), Organochlorine Pesticides (OCPs) and Polychlorinated Biphenyls (PCBs), all recognized as priority marine pollutants and frequently associated with plastics [8,9,24]. Ecotoxicity of plastic leachates was evaluated through a battery of bioassays applying serial dilutions to test a range of concentrations (from 100 to 0.1 g/L). Measured endpoints included bioluminescence inhibition in *Aliivibrio fischeri*, algal growth inhibition of *Phaeodactylum tricornutum* and alteration of embryonic development in *Paracentrotus lividus*, which were selected for their complementary ecological roles within marine ecosystem. Moreover, ecotoxicological results were analyzed within a quantitative Weight of Evidence (WOE) model, to derive synthetic hazard indices for each plastic category, allowing a robust and straightforward comparative evaluation of the potential environmental hazard associated with leachates from beached and commercial plastics.

2. Materials and methods

2.1. Selection of plastic materials

Five categories of plastic items were selected for the preparation of leachates: i) Heterogeneous Fragments (HF), *i.e.* rigid plastic materials with different size and irregular shape; ii) Mussel Nets (MN), *i.e.* fragments of nets commonly used in mussels' farming; iii) fragments of Polystyrene Boxes (PB), used for the trade of caught fish; iv) parts of Drink Bottles (DB); v) "Rapido" Trawl Rubber (RTR). *i.e.* material typically used to protect the mouths of fishing nets (known as Rapido) during bottom trawling in the Adriatic Sea (Figure S1). These materials were chosen based on evidence from a clean-up activity, carried out in November 2022 along the Conero Riviera (Ancona, Italy, Central Adriatic Sea), specifically on a remote beach of approximately 176 m^2 (43 ° 35'11"N 13 ° 33'53"E) located between a cliff and the sea, making it inaccessible by land. Since the targeted area was neither intended for recreational use nor subject to other anthropogenic activities, it was not regularly maintained. This, combined with its geomorphology, lead to the easy accumulation of plastic litter, primarily related to the beaching of wastes from the sea. In fact, during the clean-up event, 1098 plastic objects, weighing 149 Kg were collected (corresponding to about 6.2 objects/m^2, 0.85 Kg/m^2) and subsequently divided into categories. Most of the beached plastic objects were related to "Fishing, Aquaculture & Trade in fish items" from which MN, PB and RTR were selected as the main representative of this category. DB were selected as typical everyday use objects among those collected, while HF represented a group of mixed-origin plastic debris with no identifiable source (Figures S2-S3, Table S1). The different chemical-physical characteristics such as density, color, porosity, specific surface area, and polymer type, were also taken into account for the selection of the five plastic categories. Specifically, the chemical characterization was performed using Fourier Transform Infrared spectroscopy (FTIR) in Attenuated Total Reflectance (ATR) (Text S1) revealing that HF was composed of polypropylene (PP, 73%), polyethylene (PE, 6.7%), polystyrene (PS, 6.7%), polyethylene terephthalate (PET, 6.7%,) and ethylene-propylene copolymer (EPM, 6.7%); MN consisted of PP (73%) and PE (33%); PB was made of 100% PS (expanded form, EPS); DB was entirely constituted by PET; finally RTR was composed of a black rubber made of Ethylene-propylene-diene elastomer (EPDM) and an internal fibrous material made of PET. Representative IR spectra are provided in Figure S4.

To recreate the 5 categories with commercial plastics, new objects, intended for the same commodity use as the beached ones, were purchased from the local market and analysed through FTIR spectroscopy to ensure the same polymeric composition.

Both beached and commercial plastics were manually cut with clean scissors into pieces smaller than 1 × 1 cm (Figure S5) that were used for the preparation of plastic leachates.

2.2. Preparation of plastic leachates

Leachates were obtained for each plastic category by adding plastic fragments in natural seawater in glass flasks (referred to as L1). A plastic concentration of 100 g/L was used (10 g in 100 ml), except for PB, which required a reduced ratio of 33 g/L (3.3 g in 100 ml) due to the low specific weight and large sample volume. These relatively high starting concentrations were not intended to reflect environmentally realistic conditions, which typically ranged from ng/L to µg/L [19]; instead, they were selected to enable subsequent serial dilutions for toxicity testing, supporting hazard assessment.

The starting salinity of natural seawater was adjusted to 33 PSU before use, by adding ultrapure water, to meet the specific requirements of the ecotoxicological bioassays. The seawater was then filtered with 0.22 µm cellulose acetate membrane (Sartorius Stedim Biotech). Flasks containing plastic fragments and seawater were placed on an orbital shaker at room temperature (≈20°C) under natural lighting conditions and gently agitated for 72 h, simulating wave motion and the potential release of contaminants into the marine environment. The flasks for each category were prepared in triplicate as well as for a blank sample, consisting of natural seawater (salinity = 33 PSU), which was run in parallel with the plastic samples and subjected to the same procedures. At the end of the leaching time, L1 was collected and stored in glass bottles at 4°C. For the chemical analyses, samples were processed and analyzed within 48 h to preserve stability. For ecotoxicological bioassays, leachates were filtered through a Minisart syringe filter (0.22 μm) to prevent mechanical disturbance from plastic particles during the bioassays, which were performed shortly after storage, following the timing required by the experimental protocols.

The extraction of L1 using seawater as medium enables the assessment of chemical compounds loosely adsorbed on the plastics' surface, which could easily leach into the marine environment under natural hydrodynamics and solar radiation conditions. However, contaminants more strongly bound to plastic fragments surface may require different physicochemical conditions for release. To verify whether the seawater extraction was sufficient to recover analytes associated with the plastic fragments, a sequential extraction (referred to as L2) was performed using nitric acid for metals or methanol for organic compounds, while preserving the integrity of the plastic polymers. Specifically, after collection of L1, HNO_3 (20% in ultrapure water) and pure methanol (CH_3OH) were added to the flasks that still contained plastic fragments: the same volume used for the seawater in L1 (100 ml) was applied, with HNO_3 added to one set of flasks for the analysis of trace metals and CH_3OH to the remaining flasks for organic compounds analysis. During a leaching time of 4 h, flasks were kept in motion as previously described. At the end of the leaching process, L2 was recovered and maintained at 4°C in glass bottles. As for L1, chemical analyses of L2 were performed within 48 h.

2.3. Chemical analysis of plastic leachates

L1 and L2 of each sample were analysed to identify the occurrence of trace metals, PAHs, AHs, OCPs and selected PCBs. These contaminant classes were chosen for their relevance in the marine environment and, despite the limited solubility of some compounds (e.g., PAHs and PCBs), their documented occurrence in aquatic ecosystems and strong affinity for plastic surfaces make them suitable targets for assessing the potential of plastics to carry and release contaminants. According to the target analytes, different analytical techniques were employed. Determination of trace metals, including aluminium (Al), arsenic (As), cadmium (Cd), chromium (Cr), iron (Fe), manganese (Mn), mercury (Hg), nickel (Ni), lead (Pb), copper (Cu), vanadium (V) and zinc (Zn), were conducted by atomic absorption techniques as previously described [25].

Analytical determinations of polycyclic aromatic hydrocarbons (PAHs: 1-methylnaphthalene, 2-methylnaphthalene, 7,12-dimethylbenzo[*a*]anthracene, acenaphthene, acenaphthylene, anthracene, benzo[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*g,h,i*]perylene, benzo[*k*]fluoranthene, chrysene, dibenzo[*a,h*]anthracene, fluoranthene, fluorene, indeno[1,2,3-cd]pyrene, naphthalene, phenanthrene and pyrene) were performed by high performance liquid chromatography (HPLC) techniques following previous validated method [26].

The analysis of aliphatic hydrocarbons (C10-C40) was carried out by gas chromatography (GC) with flame ionization detector (FID), following a previous validated technique [26].

Finally, the determination of EPA 8081 OCPs and selected PCBs were analysed by gas chromatography-mass spectrometry (GC-MS) techniques as previously described elsewhere [27]. Analytes includes Aldrin, a-Chlordane, g-Chlordane, p,p'-DDD, p,p'-DDE, p,p'-DDT, Dieldrin, Endosulfan I, Endosulfan II, Endosulfan sulphate, Endrin, Endrin aldehyde, Endrin ketone, Heptachlor, Heptachlor epoxide, a-Lindane, b-Lindane, D-Lindane, g-Lindane, Methoxychlor, Dichlorobenzidine, Hexachlorobenzene, Hexachlorobutadiene, Mirex, and a series of PCBs of primary interest (EPA 8082 A).

Results on plastic leachates were obtained from 3 experimental replicates and expressed in µg/g (dry weight) of sample for trace metals and AHs and in ng/g (dry weight) for organic compounds. In the blank samples, no organic compounds were detected, and metal concentrations were negligible; values are therefore not reported as they did not contribute to plastic samples results. A complete description of analytical methods is provided in the Supplementary Materials (Text S2).

2.4. Ecotoxicity of plastic leachates: battery of bioassays

The toxicity was assessed for different concentrations, including undiluted plastic leachates (100 g/L for all samples, 33 g/L for PB) and a series of sequential dilutions (20, 10, 1, 0.1 g/L). The goal of preparing serial dilutions from a single high-concentration leachate is to generate a concentration–response gradient to support hazard assessment. This approach also ensures comparability between treatments and minimizes batch-to-batch variability, which is particularly important given the compositional complexity of plastic materials. Ecotoxicological effects were evaluated through a battery of standardized bioassays. The bioluminescence inhibition test was performed on the species *Aliivibrio fischeri* using Microtox® photometer and lyophilized bacteria purchased by *BioLight,* Aqua Science, following UNI EN ISO 11348–3:2019 protocol [28]. The algal growth inhibition assay was conducted using the unicellular marine microalgae *Phaeodactylum tricornutum* according to UNI EN ISO 10253:2016E protocol [29]. The sea urchin embryotoxicity assay with *Paracentrotus lividus* were performed following methods described by ISPRA [30]. Abnormalities of malformed embryos were classified according to a severity scale of alteration levels (Figure S6): 0, "Normal development" (pyramid-shaped embryos at pluteus stage); 1, "Arms" (incorrect location of skeletal rods: crossed tip, separated tip, fused arms); 2, "Skeleton/Shape" (incomplete or absent skeletal rods, abnormal shape, folded tip, fractured ectoderm); 3, "Undeveloped" (pre-pluteus, undeveloped stages). For each bioassay, the blank sample was tested in parallel with the plastic leachates under identical experimental conditions and observed effects were consistently below 10% in all assays. All experimental data were then expressed as percentage values normalized to the corresponding control using five experimental replicates.

2.5. Statistical analyses and weight of evidence (WOE) elaboration

Analysis of variance (ANOVA) was performed on chemical and ecotoxicological data to highlight statistically significant differences between categories (HF; PB; MN; RTR and DB) in beached and commercial plastic leachates, analysed separately (level of significance at the 95%); homogeneity of variance was tested by Shapiro Wilk-test, and post-hoc comparisons (Tukey test) were used to compare means of values. T-test student was applied to chemical data to analyse significant differences between beached and commercial leachates of the same category of sample.

Non-metric Multidimensional Scaling (nMDS) was used to perform a multivariate ordination analysis to visualize the similarities among plastic leachates based on their chemical composition and ecotoxicological effects. The analysis was conducted using the metaMDS function from the vegan package [31] in R software (v. 4.4.1). The analysis was performed using Bray-Curtis dissimilarity on square-root transformed and standardized data. nMDS was applied to a dataset including both chemical parameters (metals, AHs, PAHs) and ecotoxicological

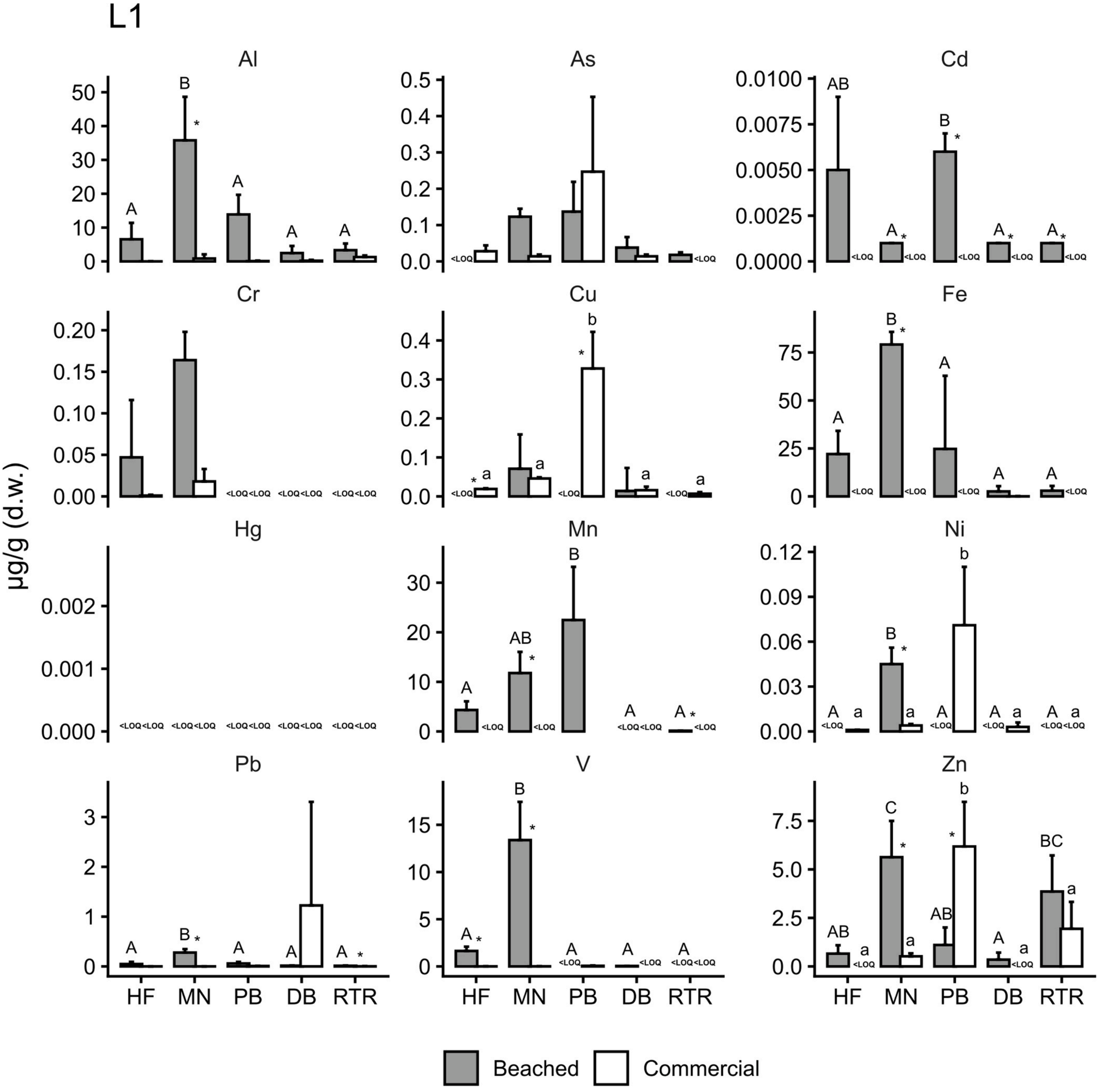


Fig. 1. Concentrations (µg/g, d.w.) of metals in Leachate 1 (L1, ASW) of beached and commercial plastics. Asterisk (*) indicates statistically significant differences ($p < 0.05$) (*t*-test) between beached and commercial samples. Uppercase letters highlight differences ($p < 0.05$) among samples within beached plastic leachates; lowercase letters highlight differences ($p < 0.05$) among samples within commercial plastic leachates. HF=heterogeneous fragments; MN= mussel nets; PB= polystyrene boxes; DB= drink bottles; RTR= “Rapido” trawl rubber. For statistical analyses, values <LOQ were replaced with LOQ/2.

endpoints (bacterial bioluminescence inhibition, algal growth inhibition, and sea urchin embryotoxicity) for all plastic categories (HF, PB, MN, RTR and DB), distinguishing between commercial and beached samples.

The results from the entire battery of ecotoxicological bioassays were further elaborated through a quantitative Weight of Evidence (WOE) model to summarize, in specific levels of hazard, leachates' effect of beached and commercial plastic categories (HF; MN; PB; DB and RTR). The weighted integration of results was carried out using the validated WOE approach [32], which has previously been applied in several case studies for environmental risk assessment [33-36]. Precisely, criteria to elaborate results from ecotoxicological bioassays were based on specific thresholds and weights assigned to each bioassay depending on the biological endpoint, tested matrix, and time of exposure [32]. A cumulative Hazard Quotient ($HQ_{Battery}$) is obtained by the summation (Σ) of the weighted effects ($Effect_w$), i.e., the variation measured for each test compared to specific threshold, corrected for the statistical significance of the difference, the biological importance of the endpoint and exposure conditions (w2). $HQ_{Battery} = \sum_{k=1}^{N} Effect_w(k) \bullet w_2$

Finally, the $HQ_{Battery}$ was assigned to 1 of 5 classes of hazard [27]: Absent, Slight, Moderate, Major, Severe. Whole calculations and assumptions were detailed elsewhere [32].

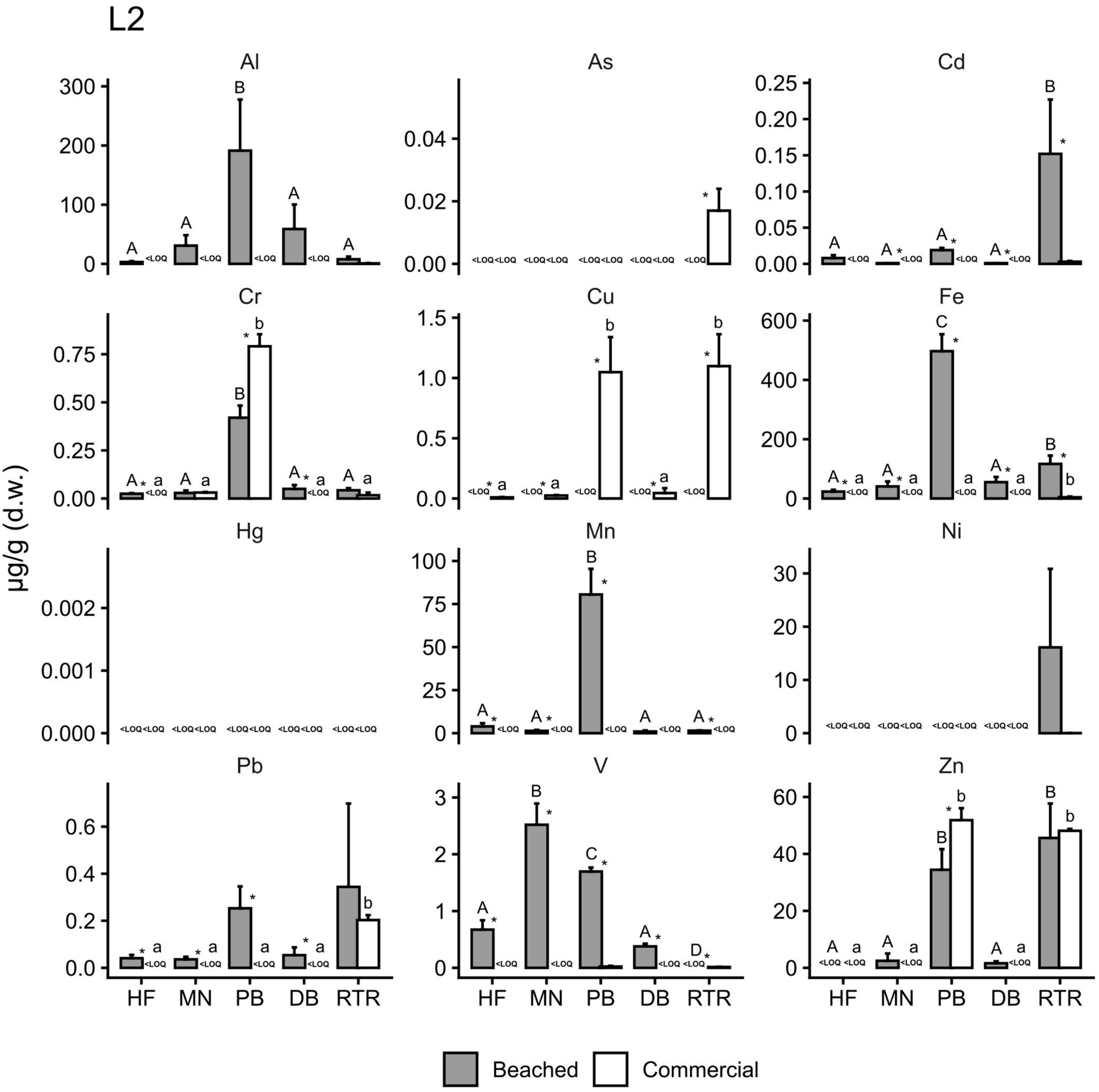


Fig. 2. Concentrations (µg/g, d.w.) of metals in Leachate 1 (L2, ASW) of beached and commercial plastics. Asterisk (*) indicates statistically significant differences ($p < 0.05$) (*t*-test) between beached and commercial samples. Uppercase letters highlight differences ($p < 0.05$) among samples within beached plastic leachates; lowercase letters highlight differences ($p < 0.05$) among samples within commercial plastic leachates. HF=heterogeneous fragments; MN= mussel nets; PB= polystyrene boxes; DB= drink bottles; RTR= "Rapido" trawl rubber. For statistical analyses, values <LOQ were replaced with LOQ/2.

3. Results

3.1. Chemical analyses

3.1.1. Trace metals

In L1 from beached plastics, Al, Cd, Fe, Pb, and Zn were detected across all plastic categories. Among these, Al and Fe were the most abundant, reaching significantly higher levels in MN (35.78 ± 12.86 μg/g and 79.16 ± 6.59 μg/g, respectively) compared to the other categories. Zn was detected at lower concentrations, peaking in MN (5.63 ± 1.87 μg/g) and RTR (3.86 ± 1.86 μg/g). Pb reached a maximum concentration of 0.279 ± 0.069 μg/g in MN, whereas Cd were at negligible levels in all plastics (0.001–0.006 μg/g). Mn and V were notable in PB (22.47 ± 10.72 μg/g) and MN (13.38 ± 4.04 μg/g), respectively. Commercial plastics, in contrast, showed very low metals concentrations, mostly close to or below the instrumental detection limit (LOD). An exception was PB, which exhibited relatively higher levels of Zn (6.18 ± 2.30 μg/g), Cu (0.328 ± 0.094 μg/g) and Ni (0.071

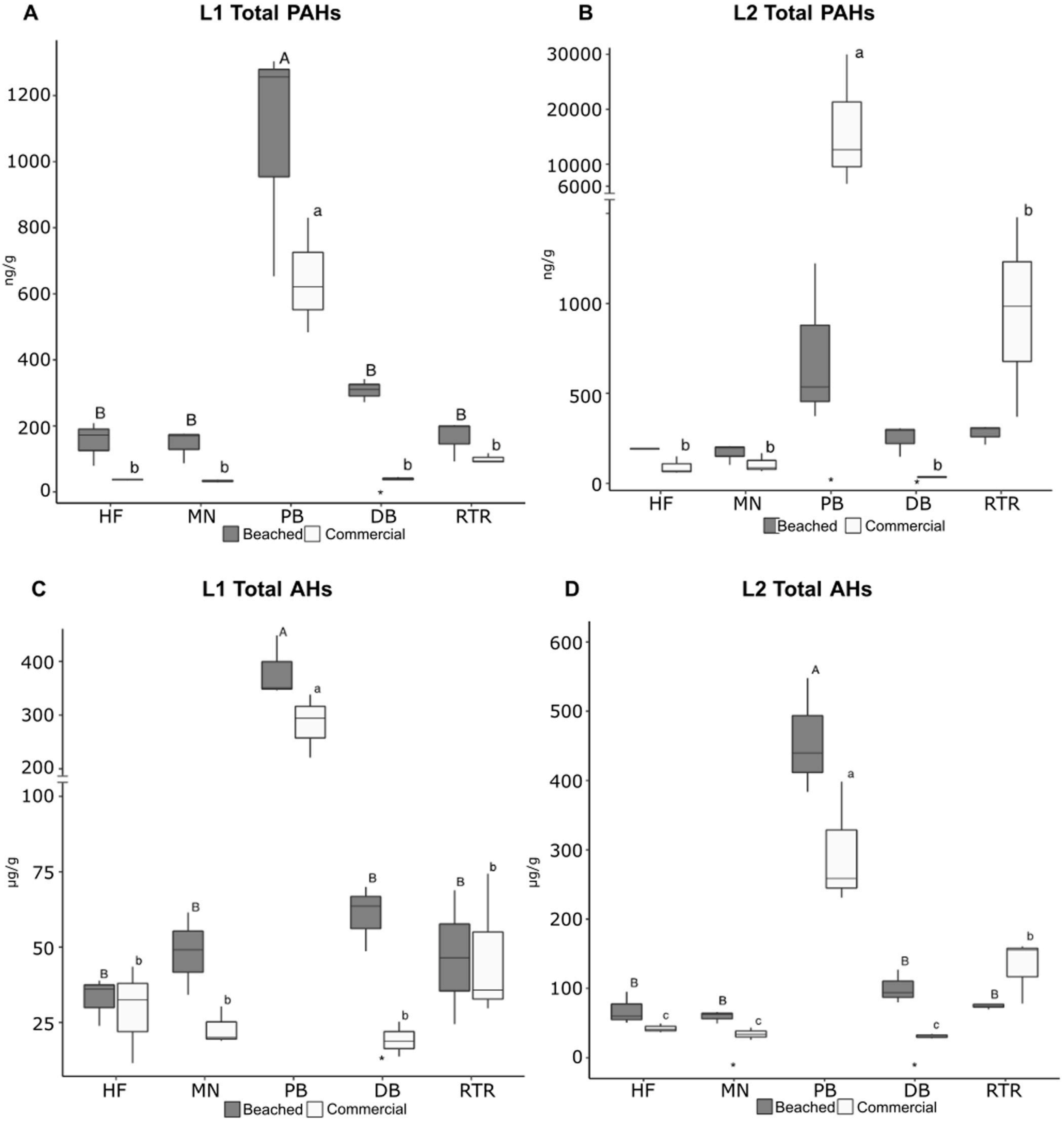


Fig. 3. Concentrations of (A-B) total PAHs (ng/g d.w.) and (C-D) total AHs (μg/g d.w.) found in leachate 1 (L1) and leachate (L2) of beached plastic samples (grey) compared to commercial plastic ones (white). Asterisk (*) indicates statistically significant differences ($p < 0.05$) (*t*-test) between beached and commercial samples. Uppercase letters highlight differences ($p < 0.05$) among samples within beached plastic leachates; lowercase letters highlight differences ($p < 0.05$) among samples within commercial plastic leachates. HF=heterogeneous fragments; MN= mussel nets; PB= polystyrene boxes; DB= drink bottles; RTR= "Rapido" trawl rubber.

± 0.039 μg/g) compared to the other categories (Fig. 1 and Table S2).

Al, Cd, Fe, and Zn were detected also in L2 from beached plastics across all categories, but at concentrations approximately one order of magnitude higher. Al and Fe remained the most abundant metals, reaching elevated concentrations in PB (191.33 ± 86.35 μg/g and 496.84 ± 57.20 μg/g, respectively). Although present at negligible levels, Cd was highest in RTR (0.15 ± 0.07 μg/g) while Zn showed notable levels in both PB and RTR (34.43 ± 7.23 μg/g and 45.60 ± 12.08 μg/g, respectively). PB remained the plastic category whose leachates exhibited the highest levels of Mn, reaching in L2 a concentration of 80.53 ± 14.89 μg/g. Similarly, MN showed the highest levels of V, although at concentrations approximately one order of magnitude lower than those observed in L1. Even for L2, commercial plastics showed very low trace metal values and mostly below the LOD, except for Zn which was found at appreciable levels in PB (51.85 ± 4.196 *μ*g/g) and in RTR (48.129 ± 0.614 *μ*g/g). The same plastic categories also exhibited higher Cu concentrations compared to the other materials, with values of 1.048 ± 0.291 μg/g and 1.098 ± 0.265 μg/g, respectively (Fig. 2 and Table S3).

3.1.2. *Organic compounds*

Concentrations of OCPs and PCBs were under the limit of detection of the instrument for all the analysed samples. PAHs and AHs were detected in L1, with generally higher concentrations in beached plastics than in commercial ones. However, statistically significant differences were observed only for DB (Fig. 3A and C). In this category, PAHs were higher in beached samples (307.613 ± 34.721 ng/g vs 46.214 ± 5.348 ng/g), whereas AHs showed the opposite trend, with higher concentrations in commercial DB (60.73 ± 22.19 μg/g vs 19.28 ± 5.7 μg/g). Among categories, PB exhibited significantly higher concentrations of both PAHs and AHs compared to the others, in both beached and commercial leachates. Specifically, in beached PB samples, PAHs and AHs reached 1070.60 ± 362.74 ng/g and 381.88 ± 57.87 μg/g, respectively, whereas in commercial PB they were 644.49 ± 174.90 ng/g and 285.14 ± 59.18 μg/g. In L2, hydrocarbon levels were, in some cases, approximately one order of magnitude higher than those measured in L1 (Figs. 3B and 3D). PAHs showed particularly elevated levels in commercial PB (16350.95 ± 12208.33 ng/g) and RTR (944.72 ± 555.51 ng/g), while AHs in commercial RTR (131.33 ± 46.28 μg/g). Consistent with L1, PB exhibited the highest hydrocarbons concentrations among categories, with statistically significant differences observed only in beached samples for PAHs (16350.95 ± 12208.33 ng/g) and in both beached (457.006 ± 83.3 μg/g) and commercial (297.04 ± 89.67 μg/g) samples for AHs

Concentrations of all the analysed PAHs and AHs are available in Tables S4-S7.

3.2. *Ecotoxicological bioassays*

The results of bioluminescence inhibition test on the bacteria *A. fischeri*, expressed as percentage (%), showed no toxic effects from the exposure to beached plastic leachates, with bioluminescence inhibition values remaining below 20%, even for the highest tested concentration (100 g/L for all samples, 33 g/L for PB) (Fig. 4A). Even for commercial plastics the exposure to leachates did not cause adverse effects with the only exception of 'RTR', which lead to a significant bioluminescence inhibition compared to the other categories at the concentrations of 10, 20 and 100 g/L, with percentages of 44.81%, 72.93% and 91.54%, respectively (Fig. 4B).

The results on the growth inhibition test on the algae *P. tricornutum*, expressed as percentage (%), revealed that beached plastic leachates did not cause relevant toxic effects, with values of inhibition below 30% for all the samples tested, even for the highest concentration (100 g/L for all samples, 33 g/L for PB). Despite this, the major effect of algal growth inhibition was related to HF sample (26.87%) for the leachate's concentration of 10 g/L. Conversely, a biostimulation effect (55%) was measured for DB sample for the leachate's concentration of 100 g/L (Fig. 4C). Even for commercial plastics, the toxic effect of leachates appeared to be limited, with inhibition percentages ranging from 13.39% (DB, 1 g/L) to 37.80% (DB, 100 g/L). The only exception was RTR, which caused significant higher toxic effects compared to the other plastic samples already at the leachate's concentration of 1 g/L (95.56%) reaching 99.42% of inhibition at 100 g/L (Fig. 4D).

The results on *P. lividus* embryotoxicity test were reported as percentage of malformed embryos after 48 h of exposure to beached and commercial plastic leachates. Considering beached plastics, it was generally observed that less concentrated leachates (0.1, 1, 10 g/L) did not cause relevant toxic effects, with malformed embryos percentages below 35% for all the samples tested, except for RTR (50.95% at 10 g/L). In contrast, at higher concentrations (20 g/L and 33 or 100 g/L), leachates induced a significant increase in abnormalities across samples, with malformed embryos percentages reaching up to 100% for HF (100 g/L), MN (20 and 100 g/L) and RTR (100 g/L). Focusing on specific embryo abnormalities, the level of alteration (1 "Arms", 2 "Skeleton/Shape", 3 "Undeveloped") increased in a dose-dependent manner with higher leachates concentrations. In fact, at concentrations up to 10 g/L, levels 1 and 2 accounted for almost all the abnormalities, while

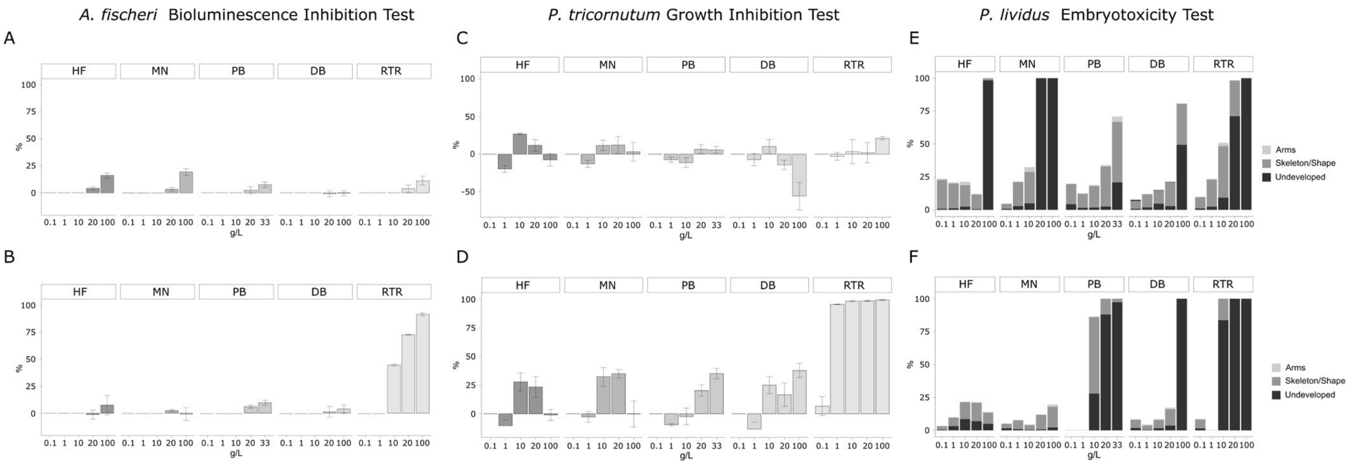


Fig. 4. Percentages of effect expressed as means ± standard deviations (n = 5) of the ecotoxicological responses in *Aliivibrio fischeri, Phaeodactylum tricornutum* and *Paracentrotus lividus* exposed to undiluted leachates (100 g/L for all samples, 33 g/L for PB) and a series of sequential dilutions (20, 10, 1, 0.1 g/L) obtained from (A-C) beached and (D-F) commercial plastics. HF=heterogeneous fragments; MN= mussel nets; PB= polystyrene boxes; DB= drink bottles; RTR= "Rapido" trawl rubber.

at higher concentrations the percentage of these two decreased and the contribution of “undeveloped” embryos (level 3) increased, reaching 100% in some cases (MN, RTR) (Fig. 4E).

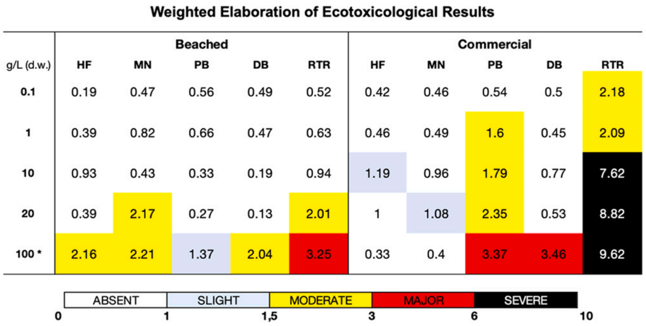
Regarding commercial plastic leachates, HF and MN samples did not cause relevant toxic effects, with malformed embryos percentages remaining below 25% for all the exposure concentrations. However, nearly all embryos showed Skeleton/Shape alterations (level 2). On the other hand, PB, DB, RTR samples exhibited negligible percentages of malformed embryos at lower concentrations (0.1, 1 g/L) but caused a statistically significant increase in abnormalities at higher concentrations (10, 20, 33 or 100 g/L) reaching up to 100%, with “undeveloped” embryos comprising nearly all the larvae (Fig. 4F). Figure S7 shows microscopic photographs of embryos after the exposure to undiluted and sequential dilutions of RTR leachates, which exhibited toxicity regardless beached and commercial samples.

3.3. *Weight of evidence elaboration and nMDS analysis*

The WOE elaborated and integrated results of the entire battery of bioassays (*A. fischeri*, *P. tricornutum* and *P. lividus*) providing an integrated level of hazard for each treatment (Fig. 5). Considering beached plastics’ leachates, the hazard level was summarized as Absent for all samples at concentrations from 0.1 g/L to 20 g/L, except for MN and RTR samples, which showed a Moderate ecotoxicological hazard level at 20 g/L. On the contrary, the highest concentration of leachates (100 g/L) resulted in Moderate hazard level for HF, MN and DB and Major for RTR. For PB, the hazard level remained Slight, even at the highest concentration (33 g/L).

Regarding commercial plastics, the leachates from HF and MN samples were classified with Absent or Slight levels of ecotoxicological hazard. For DB, a Major hazard level was assigned only to the highest concentration of leachate (100 g/L). A Moderate hazard level was calculated for PB at 1–20 g/L and for RTR at 0.1–1 g/L, increasing in a concentration-dependent manner to Major at 30 g/L for PB and Severe at 10–100 g/L for RTR.

The non-metric multidimensional scaling (nMDS) plot (Fig. 6) highlighted clear differences in the overall chemical composition and ecotoxicological effects between beached and commercial plastic leachates. A consistent clustering by item type and origin is evident. In particular, beached plastic samples (orange dots) are generally distributed on the left side of the ordination space, together with most of the investigated chemicals: metals, PAHs and AHs, while commercial samples (blue dots) tended to cluster on the right. The observed ordination suggests that both plastics origin (beached vs commercial) and item category played a major role in determining the contamination profile of plastic leachates. Furthermore, the nMDS analysis highlighted the different sensitivity to leachates exposure of the three biological endpoints, with embryotoxicity test resulting the most affected among the battery of bioassay by both beached and commercial plastic. The relevance of RTR sample was, moreover, emphasized, which not only appeared to have the highest value in terms of percentage on the total amount of marine litter collected during the survey but also exerted the higher toxic effect.



Weighted Elaboration of Ecotoxicological Results

	Beached					Commercial				
g/L (d.w.)	HF	MN	PB	DB	RTR	HF	MN	PB	DB	RTR
0.1	0.19	0.47	0.56	0.49	0.52	0.42	0.46	0.54	0.5	2.18
1	0.39	0.82	0.66	0.47	0.63	0.46	0.49	1.6	0.45	2.09
10	0.93	0.43	0.33	0.19	0.94	1.19	0.96	1.79	0.77	7.62
20	0.39	2.17	0.27	0.13	2.01	1	1.08	2.35	0.53	8.82
100 *	2.16	2.21	1.37	2.04	3.25	0.33	0.4	3.37	3.46	9.62

Fig. 5. Weighted elaboration of collected results for each sample at different sequential dilutions of beached and commercial plastic leachates (*33 g/L for PB sample). “Hazard Quotient” (HQ, 0–10) of the entire battery were reported, for each dilution of the various sample. “Level of hazard” assigned to the entire battery was reported distinguishing by colour 5 classes of hazard: Absent, white; Slight, light blue; Moderate, yellow; Major, red and Severe, black. HF=heterogeneous fragments; MN= mussel nets; PB= polystyrene boxes; DB= drink bottles; RTR= “Rapido” trawl rubber.

4. Discussion

4.1. *Chemistry of plastics’ leachates*

In the present study, a plastic-to-water ratio of 100 g/L (30 g/L for Polystyrene Boxes, PB) was used to generate leachates from beached and commercial plastics.

Clear differences emerged both between weathered and new plastics and among material categories.

Metals, in particular, were more readily leached from beached plastics compared to commercial ones. This may reflect environmental adsorption rather than contributions derived from intrinsic polymer additives, such as metal-based catalysts, stabilizers, colorants, or flame retardants [37]. Indeed, because of plastic additives are not chemically bound to the polymer matrix, they can easily diffuse into seawater during the first weeks [9,38]. However, aging may simultaneously promote the release of these compounds and the uptake of metals from the environment, as aged plastics have been reported to sorb up to five times more heavy metals than new plastics [39]. Therefore, detected substances may originate from a combination of additives leaching and environmental adsorption.

Regardless of chemicals origin, beached plastics exhibited enriched chemical profiles, supporting the role of stranded plastics as reservoirs and sources of contaminants in coastal ecosystems. This is particularly concerning for “Mussel Nets” (MN), which exhibited both the highest number and concentrations of detected metals among the investigated plastic categories. Although harmonized European Environmental Quality Standards (EU-EQS) are unavailable for Al, Fe, Zn, and V, levels detected in MN are comparable to those reported in beached plastic debris [40], abandoned fishing gear [37], and plastics subjected to prolonged environmental exposure [41]. Considering their extensive use, prolonged immersion, and large exposed surface area in aquaculture settings, MN may represent chronic local sources of metals release, which may induce multiple physiological and biochemical impairments when accumulated by marine organisms [37,42]. Among regulated priority substances, Pb was detected in MN at 0.279 μg/g, corresponding to ≈ 0.279 μg/L in leachates. This concentration is well below the maximum allowable concentration (MAC-EQS) of 1.3 μg/L established for transitional and coastal waters under the Water Framework Directive (WFD, 2000/60/EC) and its daughter Directives (2008/105/EC; 2013/39/EU), suggesting limited Pb-related ecotoxicological risk under experimental conditions.

Polystyrene Boxes (PB) stood out for releasing the highest concentrations of both PAHs and AHs, regardless the weathered status, identifying them as a major contributors to hydrocarbons leaching from coastal litter. Widely used in the fishery sector for fish storage and transport, PB are among the most prevalent components of marine litter, both at sea and along coastlines [43]. Consistently, they accounted for 51% of collected items during our beach clean-up (Figure S2), emphasizing their relevance in driving local contamination. Their elevated release is probably primary driven by intrinsic material properties, characterized by low volume weight ratio, high porosity, and large specific surface area, which enhance the water-plastic interface and facilitate both release and adsorption of compounds. Supporting this interpretation, Hüffer et al. [44] reported that artificially aged polystyrene microplastics showed significantly lower adsorption of organic compounds than pristine particles, indicating that aging does not

Fig. 6. Non-metric multidimensional scaling (nMDS) ordination of beached (orange) and commercial (blue) plastic items based on their chemical composition (metals, PAHs, AHs) and ecotoxicological effects. The size of the dots indicates the relative percentage weight of each item category.

necessarily enhance the uptake of environmental contaminants, as polymer oxidation can reduce hydrophobicity despite surface cracking. It should be note that AHs and PAHs detected in L1, particularly the lower molecular weight fractions, may partially originate from polymer degradation. In beached plastics, environmental weathering promotes both mechanical and molecular fragmentation, generating leachable, soluble and/or volatile byproducts [45-50]. Although commercial plastics have not undergone environmental aging, low-molecular-weight hydrocarbons may still derive from residual monomers or additives [15]. Therefore, polymer-related sources cannot be excluded for either plastic type. When converted to leachate concentrations (μg/L), PAHs released from PB (beached and commercial) exceeded the EU EQS values. Naphthalene (4.65 and 5.61 μg/L), Benzo[*a*]pyrene (0.38 and 0.24 μg/L), ΣBenzo[*b*]fluoranthene + Benzo[*k*]fluoranthene (0.79 and 0.6 μg/L), and ΣBenzo[ghi]perylene + Indeno[1,2,3-cd]pyrene (0.7 and 0.3 μg/L) (Table S4) were markedly above regulatory thresholds (1.2, 0.05, 0.03, and 0.002 μg/L, respectively), highlighting the high leaching potential of these compounds under the experimental conditions.

However, it should be emphasized that the detected levels of inorganic and organic compounds in L1 should not be directly extrapolated to environmental conditions, as the applied plastic-to-water leaching ratio (100 g/L or 33 g/L) are much higher than typical environmental concentrations and thus represent a worst-case scenario. In the marine environment, dilution, biodegradation, and geochemical processes are likely to substantially reduce the concentration and bioavailability of both metals and hydrocarbons [51], mitigating, but not entirely eliminating, the potential ecotoxicological risk.

The second sequential extraction (L2) pointed out as contaminants mobilization can be strongly medium-dependent and plastic-specific [52-55]. Compared to L1, the more aggressive conditions enhanced Fe and Zn release, particularly from beached PB and RTR, by mobilizing less labile fractions not readily released under seawater conditions. This indicates that L2 reflects a potential maximum release rather than typical environmentally exposure. Nonetheless, in natural marine environments, processes such as ocean acidification, natural oxidation, and plastic degradation can create conditions approaching nitric acid leaching [56], potentially increasing metal mobility and plastic toxicity over time [57]. The elevated Fe and Zn concentrations can be attributed to the combined effects of slow metals migration in seawater [37], the strong oxidizing power of nitric acid, which particularly promotes the release of these metals [58], and the physical properties of the plastic materials [59]. In particular, the rubbery nature of RTR and the porous structure of PB likely facilitate the leaching compared to the rigid nature of HF, MN, and DB [59]. Also, PB and RTR samples, specifically the commercial ones, exhibited a significant increase in PAHs levels. While this is partly attributable to the high extraction efficiency of methanol, due to its polarity and ability to dissolve a wide range of organic compounds, these results may also reflect the synergic action of natural processes such as photodegradation, oxidation, hydrolysis, wave action, sand abrasion, and biological activity, which progressively enhance organic contaminants release from plastics in the marine environments.

4.2. *Ecotoxicity of plastics' leachates and hazard assessment*

Following a common approach in ecotoxicological studies, a high initial plastic-to-water ratio was used in this study followed by serial dilutions. From a toxicological perspective, this enabled the generation of a concentration-response gradient suitable for toxicity tests, accounting for dilution processes in aquatic environments and allowing evaluation of biological responses under progressively more environmentally relevant conditions. Similar 1:10 ratios have been adopted in previous leachate-based ecotoxicological studies [15,22,60-62].

Overall, a clear differential sensitivity among trophic levels and endpoints was highlighted. Embryotoxicity in *P. lividus* emerged as the most sensitive and ecologically relevant indicator, compared to acute bacterial bioluminescence inhibition in *A. fischeri* and algal growth inhibition in *P. tricornutum,* in line with findings reported in previous studies on plastic leachates [33,63,64]. Part of the embryotoxicity may be attributed to zinc, which is known to be toxic to sea urchin larvae likely due to a disruption of calcium homeostasis [65]. Accordingly, leachates with the relatively higher zinc concentrations, *i.e.* commercial and beached RTR, commercial PB and beached MN leachates, elicited the most pronounced effects.

Focusing on RTR, this category displayed the strongest and most coherent concentration-response relationship across the bioassays tested, indistinctly for beached and commercial plastics. It was the only material inducing marked acute toxicity in *A. fischeri,* very high growth inhibition in *P. tricornutum* already at 1 g/L, and severe embryotoxic effects in *P. lividus*, with complete developmental arrest at higher concentrations (20 and 100 g/L). These findings align with previous studies on similar elastomers, such as car tire rubber (CTR), which contain elevated zinc concentrations and exhibit greater toxicity to marine biota compared to thermoplastics, including developmental effects to sea urchin and reduction of gamete quality in other invertebrates [16,20,24,66-70]. This consistency suggests the presence of particularly hazardous additives or degradation products, highlighting how polymer formulation of RTR, rather than environmental weathering alone, can drive its ecotoxicological outcomes. Indeed, rubber materials typically contain complex mixtures of substances, including vulcanization agents, accelerators, antioxidants, plasticizers, fillers (e.g. carbon black, clays, silicas, calcium carbonate) and various processing aids. Also, PAHs, phthalates, sulfenamides, guanidines, thiazoles, thiuams, dithiocarbamates, sulfur donors, phenolics, phenylenediamines and heavy metals are associated with CTR [24,55,66,71-74]. All these chemicals may contribute to toxicity. Gambardella et al. [33] had already demonstrated the high toxicity of RTR leachates in term of EC50s towards marine invertebrate species (*i.e.* jellyfish, crustaceans, sea urchins), reinforcing the direct link between elastomer chemical composition and ecotoxicological impact. This specific rubber is widely used in the Adriatic Sea during the so-called “Rapido” trawl fishery, which targets flatfish on inshore muddy bottoms, or pectinids on offshore sandy bottoms [75]. In this context it serves both as a weight to drag the net along the seabed and as protection against abrasion. Mechanical stress during fishing operations, can cause these rubber to get stuck on the bottom, or break and be transported by storms, finally settling on the shoreline where they are frequently found, particularly in the northern and central Adriatic. Indeed, RTR accounted for approximately 33% of collected marine litter by both weight and number during our beach clean-up (Figures S2 and S3). Given its widespread use, frequent loss, and confirmed toxicity, RTR represents not only a physical pollutant but also a significant chemical hazard in trawled areas.

HF and MN beached plastics caused relevant effects on algal growth and sea urchin embryos compared to commercial counterparts, but only for the higher concentrations tested (20 and 100 g/L). Composed mainly of PP and PE, their leachates likely act via polymer degradation products (e.g. monomers or parts of carbon chains) and adsorbed environmental contaminants rather than intrinsic toxicity of the pristine polymers. Moreover, once at the sea, thermoplastics quickly release those additives that make them ductile and elastic, becoming rigid until they slowly crumble [76], progressively promoting nanoplastics formation. Nanoplastics with diameter $< 0.22\ \mu$m (corresponding to the filter’s porosity used to treat leachates) could therefore be present in the tested leachates, potentially further contributing to the observed biological effects. Interactions between nanoplastics and marine biota, including phytoplankton and zooplankton such as echinoderm larvae [77,78] and crustaceans [79], have been previously demonstrated, whereas the direct role of plastic additives in these specific effects appears comparatively limited.

Conversely, some commercial plastics, including PB, DB and RTR elicited elevated effects than their beached counterparts. This suggests that the toxicity of leachates was not strictly related to the investigated chemicals (metals, PAHs and AHs), but rather to other unknown plastic-associated compounds. This deduction is in accordance with Gardon et al. [22] who observed stronger toxicity of new plastics compared to aged ones, likely due to the higher concentration of phthalates measured in their respective leachates.

The Weight of Evidence (WOE) integration further clarified these trends. For beached plastics, hazard levels were mostly classified as Absent or Slight up to 20 g/L, with Moderate to Major hazard emerging only at the highest concentrations, particularly for RTR. For commercial plastics, hazard classification reflected a more heterogeneous scenario: HF and MN remained within Absent–Slight, whereas PB and especially RTR exhibited concentration-dependent increases from Moderate to Major or even Severe. This integrated assessment confirms that hazards are strongly concentration-dependent and sample-specific, and that significant environmental risk is primarily associated with high exposure scenarios or specific formulations.

Taken together, the concentration–response analysis supports two key considerations: (i) under environmentally realistic dilution conditions, most plastic leachates showed limited acute ecotoxicity toward microorganisms and algae; however, (ii) early life stages of marine invertebrates are particular sensitive and can reveal sublethal and lethal effects.

It should be noted that initial leachates were prepared using relatively high plastic-to-water ratio (100 g/L or 30 g/L), exceeding typical environmental concentrations. While this enabled robust hazard assessment under conservative conditions, actual exposure is likely lower, highlighting the relevance of testing lower and more representative concentrations when extrapolating to real-world risk; in this context a solid-to-liquid ratio up to 1–10 g/L is recommended [19,21,33,80,81].

Overall, using a multi-trophic battery and WOE integration provides a more precautionary and ecologically relevant assessment of plastic leachates, especially in worst-case scenarios such as confined or poorly flushed marine environments.

5. Conclusions

The study showed that metals, PAHs and AHs can be released from plastic items into different solutions (L1 and L2), confirming the role of plastic as a reservoir for harmful chemicals, which, once desorbed, may further contribute to seawater pollution. However, chemical enrichment did not consistently predict ecotoxicity, which was strongly material- and endpoint-specific. The bioluminescence test (*A. fischeri*) and algal growth test (*P. tricornutum*) were affected only by the exposure to the leachate from RTR commercial sample, while the generally more sensitive *P. lividus* embryonic development test respond to both beached and commercial plastics leachates, particularly at the highest concentrations. This pattern likely suggests that embryotoxicity is primarily driven by a combination of metals (e.g., zinc), polymer degradation products, and adsorbed environmental contaminants, while bacterial and algal responses are triggered only by more chemically enriched or additive-rich plastics, such as RTR.

The WOE elaboration of the ecotoxicological results revealed that HF and MN showed higher hazard in beached samples, likely due to degradation products and adsorbed environmental contaminants. Conversely, PB, and especially DB and RTR posed higher hazard in commercial plastics, reflecting their intrinsic material properties (porosity in PB and rubbery in RTR) as well as the presence of uncharacterized associated chemicals. MN, PB, and RTR, all linked to fishing activities and the most prevalent among marine litter, emerge as key categories to consider due to their potential for chemical release and ecotoxicity.

Environmental implication

The study compared the leaching potential of weathered (beached) and unweathered (commercial) plastics in seawater by characterizing their chemical profiles and assessing ecotoxicological hazards. Weathering appeared to enhance metal release from beached plastics, notably Al and Fe, while both beached and commercial polystyrene boxes leached high levels of organic contaminants, exceeding EU Environmental Quality Standards thresholds for compounds such as naphthalene and benzo[*a*]pyrene, indicating that their porous structure, rather than aging, primarily drives release. Heterogeneous fragments and mussel nets cause significant ecotoxicological effects, such as bioluminescence inhibition, reduced algal growth, and developmental issues in sea urchin embryos. Integrating ecotoxicological results within a Weight of Evidence hazard assessment, commercial rubber plastics, commonly used in fishing equipment, emerged as the most hazardous material. For this plastic category, hazard levels were classified as Moderate even at low leachate concentrations (0.1–1 g/L) and reached Severe at higher concentrations (10–100 g/L), emphasizing their potential ecological risk in fishing-impacted areas. Overall, combining chemical and ecotoxicological analyses provides a more comprehensive evaluation of the environmental hazards posed by weathered and commercial plastics, supporting informed risk assessment and management.

CRediT authorship contribution statement

Carola Mazzoli: Conceptualization, Formal analysis, Investigation, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Lucia Pittura:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Giuseppe d' Errico:** Formal analysis, Investigation, Data curation, Writing – review & editing. **Federica Iezzi:** Formal analysis, Investigation. **Daniele Fattorini:** Investigation, Supervision, Writing – review & editing. **Marta Di Carlo:** Investigation, Validation, Writing – review & editing. **Alessandro Nardi:** Resources, Writing - review & editing. **Marica Mezzelani:** Writing - review & editing. **Maura Benedetti:** Supervision, Writing – review & editing. **Stefania Gorbi:** Conceptualization, Project administration, Resources, Supervision, Funding acquisition, Writing – review & editing. **Francesco Regoli:** Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

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

Acknowledgements

This work was supported by the project “MicroPLASTICs in edible aquatic organisms: ecotoxicological effects, transfer of chemical and biological CONtaminants and susceptibility to bacteria biodegradation” (PLASTICON; RF-2019–12370587), funded by the Italian Ministry of Health, Progetti ordinari di ricerca finalizzata, 2019, and developed under the framework of the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of December 16, 2021, rectified by Decree n.3175 of December 18, 2021 of MUR by the European Union – NextGenerationEU (Award number: Project code CN_00000033, Concession Decree No. 1034 of June 17, 2022 adopted by the MUR and Research, CUP I33C22001300007, Project title “National Biodiversity Future Center—NBFC”).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2026.142043.

Data availability

Data will be made available on request.

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