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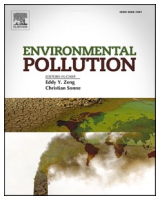

Integrative oxidative stress biomarkers in gills and digestive gland of the combined exposure to citalopram and bezafibrate with polyethylene microplastics on mussels *Mytilus galloprovincialis*☆

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A B S T R A C T

Pharmaceutical active compounds (PhACs) and microplastics (MPs) have been detected in different marine compartments from coastal areas, raising concerns due to their simultaneous discharge through wastewater treatment plants (WWTPs) and the role of MPs as vectors of pollutants for marine organisms. This study investigates the biochemical effects of citalopram (CIT) and bezafibrate (BEZ) on the mussel *Mytilus galloprovincialis*, at environmentally relevant concentrations, and their co-exposure with high-density polyethylene (HDPE) MPs. MPs accumulated in gills and digestive glands during exposure, but they were rapidly eliminated after depuration, except for a small fraction of the smallest MPs in gills. This study evaluated the biological effects in gills and digestive gland, and confirmed CIT induced oxidative stress in both tissues, exacerbated by the presence of MPs. BEZ, despite not being detected at high concentrations in the mussel tissues, activated an antioxidant response in gills and increasing the transcription of the genes *Se-gpx* and *gst-pi* in digestive gland. Both PhACs impaired the cholinergic pathway long-term, even after the depuration period, as indicated by decreased AChE levels in the gills, suggesting potential neurotoxic effects after prolonged exposure. Consequently, adverse effects were provoked by both PhACs with (CIT) and without (BEZ) significant bioaccumulation capacity.

1. Introduction

Marine ecosystems are vulnerable as a consequence of numerous pollutants inputs from diverse sources. Wastewater treatment plants (WWTPs) effluents have been identified as a major route for release, particularly of contaminants of emerging concern (CECs) such as pharmaceuticals (PhACs), typically measured at concentrations ranging from ng/L to ug/L (Mezzelani et al., 2018). More than 600 PhACs have been reported in several environmental matrices from 71 countries (aus der Beek et al., 2016), with generally higher concentrations in freshwaters than in marine systems (Rodríguez-Mozaz et al., 2017). Although the majority of PhACs are degradable, their continuous use and release into the environment confer to these molecules a pseudo-persistence behaviour (Barceló and Petrovic, 2007; Fabbri et al., 2023), causing their widespread occurrence in seawater (Alygizakis et al., 2016; Castaño-Ortiz et al., 2023a; Gros et al., 2012; Lolić et al., 2015; Moreno-González et al., 2015), sediments and aquatic organisms (Castaño-Ortiz et al., 2023a; Moreno-González et al., 2015, 2016; Grabicova et al., 2015; Klosterhaus et al., 2013; Mezzelani et al., 2016; Palma et al., 2020) and sorbed to floating plastics in coastal areas (Castaño-Ortiz et al., 2024; García-Pimentel et al., 2023).

Common classes of PhACs occurring in marine ecosystems and accumulated by non-target species include non-steroidal anti-inflammatory drugs (NSAIDs) (Mezzelani et al., 2016; McEneff et al., 2014; Cunha et al., 2017), psychiatric (Moreno-González et al., 2016; Álvarez-Muñoz et al., 2015; Martínez Bueno et al., 2014; Maruya et al., 2014; Silva et al., 2017; Wille et al., 2011) and cardiovascular drugs (Álvarez-Muñoz et al., 2015; Ali et al., 2018; Martínez-Morcillo et al., 2020), and antibiotics (Castaño-Ortiz et al., 2023a; Liu et al., 2018; Wu et al., 2021, 2022). Among these PhACs, antidepressants are widely consumed in developed countries (OECD, 2017), being specifically designed to target neurotransmission in the human brain (Di Poi and Bellanger, 2014). Previous data suggested that these drugs, even at environmentally relevant trace concentrations, may threaten the reproduction, development, behaviour, and survival of various aquatic organisms (Christen et al., 2010; Silva et al., 2015), but their toxicity

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mechanisms are still unclear (Silva et al., 2015; Miller et al., 2018).

In marine biota, mainly bivalves such as mussels and oysters, antidepressant concentrations varied from few ng/g up to 200 ng/g, with selective serotonin reuptake inhibitors (SSRIs) (sertraline (SER), fluoxetine (FLX), and citalopram (CIT)) and serotonin-norepinephrine reuptake inhibitors (SNRIs) (venlafaxine) as the most frequently detected compounds (Álvarez-Muñoz et al., 2015; Fong and Ford, 2014; Ojemaye and Petrik, 2019a,b)).

Lipid regulators (e.g. fibrates and statins) consumption has also been increasing in recent decades, but their environmental concentrations have not been thoroughly surveyed. Fibrates are widely used lipidemic-modulating drugs, being the most common the fibric acids bezafibrate (BEZ) and gemfibrozil (GF), and the fibrate esters fenofibrate (FF) and clofibrate (CLF) (Ido et al., 2017). This pharmacological class of compounds is characterized by their common capacity to provoke peroxisome induction in sensitive species (*Gambusia holbrooki)* (Nunes et al., 2008).

Other group of CECs are microplastics (MPs), which due to their hydrophobic nature and high surface area, can sorb organic contaminants (Atugoda et al., 2021). This interaction depends on MPs characteristics (e.g. polymer type, aging MPs, etc.), environmental conditions (e.g. pH, salinity, temperature, etc …) and compound specific properties, such as pKa and octanol-water partition coefficient (*Kow*) (Llorca et al., 2020; Razanajatovo et al., 2018; Santos et al., 2021). The occurrence of PhACs has also been recently confirmed in floating plastics sampled from different coastal areas (García-Pimentel et al., 2023).

The co-occurrence of PhACs and MPs has received increasing attention in recent years, mainly due to the simultaneous discharges through WWTPs and the potential role of MPs as vectors of pollutants and modulator of their bioaccumulation and ecotoxicological effects. Previous studies have found evidence of toxic interactions between microplastics and pharmaceuticals in bivalves, indicating that the combination of these two substances is more toxic than each component separately, leading to feeding inhibition, neurotoxicity, oxidative stress, and cellular damage in these organisms (Guilhermino et al., 2018; Shi, 2020; Webb et al., 2020; Álvarez-Ruiz et al., 2021).

Since the oxidative system of bivalves is considered as one of the main targets for various pollutants, the present work aimed to examine the effects of polyethylene MPs in combination with the antidepressant citalopram or the lipid regulator bezafibrate in the Mediterranean mussels *(Mytilus galloprovincialis*). The two selected PhACs have relatively hydrophobic properties (log Kow >3), and an increasing number of studies has demonstrated their presence in river and coastal waters (Nödler et al., 2014; Fernandes et al., 2020; Fernández-Rubio, 2019; de Souza et al., 2021), sediments (Moreno-González et al., 2015; Fernandes et al., 2020) and biota (Castaño-Ortiz et al., 2023a; Moreno-González et al., 2016; Castaño-Ortiz et al., 2024; Álvarez-Muñoz et al., 2015; Martínez-Morcillo et al., 2020; Mello et al., 2022). Previous studies have demonstrated that antidepressants induce the ROS production, cause oxidative modifications of cellular components and decrease the acetylcholinesterase activity in *D. magna* (Yang et al., 2018; Duan et al., 2022). Additionally, they inhibited the stress-related swimming behaviour of zebrafish (Zindler et al., 2020; Bachour et al., 2020) and provoked anxiolytic behaviours in crayfish (Buřič et al., 2018). Fibrates also affected the haemocyte functional parameters, including phagocytosis (Gagné et al., 2006), and provoked mRNA changes of enzymes and other proteins involved in the prevention from protein damage in freshwater bivalves (Contardo-Jara et al., 2011).

However, their eco-toxicological effects have been scarcely investigated in marine species. In this context, a controlled laboratory experiment with mussels *M. galloprovincialis* was carried out for 21 days at environmental realistic concentrations followed by 7 days of depuration, to study: i) the potential of PhACs to induce oxidative stress and neurotoxic effects in the digestive gland and gills ii) the influence of MPs on the effects of PhACs on mussels and iii) the mussel potential for recovery after depuration period. Bioaccumulation and metabolomic effects of this experiment have been previously published and discussed, evidencing the bioaccumulation of citalopram but not for bezafibrate in mussels (Castaño-Ortiz et al., 2023b), while oxidative stress has been characterized in this study as part of a large experimental design. All analysis (Castaño-Ortiz et al., 2023 and this study ones) corresponded to the same experiment.

2. Materials and methods

2.1. Chemicals

Citalopram, CIT (*CAS* 59729-32-7) and bezafibrate, BEZ (CAS 41859-67-0) standards were purchased from Sigma-Aldrich with a high purity grade (>95%). The microplastic consisted on a micronized powder of virgin high-density polyethylene (HDPE) obtained from Micro Powders Inc. (www.micropowders.com, reference MPP-635XF). The provider indicated that polyethylene (PE) was composed by non-uniform particles with maximum size of 22 μm, mean size of 4–6 μm, and density at 25 °C of 0.96 g cm^3. The MPs characterization (Fig. 1S) was previously published by Fernández and Albentosa (2019a). The HDPE particles were subjected to short-term aging in seawater by exposition to environmental conditions (lab terrace: sunlight and temperature, with one manual agitation per day except in weekends) in transparent quartz Erlenmeyers during one month. The objective of this short-aging process in seawater was to get MPs similar to those commonly found in the marine environment, reducing the adverse effects of the possible high and fast leaching of plastic additives from virgin plastics. The size and shape were evidenced by the scanning electron microscopy image (Fig. 2S).

2.2. Animals collection and experimental design

Animal collection and exposure conditions have been detailed elsewhere (Castaño-Ortiz et al., 2023b). Briefly, mussels *M. galloprovincialis* (5 ± 1 cm shell length) were obtained from a farm located in the Mediterranean Sea in Benalmádena (Andalucia, Spain). 1026 mussel were distributed into 18 aquariums of 15L, equitable based on size (57 mussels per tank) and acclimatized for 7 days to laboratory conditions with aerated seawater (0.45 μm-filtered), at 16.5 ± 1 °C, salinity 37.5 ± 0.5 practical salinity units, natural photoperiod regime (12 h light:12 h dark) and daily fed on the *T-isochrysis* microalgae (4–8 μm) in a ration equivalent to 1% of mussels dry weight. During acclimatization and the experiment seawater was entirely renewed three times per week. The experimental design included 6 treatments tanks per triplicate (18 tanks) with organisms daily exposed for 21 days to: 1) 500 ng/L of BEZ (BEZ); 2) 500 ng/L of CIT (CIT); 3) 500 ng/L of BEZ with PE (1 mg/L, PEBEZ); 4) 500 ng/L of CIT with 1 PE (1 mg/L, PECIT); 5) 1 mg/L of PE without pharmaceutical compound sorbed; and 6) Control mussels (CTRL). Concentrations of PE, pharmaceuticals and solvent were daily dosed for 21 days. At the end of the exposure period, mussels were maintained for additional 7 days in contaminants-free seawater, intended as a depuration period. In total the experiment lasted 28 days. The selected pharmaceuticals concentration (500 ng/L) was slightly higher than those reported in coastal waters (generally in 1–100 ng/L range) but around or below the established toxicity thresholds for citalopram and bezafibrate (Castaño-Ortiz et al., 2023b). The stock solutions of BEZ and CIT (100 and 110 mg/L) were prepared in methanol and stored al 4 °C during the experiment. Working PEBEZ and PECIT solutions (1 mg/L, CIT-BEZ) were daily prepared by diluting the stock BEZ and CIT solutions in seawater with PE, and incubating them for 24 h under continuous agitation at 16.5 ± 1 °C. To ensure similar exposure conditions in PE and solvent control tanks (CTRL) methanol was added at the same concentration used in the rest treatments (0.0005%). Less than 3% mortality was observed during the experiment.

At day 10, 21 and 28, mussels were sampled, collecting at each sampling time. 16 individuals for each tank (a total of 48 specimens for

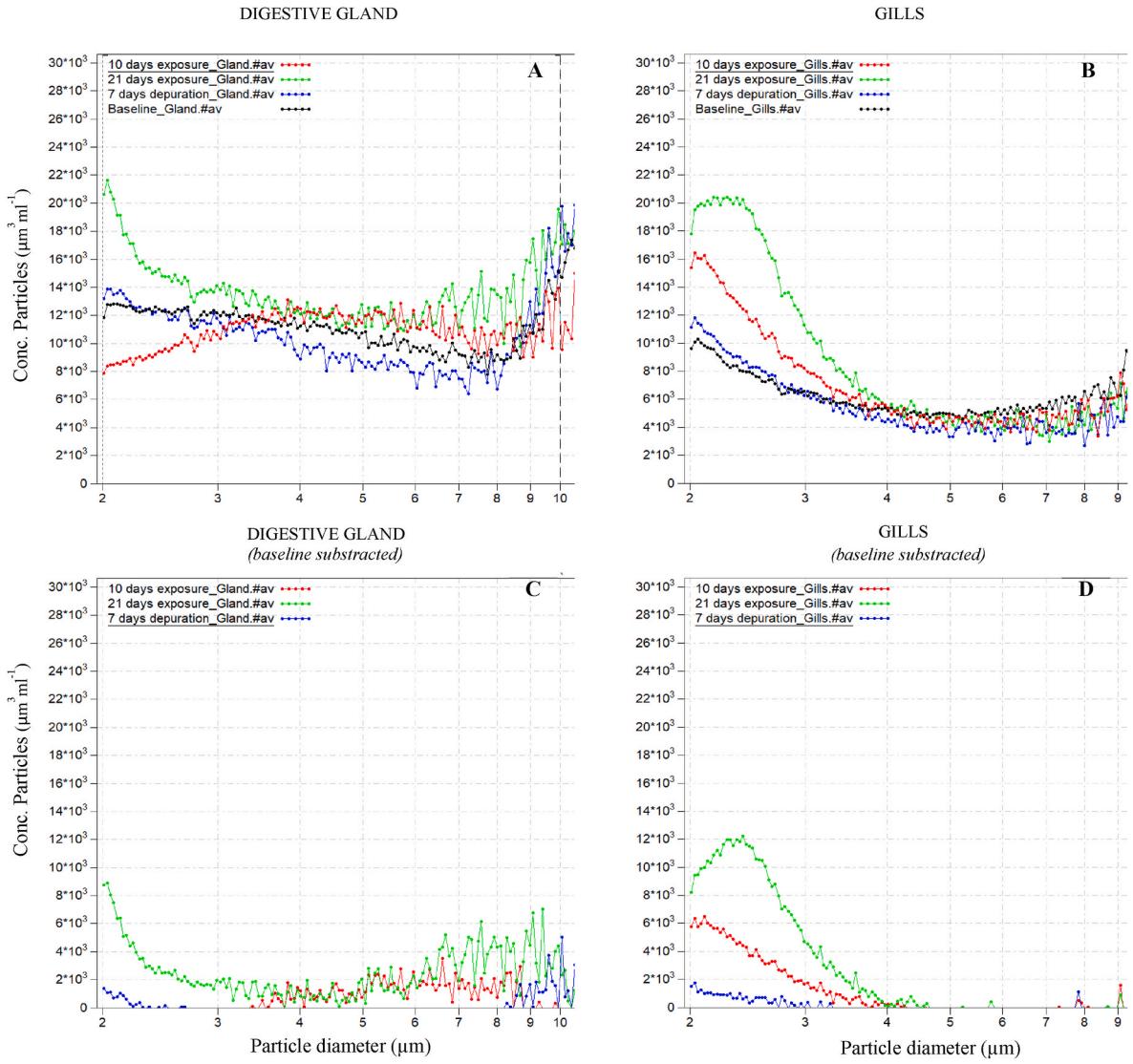

Fig. 1. MPs estimated accumulation in mussel's tissues: A, C) Digestive gland; B, D) Gills. Red line detection particles after 10 days of exposure. Green line detection particles after 21 days of exposure. Blue line detection particles after 7 days of depuration. The baseline obtained from particles measured in control organisms has been subtracted (C, D). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

each treatment) at each sampling time. Whole body of six individuals were pooled, freeze-dried and kept at − 20 °C until chemical analysis whereas ten individuals were dissected on ice for different purposes: MPs analysis of gills and digestive gland (4 indiv.) and metabolomic and biomarker analyses in gills and digestive gland (6 indiv.). The collected tissues were individually frozen in liquid nitrogen (−196 °C) and stored at − 80 °C. Biometric information was recorded upon dissection, namely mussel length (with valve) and wet weight (without valve).

2.3. Chemical analyses on pharmaceuticals accumulation in *M. galloprovincialis* and seawater

Bioaccumulation of CIT and BEZ in mussels was determined on 3 replicates per treatment, each constituted by whole tissues of 6 specimens. Pool obtained were freeze-dried, powdered and homogenised with a ceramic mortar. The methods of extraction and analysis as well as the results of CIT and BEZ bioaccumulation have been previously published by Castaño-Ortiz et al., (2023b).

The detailed methods for the analysis of PhACs in seawater, along with the results, are explained by Castaño-Ortiz et al. (2023). The concentrations of pharmaceuticals in seawater were analysed throughout the experiment, confirming that CIT, due to its removal from the water column by the organisms, among other factors, maintained the desired concentration of 470 ± 144 (CIT) and 505 ± 198 ng/L (PECIT) thanks to the daily dosing. In contrast, the concentration of BEZ, not being accumulated by the organisms nor subjected to other dissipative factors (degradation, volatilization, etc) that would cause significant removal, varied moderately along the experiment, being BEZ concentration in seawater higher and variable than initially expected: 1074 ± 505 (BEZ) and 1232 ± 797 ng/L (PEBEZ).

2.4. Concentration of MPs in digestive gland and gills

To test the uptake of PE by mussels in exposure tanks (PE, PEBEZ, PECIT) the clearance of PE particles from water (μm^3/mL, 0–60 min) was tested by means of a Coulter-Counter Beckman model Multisizer™ III (MSIII).

The concentration of PE particles (μm^3 mL^{-1}) was estimated in digestive gland and gills homogenates from exposed mussels. Although this is not a specific method for the MPs analysis, it is useful for the estimation of their occurrence in the different tissues of exposed mussels, considering the non-MPs exposed mussels as control and baseline at

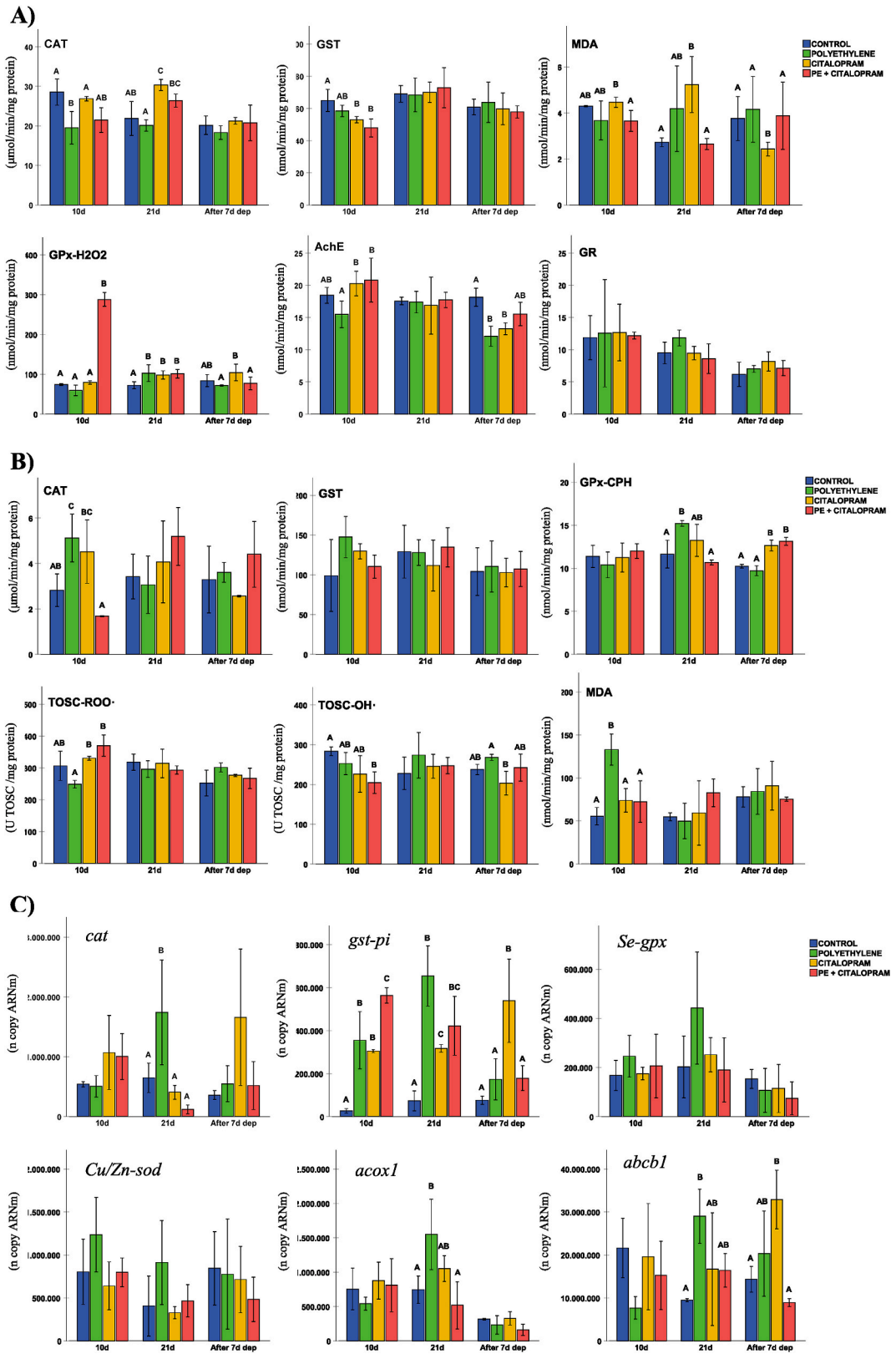

Fig. 2. CITALOPRAM: Entire set biomarker analyses in gills (A); Entire set biomarker analyses in digestive gland (B); Entire set gene expression analyses in digestive gland (C); Data are given as mean values ± standard deviation. Superscript letters indicate statistically significant differences ($p < 0.05$, ANOVA with Tukey's post hoc test) among group means. Graphs without superscript letters indicate no statistically significant differences between treatments. Sampling time code: 10 days exposure (10d); 21 days exposure (21d); 7 days depuration (after 7d dep).

lab-controlled conditions to estimate the presence of other particles, which could interfere with this analysis. MPs quantification was performed on 6 replicates samples per treatment, each one constituted by the tissues (gills and digestive gland) of 2 specimens. The methodology used followed the protocol described by Fernández and Albentosa, (2019a,b) with minor modifications. The detailed protocol is provided in Supplementary Material (SM). Briefly, the different particles size ranges were quantified using the MSIII with an aperture tube of 100 μm, which made measurements from 2 to 60 μm. Mean baselines for digestive gland and gills homogenates of control mussels (feed on microalgae) were subtracted from those obtained from mussels exposed to PE (PE, PEBEZ, and PECIT). No statistically significant differences were found between control samples throughout the experiment and, consequently, a mean particle baseline was established for each tissue using data from all control cases (mussels not exposed to MPs along the experiment) (Fig. 4S). The PE concentrations (μm^3 mL^{-1}) estimated with the MSIII were subsequently transformed into mm^3 per mg^{-1} and μg MPs per mg^{-1} of wet tissue using the PE density.

2.5. Biomarker analyses

Standardized methods were used for biomarkers analyses. All the detailed protocols applied for the analysis are given in SM and Table 1S. Digestive gland and gills were analysed for lipid peroxidation by measuring malondialdehyde (MDA). The antioxidant enzyme activities determined in gills were catalase (CAT), glutathione S-transferases (GST), glutathione reductase (GR), glutathione peroxidases (GPx) and acetylcholinesterase activity (AChE). The analyses of CAT, GST, GPx in digestive gland were integrated with the Total Oxyradical Scavenging Capacity Assay (TOSCA) toward peroxyl (TOSC ROO•) and hydroxyl radicals (TOSC HO•) and with molecular analyses for quantification of mRNA levels of the following target genes: catalase (cat), glutathione S-transferase pi-isoform (gst-pi), selenium-dependent glutathione peroxidase (Se-gpx), Cu, Zn superoxide dismutase (Cu/Zn-sod), Acyl-CoA oxidase 1 (acox1) and p-glicoprotein, multidrug resistance protein, (*abcb1*).

2.6. Statistical analysis

Statistical analysis was performed using SPSS statistical package (v. 15. 0). All data were tested for normality and homoscedasticity through the Shapiro-Wilk and Levene's test, respectively. The existence of significant differences in the results obtained between exposures were tested/verified by comparing them with the control of their sampling time, by ANOVA test and Tukey post hoc analyses (Annex I and Annex II in Supplementary Material). A minimum significance level of 95% was applied for all analyses ($p < 0.05$).

3. Results

3.1. MPs bioaccumulation

In the present study the clearance of PE particles by mussels in exposure tanks (PE, PEBEZ, PECIT) was monitored by measuring the concentration of particles in water after the dosage (μm^3 per mL; 0,15, 30, 45 and 60 min). PE particles were totally cleared from the water column in the experimental tanks in 15 min (Fig. 3S).

The estimated concentration of MPs (μm^3 per mL) in gills and digestive gland homogenates and the accumulation of MPs (μg per mg wet tissue) in tissues of mussels exposed to PE after 21 days ranged 0.032–0.035 μg/mg mussel (Fig. 1 and Table 2S). The estimated concentration of MPs in gills and digestive gland increased during the exposure, detecting the highest presence of MPs in both tissues for the three PE exposed groups (≈0.050 μg per mg wet tissue) after 21 days. In fact, the particle concentrations were significantly higher in gills and digestive gland of exposed mussels than those found in controls ($p < 0.05$) analysed at 10 days in the same size range than PE MPs (Table 2S). However, these differences were not statistically significant at 21 days at $p < 0.05$ level due to a higher variability in the obtained results. No significant differences on the MPs levels were detected among organisms exposed to different treatments containing PE particles at any time ($p < 0.05$). After the depuration period the MPs concentrations were low but still detectable in gills and digestive gland (0.011 and 0.006 μg per mg wet tissue). Therefore, mussels were able to eliminate MPs from their tissues.

It should be noted that the profile of the PE particles accumulated in gills and digestive gland was different (Fig. 1). The majority of PE particles estimated in digestive gland ranged between 4 and 10 μm, resembling the profile of stock PE particles (Fig. 1-A). In contrast, the majority of PE particles detected in gills were below 3 μm in diameter (Fig. 1-B).

3.2. Biomarkers in Gills

Results on biomarkers measured at cellular levels in mussels exposed to CIT, BEZ and PE are reported in Table 3S and Fig. 2-A and 3-A. GPx activity in gills significantly increased compa red to the control after the exposure to PE and CIT for 21 days. However, the combination of PE and CIT (PECIT) markedly induced this activity after 10 days and remained high after 21 days. This response observed for CIT was accompanied by a significant induction of CAT activity and increase in lipid peroxidation after 10 days, which remained higher than the controls at 21 days. Interestingly, PECIT exposure did not alter both MDA and CAT activity. However, in PE treatment an inhibition of CAT activity was measured after 10 days of exposure.

The GST activity levels, associated with the phase II metabolism of xenobiotic compounds, decreased at 10 days following exposure to both CIT and PECIT. However, the presence of PE alone did not affect this activity. Regarding GR enzyme, no changes were observed in any treatment.

Exposure to BEZ triggered antioxidant defences, primarily through modulation of GPx, which activity showed a significant increase compared to the controls after 10 days of exposure to both BEZ and PEBEZ. This increase was notable, particularly in the former case, rising approximately four-fold compared to control values. However, GPx returned to similar levels to those of the controls in organisms exposed to BEZ after 21 days. Conversely, in the PEBEZ treatment, a clear inhibition of GPx was found after 21 days. On the other hand, CAT, GR, and GST levels did not exhibit significant differences compared to control during the exposure (7 and 21 days) for both BEZ and PEBEZ exposures. Lipid peroxidation results revealed the ability of BEZ to damage cell membranes, as evidenced by the elevated levels of MDA after 10 days of exposure to BEZ and at both exposure sampling times (10 and 21 days) in the PEBEZ treatment.

After the depuration period CAT, GPx, GST, and GR activitiy levels were equivalent to those of the control group for CIT, PECIT, and PEBEZ. However, GPx and GST activities significantly decrease in mussels exposed to BEZ after 7 days of depuration compared to the control groups. Despite the inhibition in these antioxidant defence enzyme levels, lipid peroxidation levels, and consequently, the ability of ROS to affect lipids, were significantly lower than control values for both CIT and BEZ treatments.

After 10 and 21 days of exposure, AChE showed no statistically significant differences compared to the control throughout all treatments involving CIT, PECIT, BEZ, or PE. However, a significant increase was observed for PEBEZ after 10 days of exposure, followed by a significant decrease after 21 days. After the depuration period, organisms exhibited a clear inhibition of this enzymatic activity throughout all treatments, with significant differences to the control for CIT, BEZ, and PE.

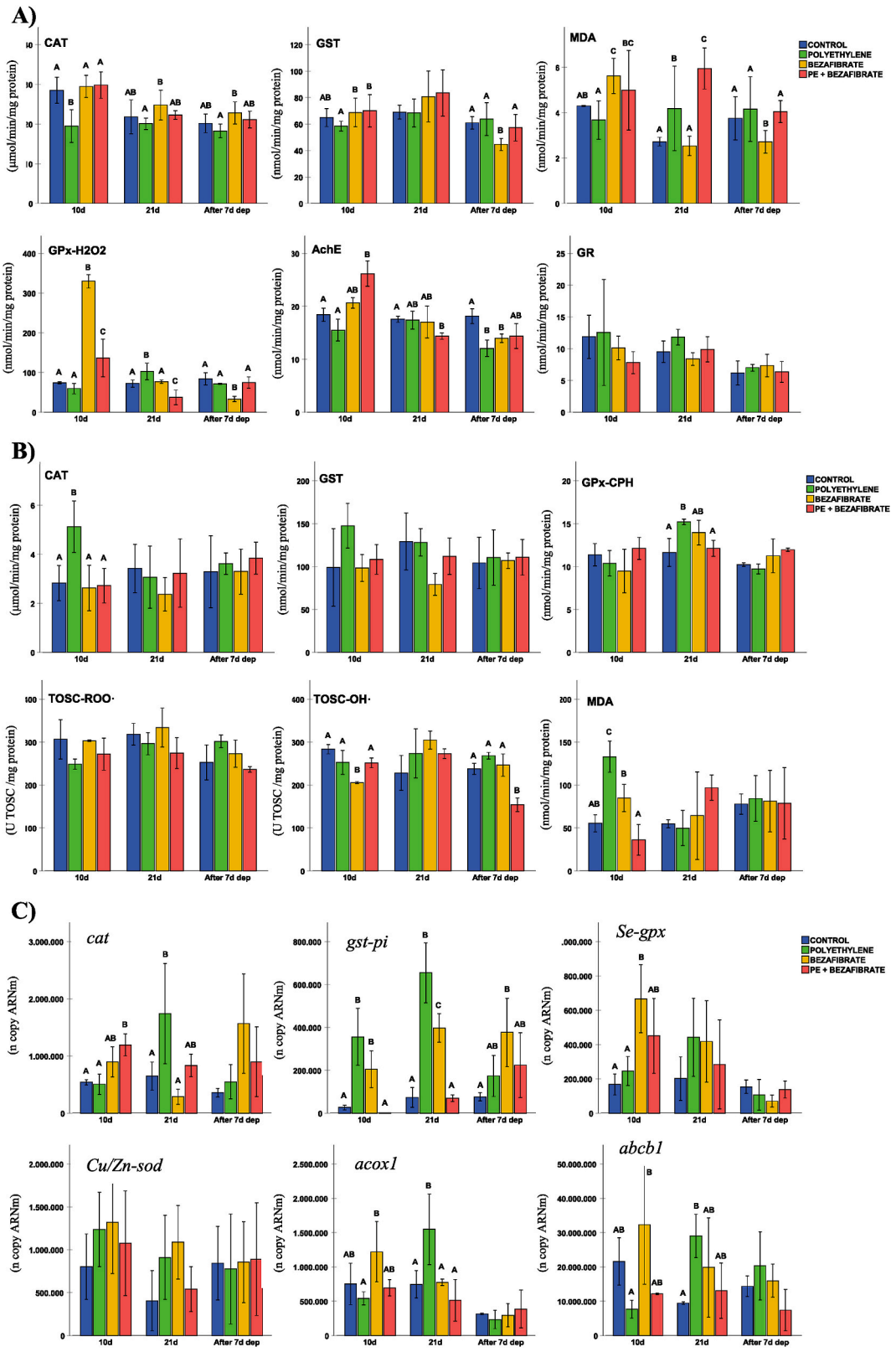

Fig. 3. BEZAFIBRATE: Entire set biomarker analyses in gills gland (A); Entire set biomarker analyses in digestive (B); Entire set gene expression analyses in digestive gland (C); Data are given as mean values ± standard deviation. Superscript letters indicate statistically significant differences ($p < 0.05$, ANOVA with Tukey's post hoc test) among group means. Graphs without superscript letters indicate no statistically significant differences between treatments. Sampling time code: 10 days exposure (10d); 21 days exposure (21d); 7 days depuration (after 7d dep).

3.3. Biomarkers in digestive gland

Results on biomarkers measured at cellular and molecular levels in mussels exposed to CIT and PE are reported in Table 4S and Fig. 2-B and 2-C. Analyses on single antioxidants enzymes highlighted a significant induction of CAT activity in mussels exposed to single stressors (PE and CIT) after 10 days of exposure. However, limited and not significant variations compared to CTRL organisms were detected at both the end of the exposure and depuration periods. Similarly, high variability characterized the activity of GST throughout the entire experiment, with lack of significant changes among treatments. Average highest values of GPx activity were found in organisms exposed to single stressors (PE and CIT) at the end of exposure period (21 day); such variation persisted even after the end of depuration period for CIT exposed organisms, when also a statistically significant induction in PECIT-treatment was recorded. After 10 days of exposure, the highest average levels of the Total Oxyradical Scavenging Capacity toward peroxyl (ROO•) were observed for CIT-exposed mussels (CIT and PECIT), in parallel to the decrease of these mussels' capability to neutralize hydroxyl (HO•) radicals. Limited variation of both parameters (TOSC ROO• and HO•) was found in exposed organisms compared to CTRL, at the end of both exposure and depuration phases. After 10-days of exposure, PE-exposed mussels displayed a significant increase of MDA levels, while no significant changes were detected for all treatments at the end of exposure and depuration phases (Fig. 2-B).

Transcript levels of selected genes showed variable responses (Fig. 2-C). Although not significant, *cat* transcription increases in CIT-exposed mussels (CIT and PECIT) after 10 days of exposure, and persisted only in CIT even after the end of depuration period. A similar pattern of variation was detected for mussels treated with PE alone after 21 days. A significant increase mRNA levels of *gst-pi* was found for all treatments compared to CTRL at 10 and 21 days of exposure and even after the depuration period. No significant differences were detected for *Se-gpx*, *Cu/Zn-sod*, while an increase in *acox1* and *abcb1* was observed after 21 days of exposure in PE-exposed mussels, this trend was found for *abcb1* in CITPE even at the end of depuration period.

Results on biomarkers measured at cellular and molecular levels in mussels exposed to BEZ and PE are reported in Table 4S and Fig. 3-B and 3-C. Analyses on single antioxidants enzymes highlighted a significant induction of CAT activity in mussels exposed to PE for 10 days; no significant variations compared to CTRL was measured at both the end of the exposure and depuration periods. Similarly, in mussels exposed to PE, the induction of GST and GPx activities was confirmed after 10 and 21 days of exposure, respectively. Limited effects on Total Oxyradical Scavenging Capacity toward peroxyl (ROO•) and hydroxyl (HO•) radicals were detected, with the only exception for a statistically significant decrease of capability to neutralize hydroxyl (HO•) radicals in mussels exposed to BEZ alone after 10 days and to PEBEZ at the end of the depuration period (28 days). Variation on single antioxidant enzymes in PE-exposed mussels was observed in parallel to the significant accumulation of MDA after 10 days of exposure (Fig. 3-B).

Transcript levels of selected genes showed variable responses (Fig. 3-C). An increase of *cat* transcript levels was observed in BEZ-exposed mussels (BEZ and PEBEZ) compared to CTRL at days 10 and 28, while a similar trend was also found for PE at the end of the exposure phase (day 21). Similarly, a significant increase mRNA levels of *gst-pi* was detected in mussels exposed to PE and BEZ alone at days 10 and 21; BEZ effects on *gst-pi* persisted even after the depuration period. An increase in mRNA levels of *Se-gpx* was observed BEZ-exposed mussels (BEZ and PEBEZ) after 10 days-exposure. However, no significant differences were detected for *Se-gpx* and *Cu/Zn-sod*, while an overall increase in *acox1* and *abcb1* was observed during the exposure phase (10 and 21 days) in organisms exposed to PE and BEZ alone (Fig. 3-C).

4. Discussion

The study investigated the biological effects on the gills and digestive gland of mussels exposed to CIT, BEZ, MPs, and their combination. Gills serve as an interface between the external and internal environments, being the first organs exposed to contaminants through gas exchange and nutrition via the uptake of food particles (Viarengo and Canesi, 1991; Vidal-Liñán and Bellas, 2013). Digestive gland in bivalves are well-suited tissues for assessing adverse effects induced by suspended solids and colloids through ingestion (Faggio et al., 2018). These tissues perform distinct roles in various physiological processes such as the respiration or feeding, and therefore, their response might be influenced by these factors and have different implications for the health of bivalves.

4.1. MPs bioaccumulation

There is ample evidence that MPs can be ingested by mussels (Van Cauwenberghe et al., 2015; Birnstiel et al., 2019; Zhao et al., 2018; Gonçalves et al., 2019). However, the majority of experimental studies did not measure the uptake or accumulation of MPs in exposed organisms.

The rapid clearance of PE microparticles (15 min) was also observed for the same MPs by Fernández and Albentosa, 2019a,b) and other similar PE by Fernández et al. (2020). Moreover, the PE particles tested in the present study ($\leq$22 μm) were within the size range that bivalves can filter with maximum efficiency (Brillant and MacDonald, 2000; Ward and Shumway, 2004).

Once ingested, MPs may be retained in the digestive tract, eliminated as bio-deposits or translocated to other tissues (Ward and Shumway, 2004; Browne et al., 2008). The increasing accumulation of MPs observed from day 10 to day 21 agreed with the continuous exposure and uptake of PE particles by mussels. The cessation on MPs exposure for 7 days resulted on a drastic decrease on MPs levels. This indicated that at the end of depuration period the majority of microplastic particles were expelled by mussels, from both tissues, keeping, however, small residues on gills and digestive gland, as also demonstrated by previous studies (Avio et al., 2015; Ribeiro, 2017; Santana et al., 2017; Pittura et al., 2018).

The differential size accumulation of MPs in digestive gland (4–10 μm) and gills (mainly $\leq$3 μm) may imply different toxicological effects of the PE particles tested in these tissues. Several studies with mussels have reported the accumulation of MPs in gills (Setälä et al., 2016; Avio et al., 2015; Von Moos et al., 2012). The accumulation of the smallest MPs in gills suggested their translocation through the haemolymph (phagocytosis process to protect against invasion of foreign materials), as it was observed in previous studies (Fernández and Albentosa, 2019a, b; Browne et al., 2008).

The bioaccumulation of CIT and BEZ in mussels was studied previously by Castaño-Ortiz et al., (2023b), as well as the influence of MPs on this process. In this experiment, BEZ concentrations in whole mussels were approximately 300-400-fold lower than citalopram ones, evidencing the limited bioaccumulation for the lipid regulator as consequence of the acidic nature of BEZ should limit their transport through biomembranes and its retention in tissues (Castaño-Ortiz et al., 2023b). However, the accumulation of CIT was similar with and without HDPE at the end of the exposure period, being slower the accumulation kinetic for the PECIT cases (10 days).

The bioaccumulation, translocation and distribution of PE in the mussel tissues could explain the differences in the bioaccumulation of CIT over exposure time, specially at short time, due to their capacity to bind these compounds. It is noticed that after depuration period, CIT levels persisted in the tissues despite the elimination of MPs particles. In contrast, BEZ accumulation in mussel tissues were significantly lower than CIT, moreover the effect of MPs on its bioaccumulation was negligible at both sampling times.

4.2. Effects on Gills

The biomarker responses in mussels showed a significant decrease of the CAT activity after 10 days of exposure to PE, while GST and GPx levels did not shown statistical differences compared to the control levels (Table 3S). However, PE increased GPx activity after the longer exposure period of 21 day. The response of GPx in this study agrees with previous findings on the role of MPs as oxidative stressors, which might be related to differences in MPs' exposure concentration, size, and duration (Zhou et al., 2022; Li et al., 2022a). The CAT inhibition may be related to a possible saturation of enzymatic defences and oxidative stress such as suggested by other authors (Pampanin et al., 2005; Regoli and Principato, 1995; Vlahogianni and Valavanidis, 2007). This inhibition in gills has also been observed in other bivalves (*R. philippinarum* and *T. granosa*) after their exposure to PS (Zhou et al., 2022).

The exposure of mussels to CIT and BEZ, regardless of the presence of PE, led to an increase in GPx levels, indicating the ability of these drugs to induce oxidative stress in gills. GPx is the most important peroxidase related to detoxification hydroperoxides, catalyzing the glutathione dependent reduction of H_2O_2 and organic hydroperoxides (Hampel et al., 2016). Several studies showed an increased activity in relation to oxidative stress due to the accumulation of organic and metallic contaminants (Solé et al., 1994; De Almeida et al., 2004; Gwoździński et al., 2010), and antidepressant like FLX and CIT in whole soft tissues of *D. polymorpha* (Magni et al., 2017).

Our results showed that CAT activity increased the antioxidant defence of the mussels exposed to CIT after 21 days of exposure, while BEZ did not affect its gills values during the exposure and depuration period. A previous study did not detect significant changes in CAT activity levels after FLX exposure in gills from *Mytilus galloprovincialis* (Gonzalez-Rey and Bebianno, 2013). However, most of the studies in bivalves reported a CAT inhibition after the exposure to PhACs, such as FLX in haemolymph from *T. granosa* (Shi et al., 2020), in soft tissues of *Crassostrea gigas* (Di Poi et al., 2016), and soft tissues from *D. polymorpha* after CIT exposure (Magni et al., 2017). These results could be due to a high production of ROS linked to certain PhACs exposure such as antidepressants.

Following exposure to CIT, the induction of both CAT and GPX activities suggested a potential increase in reactive oxygen species (ROS) production. CAT plays a complementary role with GPx in scavenging oxygen-derived radicals. The main function of the catalase is to discompose hydrogen peroxide into water and oxygen, being most effective at degrading high concentrations of hydrogen peroxide (Kehrer and Robertson, 2010). In fact, MDA levels were significantly higher after 21 days for CIT compared to control (Table 3S), suggesting that antioxidant defenses may be overwhelmed. However, MDA levels did not show an oxidative damage after the co-exposure to PE and CIT. MDA is a biomarker of oxidative damage as well as an indicator of the severity of lipid peroxidation (Ho et al., 2013). When the oxidative stress period is prolonged, free radicals created by tissue cells increase and gradually accumulate, this can lead to a constant decrease in the antioxidant properties, increasing the MDA levels and causing further histopathological lesions (Chen et al., 2023).

Oxidative stress after BEZ and PEBEZ treatments were also accompanied by an increase in MDA levels, being significant for BEZ at 10 days and for PEBEZ at 21 days (Table 3S). As previously indicated, the main antioxidant response obtained by these treatments was mediated by the GPx. The rise in lipid peroxidation levels in the PEBEZ group after 21 days of treatment was linked with the inhibition of GPx activity, which dropped below that of the control group. This fact contrasted with the observed effects on BEZ exposure, where GPx and LPO levels were comparable to those of the control. Our results agree with previous ones in bivalves which found a capacity of some PhACs such as fibrates or anti-inflammatories to increase of the LPO levels in bivalves (Aguirre-Martinez, 2021; Gonzalez-Rey and Bebianno, 2012). This process affected the balance between ROS production and elimination capacity, with elevated lipid peroxidation levels and morphological alterations in gills and digestive tubules that increased the cellular damage (Quinn et al., 2011; Russo et al., 2023).

GST enzyme is involved in important cellular processes, such as the conjugation and detoxification of organic compounds, and it also plays a protective role against oxidative stress by catalyzing selenium-dependent glutathione peroxidase activity (Prohaska, 1980; Santos et al., 2004). During the exposure, the response of this enzyme differed for the two PhACs tested. On the one hand, this enzymatic activity decreased compared to the control values after 10 days of exposure to CIT and PECIT, showing no differences at 21 days. However, BEZ did not exhibit significant differences for any of the treatments, either alone or with PE (Table 3S). The ability of CIT to reduce the GST activity is consistent with earlier findings in *D. polymorpha* exposed to CIT (Magni et al., 2017). However, the exposure to FLX induced GST activity in gills after 7 days in *M. galloprovincialis* (Gonzalez-Rey and Bebianno, 2013), and it did no provoked changes on this activity in the bivalve *Perna perna* (Cortez et al., 2019). Previous studies on the exposure of fish to certain fibrates such as clofibrate or GF showed the capacity to inhibit GST on liver tissues (Nunes et al., 2008; Falfushynska et al., 2022), where pharmaceuticals as CIT or BEZ were primarily metabolized by hepatic enzymes. Thus, our results on the lack of significant differences in GST activity seem to indicate a lack of capacity of the gills to metabolize and detoxify these compounds.

After the depuration period, the antioxidant activity levels (GST, GPX, CAT and GR) in the gills of mussels exposed to CIT and PECIT were similar between treatments and the control, evidencing the mussels' recovery capacity after exposure. Indeed, MDA levels were significantly lower than the control in organisms exposed to CIT. However, the CIT treatment exhibited significantly higher GPx activity compared to PECIT, despite both treatments showed an equal decrease in CIT concentrations in the whole soft tissues (Castaño-Ortiz et al., 2023b). Conversely, during the BEZ depuration period, GST, GPx, and MDA levels decreased significantly compared to the control (Table 3S). It is understood that the behaviour of some enzymes follows a bell-shaped curve. Initially, the increase in antioxidant enzymes indicated a balance in self-defense mechanisms in organisms, while subsequent inhibition could be linked to sustained oxidative stress throughout the exposure period, leading to the deactivation of enzymatic mechanisms and a decrease in their responses compared to the control. The observed inhibition suggested these antioxidant enzymatic activities must follow that pattern.

Our findings indicated that treatments containing PE, CIT, and BEZ were capable of inhibiting AChE activity in gills. However, this inhibition for PE, CIT and BEZ was only evident after the depuration period. Surprisingly, these inhibitions were observed when CIT concentrations in tissues decreased by approximately 70% compared to the values detected at the end of exposure and when the low concentration of BEZ was completely removed from the organisms after depuration period. As it is well-known, AChE inhibition is commonly used as a biomarker for detecting and measuring the neurotoxic potential of pollutants (Galgani and Bocquene, 1989; Bocquené et al., 1990; Lehtonen et al., 2003), as well as microplastics (Ribeiro et al., 2017; Oliveira et al., 2013). Regarding antidepressants, our results demonstrated that CIT, like other SSRIs in bivalves, could inhibit AChE activity in gills after a long exposure period (Gonzalez-Rey and Bebianno, 2013; Cortez et al., 2019; Munari et al., 2014; Franzellitti et al., 2014). However, this effect may vary among different pharmaceuticals in bivalves. For example, AChE activity significantly increased after ibuprofen exposure, in a similar way to the effect detected in this work for PEBEZ after 10 days, which some authors associated with inflamed tissues (Rodrigues et al., 2022). The inhibition of AChE can also impact on physiological processes involving the gills. In marine invertebrates, the movement of water through cilia is crucial for respiratory function and food intake. This movement is regulated by neurotransmitters such as acetylcholine, dopamine, and serotonin (Cooper and Bidwell, 2006). Consequently,

AChE inhibition after the depuration period can lead to notable physiological effects, such as a decrease in feeding efficiency observed in bivalves (Campillo et al., 2013).

4.3. Effects on digestive gland

The biological resistance to toxicity in different forms of ROS in the digestive gland was evaluated by the TOSC, based on the capacity of the cellular antioxidants to reduce the oxidation of α-keto-γ−methiolbutyric acid (KMBA) in the presence of artificially generated oxyradicals (Regoli, 2000). Our results showed that organisms short term (10-d) exposed to CIT, PECIT and BEZ appeared to be more susceptible to provoke oxidative stress with a significantly lower capability to neutralize the hydroxyl radicals. The co-exposure to PE and BEZ decreased TOSC-OH•, but at the end of the depuration period. The presence of PE decreased the response to BEZ, as the treatment with PE alone did not affect TOSC values during exposure or depuration. In contrast, the response toward peroxyl (ROO•) showed a different pattern, with a higher capacity to neutralize ROS in CIT-exposed mussels (CIT and PECIT). Previous studies (Mezzelani, 2021, 2023) with PhACs such as carbamazepine or valsartan did not showed effects in TOSC response on exposed mussels.

A significant oxidative stress in the digestive gland was observed after the exposure to PE alone (10 and 21 days). This was evidenced by the increased activity levels of CAT, GPX, and LPO, as well as elevated transcript levels of the genes *cat*, *gst-pi*, *acox1*, and *abcb1*. These results agree with previous studies demonstrating that MPs can induce oxidative stress in bivalves and their oxidative defenses (Li et al., 2022a).

In contrast to this oxidative capacity of PE, our findings did not show effects of CIT, BEZ, and PECIT treatments on CAT, GST, and GPx enzymatic activities, which were similar to those observed in the control mussels. Therefore, the co-exposure to PE with CIT appeared to alters the antioxidant effects observed for this MP (Table 4S). Thus, diverse responses have been reported in bivalves following exposure to hypolipidemic drugs, affecting their redox balance and peroxisomal function. For instance, Canesi et al. (2007) noted increased levels of GST and CAT activities in the digestive gland after BEZ and GEM exposure. However, in agreement with our findings, other pharmaceuticals, such as FF, did not alter GST and SOD in the soft tissues of *M. galloprovincialis* (Russo et al., 2023), and GST and GPx activities remained unaffected following FLX exposure in the digestive gland of the bivalve *Perna perna* (Cortez et al., 2019).

Despite the absence of significant variations observed at catalytic level in terms of GST and GPx activities in the digestive gland after PhAC exposures, remarkable transcriptional changes were observed for *gst-pi* and *Se-gpx* confirming the typical higher sensitivity of transcriptional responses which, however, it does not necessarily correspond to functional changes.

During the exposure period to CIT and PECIT, a significant increase in mRNA levels of *gst-pi* was observed for all treatments compared to controls. This trend persisted even after the depuration period. On the other hand, the gene expression of *gst-pi* and *Se-gpx* showed an up-regulation after 10 days of BEZ exposure. The same trend appeared for CAT, although should be noted that its increase was not statistically significant compared to the control (Table 4S). Furthermore, as it has already been indicated, these PhACs had the capacity to modify the biomarker responses observed after PE exposure in the digestive gland of the mussels. Indeed, the co-exposure of PE with CIT did not result in an increase of *cat, acox1* and *abcb1* genes, while BEZ co-exposure only showed a strong increase in the expression of *cat*. It is necessary to emphasise that observed effects of BEZ where provoked with very low bioaccumulation levels (300–400 fold lower than for CIT), which should be consequence of the interaction of this contaminant (present in haemolymph) with the organism tissues, without high concentration of BEZ on them.

The significant induction of *Se-gpx, cat* and *gst-pi* genes suggested that organisms exposed to BEZ regulated the excess of ROS production, although this contaminant was mainly present in seawater and in the mussel haemolymph due to is acidic nature which can limit its bioaccumulation, and activate the phase II for the biotransformation of these compounds and elimination of products generated by this radical excess in the cells. Thus, GSTs can reduce lipid hydroperoxides, through their GPx activity, and can also detoxify LPO end-products such as 4HNE. Therefore, detoxification process is crucial, especially considering the lipid peroxidation detected after exposure to PE or BEZ.

The alteration in *gst-pi* expression further suggested the involvement of this enzyme in detoxification and transformation processes of CIT and BEZ within mussel cells. CIT was accumulated and also degraded along the experiment (as preliminary tests confirmed), together with the increase of *gst-pi* expression, evidencing their role in the metabolism and elimination of CIT. Surprisingly, this response was also relevant for BEZ, considering its low bioaccumulation (Castaño-Ortiz et al., 2023b). In contrast, the co-exposure in the PEBEZ showed similar *gst-pi* expression levels than the control group, suggesting the reduction of this alteration in the presence of PE, due to the response differed from the increase expression observed in the individual treatments. Thus, our results agree with the responses reported by Contardo-Jara et al. (2011) in mussels exposed to BEZ for 7 days, where the exposure to 0.36 μg L^{-1} BEZ provoked an increase in *gst-pi* transcript levels of the digestive gland, preceded by up-regulated *p-gp* and followed by up-regulated *hsp70* mRNA levels. Other studies also confirmed the induction of GST in digestive gland and gills from bivalves exposed to FLX (Chen et al., 2015).

ACOXs is another enzyme studied in this work due to its key role in maintaining redox and lipid homeostasis in bivalve (Li et al., 2022b). Peroxisomal acyl-coenzyme A oxidases (ACOXs) exhibited a significant increase in expression compared to the control following PE exposure (21 d) and after 10 days of exposure to BEZ (Table 4S). *Acox1*, the first rate-limiting enzyme catalyzing the initial step of the β-oxidation system in the peroxisome, has been shown to participate in the inflammatory response to oxidative stress induced by environmental xenobiotics (Gu et al., 2021; Mi et al., 2018; Wu et al., 2017). Several studies have suggested that these genes may be vital in molluscan metabolism and defense systems, thereby contributing to adaptation to the stressful marine environment (Li et al., 2022b). The upregulation of *acox1* implies an overproduction of hydrogen peroxide in the cells, as hydrogen peroxide is a by-product of β-oxidation of fatty acids (Lismont et al., 2019). The up-regulation of ACOXs could be associated with the up-regulated *cat* gene observed in PE exposure since, as mentioned earlier, CAT is the best-characterized enzyme for H_2O_2 peroxisomal elimination (Lismont et al., 2019). These results disagree with the findings of Canesi et al. (2007), where BEZ decreased the activity of palmitoyl CoA oxidase, while GEM was ineffective.

P-glycoprotein (*P-gp*) increased its expression after PE exposure, during the depuration period of CIT, and after BEZ exposure, although in the latter case was no significantly higher than control (Table 4S). However, this gene expression was not affected in the co-exposure of PE with these PhACs. This crucial component of the Multixenobiotic Resistance System (MXR) acts as an active barrier against harmful xenobiotics and facilitates metabolite detoxification (Franzellitti et al., 2017). MXR is mediated by ATP-dependent ABC transporters located on cell membranes and internal organelle membranes, which actively remove both endogenous chemicals and xenobiotics from cells, thereby preventing their accumulation and toxic effects (Bard, 2000). The induction in bivalves could be a response to a wide range of biotic and abiotic stressors (Buratti et al., 2013; Fu et al., 2019; Minier et al., 2000), suggesting that these transporters may constitute a general and broad-spectrum cell protective mechanism that plays a vital role in their acclimatization, response to environmental stress, and immune defences (), (Franzellitti et al., 2019, 2020). In this study, the strong induction observed for the *abcb1* gene after PE exposure was consistent with previous results published by Franzellitti et al., (2019) and suggested its

role as a defensive response following microplastic ingestion. Besides, the high levels of *P-gp* mRNA in mussels exposed to BEZ for 10 days, and after depuration of CIT exposure, may indicate a high capacity to transport and removal these substances, decreasing their toxic effects on the cells. Thus, our results confirmed *that P-gp* played an important role in the response to this type of PhACs, characterized by an oxidative stress and increase of the Phase II biotransformation enzymes.

The concentrations of CIT in mussels at the end of the exposure period reached mean concentrations of 757 ± 155 ng/g dw and 701 ± 123 ng/g dw (Castaño-Ortiz et al., 2023b), however, BEZ concentrations were considerably lower (mean levels below quantification limit <2.7 ng/g dw) than citalopram ones. The occurrence of both PhACs was also confirmed in haemolymph, evidencing the interaction of the mussel tissues with both contaminants, although no significant bioaccumulation was observed for BEZ. These interactions are enough to provoke adverse biological effects for both PhACs, as it was also confirmed in previous metabolomic studies (Castaño-Ortiz et al., 2023b). The metabolic profiles showed effects of both PhACs, increased in some cases with PE co-exposure, and also for the PE exposure alone.

After the depuration period of 7 days, the metabolism and elimination of CIT was confirmed, with concentrations decreasing from 700 ng/g to 170 ng/g after the depuration period (Castaño-Ortiz et al., 2023b). The concentration of BEZ was lower than 3 ng/g during exposure time and it was below detection limit from the organisms after depuration period. Concerning biological effects, most of the responses measured in mussels exposed to PE and PECIT did not show differences with CTRL. However, not all the biological responses returned to the levels existing in the controls, and persisted after this period in some of these treatments. Thus, the expression of the *gst-pi* gene was significantly higher for the treatments with CIT and BEZ. In the former case, the levels of *P-gp* were also significantly higher than the controls. These data may indicate the presence of the parent compound or metabolites capable of damaging the cells, which are metabolized and eliminated. For BEZ, despite a limited bioaccumulation during the exposure, and not detected in the tissues after clearance period, the expression of the GST gene seems to be related to certain interactions with tissues without its relevant accumulation.

Despite the fact that the accumulation detected for both pharmaceuticals was very different (with two orders of magnitude of difference), alterations in biological biomarkers continued to be observed even when tissue concentrations decreased considerably for CIT or were undetectable for BEZ. Thus, these results suggest the possibility that not only substances with a high bioaccumulation capacity are the responsible of adverse effects detected in marine organisms from polluted ecosystems.

It is important to note that the concentrations of BEZ measured in the exposed organisms were similar to those reported in previous literature for marine organisms (3–5 ng/g) (Nödler et al., 2014; Mello et al., 2022; Castaño-Ortiz et al., 2023a). Therefore, the effects observed in this experiment may be representative of that produced in coastal ecosystems.

5. Conclusions

The potential of CIT and BEZ to induce significant changes related to oxidative stress, biotransformation, elimination and neurotoxicity in gills and/or digestive gland from marine mussels was confirmed. These effects were observable during a period of exposure to environmental concentrations present in coastal marine systems and, even days after the cessation of exposure, certain biomarkers, such as those related to the detoxification system or neurotoxicity, remained affected. Specifically, regarding neurotoxicity, an evident delayed effect was observed after the depuration period for both dissolved pharmaceutical treatments.

In the case of CIT, the biological effects were related to the significant bioaccumulation of the antidepressant in mussel tissues. However, a notable finding from the results of this study was that the effects were observed in the organisms regardless of the bioaccumulation of the pharmaceuticals. For bezafibrate, the bioaccumulation levels were much lower, similar to those found in organisms in the marine environment (3–5 ng/g), and were not detected after the depuration period. Nevertheless, the detoxification systems were induced in both cases. Therefore, these results demonstrate that PhACs may pose a significant risk to the health of marine organisms, and some of these effects could have important consequences for organism populations after long-term exposure. For example, the detected decrease in AChE activity could significantly affect feeding, while the increased energy demand required to activate antioxidant detoxification mechanisms could reduce the energy available for growth.

It is important to consider that the effects of PhACs on organisms will depend on various environmental factors, such as the MPs presence. MPs were able to bioaccumulate in the gills and digestive gland, triggering significant molecular responses in both tissues. Furthermore, their co-exposure with pharmaceuticals was able to modify some of the biological responses caused by CIT and BEZ, both during exposure and after the depuration period.

Therefore, these results highlight the importance of considering the environmental presence of contaminant mixtures in the assessment of environmental risks in marine ecosystems. In this case, the presence of MPs in the marine environment should be considered when using these biomarkers to evaluate the biological effects of these CECs, due to their interactions and wide environmental distribution.

CRediT authorship contribution statement

M.M. García-Pimentel: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **M. Mezzelani:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis. **N.J. Valdés:** Writing – review & editing, Methodology, Formal analysis. **M.E. Giuliani:** Writing – review & editing, Methodology, Formal analysis. **S. Gorbi:** Writing – review & editing, Validation, Supervision, Methodology. **F. Regoli:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization. **V.M. León:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **J.A. Campillo:** Writing – review & editing, Validation, Supervision, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maria del Mar Garcia-Pimentel reports financial support was provided by Spanish Institute of Oceanography. Maria del Mar Garcia-Pimentel reports a relationship with Spanish Institute of Oceanography that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.125508.

Data availability

Data will be made available on request.

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