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Filtration of biopolymer PHB particles loaded with synthetic musks does not cause significant bioaccumulation in marine mussels

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ABSTRACT

The role of the biopolymer polyhydroxybutyrate (PHB, <250 μm) as a vehicle of a synthetic musks mixture (celestolide, galaxolide, tonalide, musk xylene, musk moskene and musk ketone) to *Mytilus galloprovincialis* was investigated. For 30 days, virgin PHB, virgin PHB+musks (6.82 μg g-1) and weathered PHB+musks, were daily spiked into tanks containing mussels, followed by a 10-day depuration period. Water and tissues samples were collected to measure exposure concentrations and accumulation in tissues. Mussels were able to actively filter microplastics in suspension but the concentration of the musks found in tissues (celestolide, galaxolide, tonalide) were markedly lower than the spiked concentration. Estimated Trophic Transfer Factors suggest that PHB will only play a minor role on musks accumulation in marine mussels, even if our results suggest a slightly extended persistence in tissues of musks loaded to weathered PHB.

1. Introduction

Nowadays, submillimetre plastic particles termed microplastics (MP) are one of the main environmental concerns for marine, freshwater and terrestrial ecosystems (Huerta Lwanga et al., 2016). Ingestion of those particles was proposed as a mechanism to concentrate and transfer hydrophobic organic compounds (HOC) to aquatic organisms otherwise scarcely bioavailable due to their low water solubility (Andrady, 2011). Despite thermodynamic constraints (Gouin et al., 2011), MPs were assumed to transfer sorbed HOC to the tissues of the organisms during gut passage time (Bakir et al., 2014). The HOC-vector hypothesis currently achieved the status of paradigm, although empirical testing has shown contrasting results (reviewed by Tourinho et al., 2019), with studies supporting increased availability of HOCs in presence of MP (Bellas and Gil, 2020; Besseling et al., 2013; Browne et al., 2013; Oliveira et al., 2013), lack of relevant effects (Almeda et al., 2021; Beiras and Tato, 2019; Herzke et al., 2016; Horton et al., 2018; Koelmans et al., 2016; Lohmann, 2017; Ziccardi et al., 2016) and reduced bioavailability in presence of MP (Beckingham and Ghosh, 2017; Beiras et al., 2019; Rehse et al., 2018).

The desorption kinetics of HOC from the hydrophobic matrix of polymers depends on diffusivity of the chemical within the polymeric matrix (intraparticle diffusion) and aqueous boundary layer diffusion (Endo et al., 2013; Seidensticker et al., 2017). Therefore, desorption depends on multiple properties of the sorbent (polymer crystallinity, particle size, surface properties) and the sorbate (molecular volume, Kow), which may help to explain contrasting experimental results. In the present study, we used the biodegradable plastic polyhydroxybutyrate (PHB) and as models HOCs, the most common synthetic musks.

Synthetic musks are used as fragrance additives in a wide range of scented consumer goods, such as personal care (e.g., perfumes, soaps, deodorants, shampoo) and household products (e.g., air fresheners, cleaning agents or detergents). Among the predominant compounds in these products are the polycyclic musks (galaxolide - HHCB and tonalide - AHTN), macrocyclic musks and nitromusks (musk ketone -MK and musk xylene - MX) (Nakata et al., 2007; Rimkus, 1999). Since they were first reported in aquatic environment (Yamagishi et al., 1981), synthetic musks have been widely reported in environmental matrixes (Gatermann et al., 1999; Nakata et al., 2007; Schlumpf et al., 2010; Yamagishi et al., 1983) and frequently associated with wastewater discharges (Chase et al., 2012; Homem et al., 2015; Peck and Hornbuckle, 2004; Stevens et al., 2003). Synthetic musks have been detected in waters (typically ng L^{-1}) of all continents, including surface waters, groundwater, and even in the Antarctic region, with special mention to the

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polycyclic musk galaxolide, considered an ubiquitous contaminant, which has been detected in a broad range of water samples. These compounds are only partially biodegradable, so they are not eliminated by wastewater treatment plants (WWTPs) (Celeiro et al., 2019; Montes-Grajales et al., 2017). Moreover, these substances were found to potentially cause deleterious effects and act as endocrine disruptors (Luckenbach and Epel, 2005; Parolini et al., 2015; Van Der Burg et al., 2008) being, therefore, extremely important to understand their bioaccumulation patterns in biota.

Hence, this study aims to understand the role of a MP, in this case PHB, as a vector of synthetic fragrances to *Mytilus galloprovincialis* marine mussel, which is widely used in experiments that follow the same premises (Álvarez-Ruiz et al., 2021; Pittura et al., 2018) as well as an ubiquitous environmental monitoring species (Beiras et al., 2003; Picot Groz et al., 2014; Salgueiro-González et al., 2016; Vidal-Liñán et al., 2018). Besides, the biopolymer PHB has been lately found to cause deleterious effects to aquatic biota (Beiras et al., 2021; González-Pleiter et al., 2019; Magara et al., 2019; Straub et al., 2017). Nevertheless, no data are available on the potential of PHB to act as a vector of substances to marine biota.

2. Material and methods

2.1. Experimental solutions and materials

Non additives resins of PHB were produced by extrusion following common industrial procedures by the Plastics Technology Centre AIMPLAS (Spain), ground by means of an ultracentrifuge mill (ZM200, Retsch), and sieved to particle size < 250 μm. To test the potential effect of environmental weathering, PHB in powder form, in 12 cm diameter petri dishes, was weathered for 9 months in a low-cost pilot system under standardized, controlled conditions simulating dry environments. The system was designed to simulate solar irradiation at medium latitude, exposed to two HQI-TS 2510 W/NDL metal-halide bulbs in zenithal and ground level positions (illuminance: 12,200 lumen/m2, irradiance on air 60 ×10–2 W/m2). The content of each Petri dish was mixed every day and the locations of the dishes were interchanged each three days. More specific details are presented elsewhere (Andrade et al., 2019). Aliquots of these setups were withdrawn each fortnight to monitor weathering through FTIR spectroscopy and SEM microscopy. FTIR measurements were done using a 400 FT-IR/FT-NIR PerkinElmer Spectrometer (4000–650 cm-1, 4 cm-1 nominal resolution, Beer-Norton strong apodization, 50 scans per spectrum, background-, depth-penetration- and baseline-corrected) equipped with a horizontal one-bounce diamond crystal (Miracle ATR, Pike). SEM microscopy was done by means of a JEOL JSM 6400 device, after coating the PHB particles surface with a layer of gold. Comparisons among the surface textures of virgin and weathered PHB were done. Further characterization of virgin and weathered MPs can be found in supplementary material, with spectral profile of MPs (Fig. S1) and SEM microscopy images (Fig. S2) that indicate chemical and physical changes during the weathering process.

A stock standard solution of musks mixture (celestolide (ADBI), galaxolide (HHCB), tonalide (AHTN), musk xylene (MX), musk moskene (MM) and musk ketone (MK)) containing 1000 μg mL^{-1} of each substance, deuterated tonalide-d3 (100 μg mL^{-1}) and musk xylene-d15 (100 μg mL^{-1}) in acetone were purchased from Techno Spec (Barcelona, Spain). Stock solutions of musks mixture (100 μg mL^{-1}) and AHTN-d3 and MX-d15 (20 μg mL^{-1}) were prepared in methanol. The first solution was used for the experimental assay while the last two were used for chemical analysis. Standard working solutions at different concentrations levels were prepared in ethyl acetate. All stock solutions were stored in the dark at − 4ºC.

To obtain the fragrance-spiked samples, 11 g of MPs were accurately weighed and spiked with 25 mL of 3 μg mL^{-1} mixed standard solution (6.82 μg g^{-1}), mixing carefully for a few minutes to ensure the homogeneity of the spiking. Samples were kept in a dry place during 12 h until the solvent was completely evaporated.

2.2. Biological material

Mussels (*Mytilus galloprovincialis*) between 35 and 45 mm long were collected from a location in the outer part of Ria de Vigo (NW Iberian coast) (Vidal-Liñán et al., 2010), cleaned from epibionts, and acclimated to incubation conditions in the laboratory for one week prior to experiments. Acclimatization was made in running seawater inside an isothermal room at 16ºC in the dark, and mussels were fed as in the exposure period (see below).

2.3. Exposure conditions

Over 180 mussels were exposed for 30 d to experimental solutions containing 1.5 mg L^{-1} of MPs, followed by a 10 d depuration period. Exposure was made in 30 L glass tanks, with 12 mussels per tank and three tanks per treatment, using 1 μm filtered seawater (FSW) with oceanic characteristics (salinity 34 ± 0.5‰, dissolved oxygen > 90% of saturation, DOC 11.5 ± 2.1 μM). Incubations were made at constant temperature (16ºC), in darkness. Exposure tanks were continuously aerated with 0.22 μm filtered air and were allowed to equilibrate for 1 h before introducing the mussels. Water was renewed three times per week after mussels feeding for 1 h with a mixed diet of *Isochrysis galbana, Tetraselmis suecica* and *Chaetoceros gracilis*.

Mussels were exposed to 3 different treatments virgin PHB, virgin PHB + musks and weathered PHB + musks. Tanks were daily spiked with a stock solution of 450 mg L^{-1} MP, with musk concentration of 6.82 μg g^{-1}, dispersed in FSW and 0.2 mg L^{-1} Tween-20 (v/v) stock solutions to obtain a MPs concentration of 1.5 mg L^{-1} in seawater. After 30 days of exposure, 6 mussels per tank were removed from the tanks for chemical analysis of body tissues. The remaining mussels were transferred to tanks with clean water, in same conditions as previously described, for 10 days depuration period, and 6 mussels per tank were later collected for chemical analysis.

2.4. Filtering rates and data treatment

To record mussel's filtering rates (FR), a separate experiment was conducted, 3 conditions were tested in tanks with 15 L of FSW: virgin PHB + musks without mussels (control), and with 6 mussels per tank, virgin PHB + musks and weathered PHB + musks. Triplicate samples of water were collected immediately after treatments spiking and particle density was recorded using a Multisizer 3 Coulter Counter (Beckman). The same process was repeated 2 h later. FR (mL min^{-1}) were calculated from the reduction of suspended particles in the water according to the expression (Beiras and Tato, 2019; Coughlan, 1969):

$$FR = \frac{(\mathrm{Ln}\ C_{Ct} - \mathrm{Ln}\ C_t) \times V}{t}$$

Where V is the volume of the incubation vessels (mL), t is the incubation time (h), C_t is the particle concentrations at the end of the incubation period, and Cc_t is the control particle concentrations at the end of the incubation period.

Also, the concept of Trophic Transfer Factor (TTF), defined as the ratio of a substance concentration in an organism's tissue to its concentration in the organism's food item (DeForest et al., 2007), was used to understand substances uptake by mussels from ingested MPs. The following equation was used:

$$TTF = \frac{(CM_t - CM_{ct})}{C_{PHB}}$$

where C_{Mt} is the substance concentration in mussels of both PHB + musks treatments, C_{Mct} the substance concentration in mussels from

the control (virgin PHB treatment) and C_{PHB} the concentration of a substance on the spiked MPs.

2.5. Chemical analysis of seawater

In order to understand the presence of fragrances in water throughout the experiment, water samples (250 mL) were taken prior to spiking, after 2 and finally 24 h. Water was filtered through steel filters FM 200, with 200 μm pore size (Filter lab) and frozen until analysis. Fragrances from seawater samples (spiked with 10 μl of the deuterated standards working solution) were extracted by vortex assisted liquid-liquid microextraction (VALLME) in an agitation plate Vibrax-VXR by IKA (Staufen, Germany) with 1.5 mL of ethyl acetate. 0.5 g of sodium chloride salt were added to improve the extraction. The determination was performed by gas chromatography tandem mass spectrometry (GC-Qq-MS/MS) using a Thermo-Finnigan Trace GC chromatograph and coupled to a triple quadrupole mass spectrometer (TSQ Quantum XLS) and equipped with a Triplus autosampler and PTV injector. Separation was carried out on a DB-XLB column (60 m × 0.25 mm × 0.25 μm) (J&W Scientific, Folsom, CA, USA). Recovery percentages of this method are between 76% and 100%, with a good precision (RSD <10%) and the limit of quantification of the method were 0.03 μg L^{-1}, 0.14 μg L^{-1}, 0.05 μg L^{-1}, 0.13 μg L^{-1}, 0.01 μg L^{-1} and 0.01 μg L^{-1} for celestolide, galaxolide, tonalide, musk xylene, musk moskene and musk ketone respectively.

2.6. Chemical analysis of mussel tissues

Samples of 6 individuals were taken initially, after 30 d exposure, and after further 10 d depuration. Soft tissues were dissected, thoroughly rinsed with FSW (to eliminate to the best extent particles added to tissues) and freeze-dried for analysis. For the analysis of fragrances in mussels, 1 g of sample, spiked with 50 μl of the deuterated standards working solution, were performed by a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction method followed by GC-QqQ-MS / MS. The QuEChERS extract tubes were obtained from Agilent Technologies (Santa Clara, United States). The extraction kit contains 6 g $MgSO_4$ and 1.5 g Na Acetate and a dispersive kit contains 400 mg PSA, 400 mg C_{18}EC and 1200 mg $MgSO_4$. The determination was performed using a Thermo-Finnigan Trace GC chromatograph and coupled to a triple quadrupole mass spectrometer (TSQ Quantum XLS) and equipped with a Triplus autosampler and PTV injector. Separation was carried out on a DB-XLB column (60 m × 0.25 mm × 0.25 μm) (J&W Scientific, Folsom, CA, USA). Recovery percentages of this method are between 74% and 120%, with a good precision (RSD <10%) and the limit of quantification of the method were 7.60 ng g^{-1}, 3.30 ng g^{-1}, 11.1 ng g^{-1}, 125 ng g^{-1}, 43.8 ng g^{-1} and 33.3 ng g^{-1} for celestolide, galaxolide, tonalide, musk xylene, musk moskene and musk ketone respectively.

3. Results

Filtering rates recorded show that mussels were able to actively filter MP in suspension. After 2 h, particles in suspension decreased in orders of 18% in mussels exposed to virgin PHB + musks and 30% in weathered PHB + musks. This is reflected on the obtained FRs, 2.0 mL min^{-1} for Virgin PHB + musks and 5.4 mL min^{-1} for Weathered PHB + musks. Despite not being statistically different (t-test, $p = 0.075$), a 2.7-fold FR between treatments suggests the mussel preference for weathered PHB.

3.1. Synthetic musks in seawater

Results presented in Table 1 show that only celestolide, galaxolide and tonalide were present above their detection limits. The same substances were detected in FSW (prior to any spiking) at higher mean concentrations than those registered after 2 h and 24 h. For the quantified substances at 2 h, celestolide concentrations were the lowest, ranging 0.04–0.09 μg L^{-1} while galaxolide and tonalide were found at higher concentrations in all samples ranging 4.44–7.62 and 11.35–22.80 μg L^{-1}, respectively. After 24 h, tanks with mussels suffered a statistically significant decrease in water concentrations when compared to the values registered at 2 h (Fig. 1-A). The different conditions tested have direct influence on the decrease of these substances, with higher fragrance concentration decrease in tanks with weathered PHB (88% for galaxolide and 98% for tonalide) compared with tanks with virgin PHB (60% and 89% for galaxolide and tonalide, respectively). On the other hand, water concentrations of the detected musks seem to maintain steady throughout the 24 h period in the tank without mussels and spiked to Virgin PHB + musks.

3.2. Fragrances in mussel tissues

In tissue samples, again only celestolide, galaxolide and tonalide were present above limits of quantification (Table 2). Mussels sampled from the acclimatized mussel stock shown a galaxolide baseline level of 24.3 ng g^{-1} (T0). After 30 days, celestolide was quantified in both PHB + musks treatments while galaxolide and tonalide were ubiquitous among all MPs treatments. After the depuration period galaxolide and tonalide were still detected in organisms but variations in the concentrations are clearly depicted (Fig. 1-B) while celestolide was no longer detected. Statistically significant decrease in concentrations of galaxolide and tonalide, ranging 72% and 80% decrease, in mussels previously exposed to virgin PHB + musks. Opposing, we observed a minor decrease in weathered PHB + musks treatment for galaxolide (5% decrease) and tonalide (36%), this last being statistically significant. Only a slightly decrease in galaxolide and tonalide concentrations was registered in control organisms 8% and 19%, respectively.

Based on chemical data, TTFs were calculated for treatments with both PHB + musks correcting the values by subtracting musk levels recorded in control (Table 2). After 30 days exposure values obtained were quite low, ranging 0.002–0.005 being very similar among both MPs treatments.

Table 1
Mean musks concentration in water (μg L^{-1}) obtained after exposure and depuration. nd: not detected.

	Celestolide	Galaxolide	Tonalide	Musk xylene	Musk moskene	Musk ketone
Water (μg L^{-1})						
FSW	0.09	11.0	32.2	nd	nd	nd
2 h after spike						
Virgin Phb + Musks	0.09	7.62	22.80	nd	nd	nd
Weathered PHB + Musks	0.04	7.29	21.85	nd	nd	nd
Virgin Phb + Musks (Without Mussels)	nd	4.44	11.35	nd	nd	nd
24 h after spike						
Virgin Phb + Musks	nd	3.09	2.52	nd	nd	nd
Weathered PHB + Musks	nd	0.86	0.51	nd	nd	nd
Virgin Phb + Musks (Without Mussels)	0.04	6.55	20.28	nd	nd	nd

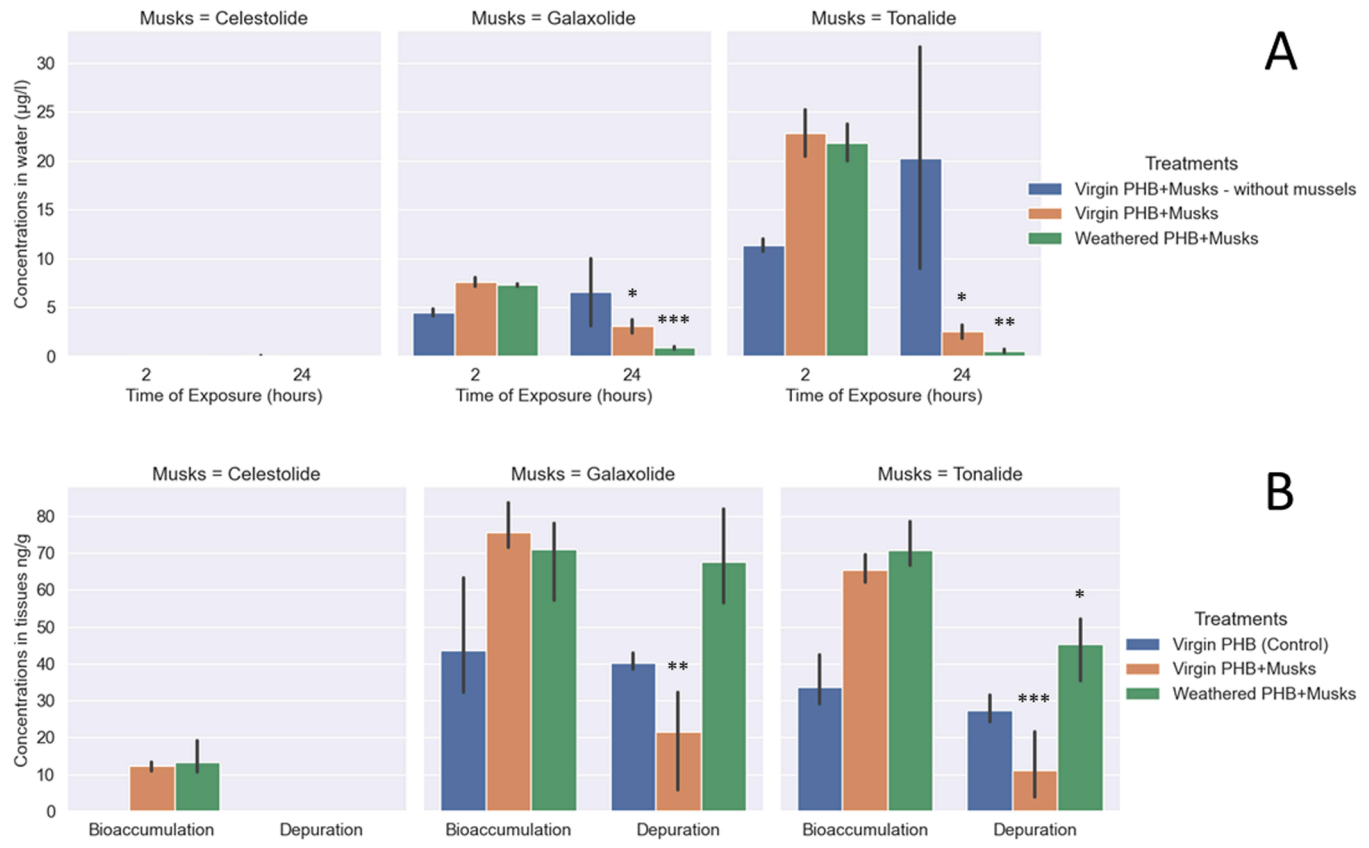

Fig. 1. Variation in time of quantified musks concentration. A) Musks concentration (µg L^{-1}) water samples during 24 h period. B) Musks concentration (ng g^{-1}) in mussel tissues at the end of bioaccumulation (30 days) and depuration (10 days) periods. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001,) indicate results of ANOVA post-hoc test.

Table 2
Mean musks concentration in mussels (ng g^{-1}) and Trophic transfer factor obtained after exposure and depuration. nd: not detected.

	Celestolide	Galaxolide	Tonalide	Musk xylene	Musk moskene	Musk ketone
Mussel (ng g^{-1})						
T0	nd	24.3	nd	nd	nd	nd
Bioaccumulation (30d)						
Virgin PHB (control)	nd	43.60	33.70	nd	nd	nd
Virgin PHB + Musks	12.20	75.57	65.57	nd	nd	nd
Weathered PHB + Musks	13.40	71.03	70.8	nd	nd	nd
Depuration (10d)						
Virgin PHB (control)	nd	40.26	27.27	nd	nd	nd
Virgin PHB + Musks	nd	21.42	12.90	nd	nd	nd
Weathered PHB + Musks	nd	67.67	45.37	nd	nd	nd
Trophic transfer factor (30 d)						
Virgin Phb + Musks	0.002	0.005	0.005	-	-	-
Weathered Phb + Musks	0.002	0.004	0.005	-	-	-

4. Discussion

In recent years, plastic debris turned out to be a scientific and social concern due to its environmental presence and accumulation in marine ecosystems. Despite the great concern, scientific research faces several challenges and uncertainties as MP impact depends on the combination of several factors like plastic properties, exposure conditions, biological parameters or contaminants transported by MP (Lusher et al., 2021). This last point is particularly relevant, not only because of additives used in plastic formulations but also due to the hydrophobic nature of plastic debris which might accumulate other hydrophobic compounds (Engler, 2012; Rochman, 2015). Using mussel as model species, several laboratory studies demonstrated that hydrophobic chemicals sorbed to microplastics could be partially transferred to mussel tissues such as fluoranthene (Stollberg et al., 2021), pyrene (Avio et al., 2015), benzo (a)pyrene (Pittura et al., 2018). Our work indicates that HOC like celestolide (log Kow = 5.7), galaxolide (log Kow = 5.9) and tonalide (log kow = 5.7) (Wong et al., 2019; PubChem, 2022) can only be transferred from MPs to mussel tissues to a very limited extent, since after 30 d exposed to MPs spiked at concentrations in the order of 10,000 ng g^{-1} the concentration in the mussel tissues was always lower than 100 ng g^{-1}, as reflected by the markedly lower TTF values (see Table 2). This is expected from the thermodynamic preference of HOCs for a synthetic hydrophobic matrix such as PHB polymer rather than the mussel tissues (Gouin et al., 2011). Some authors mention that is difficult to differentiate between bioaccumulated and adhered MPs to bivalve tissues (Kolandhasamy et al., 2018; Kuehr et al., 2022a). We try to avoid this bias by thoroughly rinsing mussel tissues with FSW but is

not possible to overcome a possible overestimation of musks accumulation due to MPs still retained in intestine. Therefore, these results should be carefully interpreted but if some, musks loaded to PHB particles are only accumulated in tissues to a very limited extent. Moreover, some studies, point out that MPs can work as depurators and this way decrease levels of HOC in mussels (Granby et al., 2018; Koelmans et al., 2016; Rios-Fuster et al., 2021), which could partially explain the musks concentration below control levels in Virgin PHB+musks treatment (Fig. 1-B), after depuration. Although, it is very difficult to corroborate this hypothesis with our experimental setup. What seems clear is that PHB will only play a minor role on the bioaccumulation of musks in marine mussel as assumed by the low TTF values obtained. This shows that the use of TTFs, seem in fact, a good option when trying to understand the role of MPs on the bioaccumulation of HOCs in opposition to the classic analysis of variance between the treatments and control in common experimental designs. Even if a significance is obtained between treatment and control, the HOC concentrations quantified in the target organism are considerably lower than those to which they were exposed.

Analysis on FSW used throughout the experiment confirmed the presence of some musks (Table 1). The water is collected directly from the Vigo bay to the laboratory facilities, thus make it impossible to control the environmental levels of these substances, which are reported to have an ubiquitous presence in coastal environment (Aminot et al., 2021; Castro et al., 2018; Lee et al., 2014, 2010). Galaxolide and tonalide have been reported in marine water samples (Aminot et al., 2019; Andresen et al., 2007; Sumner et al., 2010) and galaxolide even reported in sediments of Vigo area (León et al., 2020). Therefore, is reasonable to assume that previously and/or during the experiment, mussels were exposed to these compounds through water, which can explain the levels of some musks in control treatment with virgin PHB. Nevertheless, chemical analysis shows a decrease of musks concentration, after 2 and 24 h of the spiking (Table 1), indicating that loaded MPs will not increase the musks concentration in the seawater. Therefore, is reasonable to assume that musks concentration observed in mussels of control, after 30d exposure, are due to the contaminated waterborne exposure while on the other treatments, the slightly higher concentrations are also explained by the loaded MPs, and thus validating the TTFs obtained.

It is important to note that mussels seem to have preference to filter weathered PHB with a 2.7-fold filtering rate compared to virgin PHB, which is in agreement with previous findings on the preference by mussels and copepods to ingest weathered MPs (Bråte et al., 2018; Vroom et al., 2017). Even if this doesn't seem to affect musks accumulation by mussels, given by similar musks concentrations after 30 days, differences among treatments are then clearly spotted after the 10 days depuration period. A significant decrease of musks concentrations in mussels exposed to virgin PHB + musks is observed while, on the other hand, a low concentration variation observed on the other treatment (no decrease for galaxolide and slight decrease for tonalide) might indicate a different pattern of musks accumulation from weathered PHB particles. Works with freshwater bivalve *Corbicula fluminea* have shown that virgin micro and nanoparticles would be rapidly egested from the bivalve (Kuehr et al., 2022a, 2022b). This might explain the observed results with virgin PHB that most likely were rapidly egested and possibly indicate that musks levels measured after 30 days could be due to PHB in digestive tract. The same pattern might not happen to weathered PHB as levels did not rapidly decreased after 10 days. This reinforces the need to apply environmentally relevant conditions, as weathering, when making experiment with MPs (Beiras and Schönemann, 2020; Pannetier et al., 2019a, 2019b).

In conclusion, our results show for the first time the potential role of the biopolymer PHB as a vector of highly hydrophobic compounds accumulation in mussels although, most likely it will not play an important role on the accumulation of synthetic musks in environment. Nevertheless, further studies are certainly necessary to be carried out with other HOC or other experimental designs as still few information is available on this polymer. Moreover, the difference in results obtained when using weathered MPs in ecotoxicological assays vs pristine commercial products, is here demonstrated to reinforces the need to use the firsts to achieve a greater ecological significance of the findings reported. Finally, the use of the TTFs is suggested to be used in bioaccumulation experiments of MPs and HOC to properly understand the accumulation patterns in target organisms and therefore identify critical issues or problematic substances.

CRediT authorship contribution statement

Leticia Vidal-Liñán: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft. **Carmen Moscoso**: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Filipe Laranjeiro**: Validation, Formal analysis, Writing – original draft. **Soledad Muniategui-Lorenzo**: Conceptualization, Methodology, Validation, Formal analysis, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Ricardo Beiras**: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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.etap.2023.104092.

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