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Polyethylene microplastics as a vector of tributyltin in *Mytilus galloprovincialis* and their consequences

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ABSTRACT

Microplastic pollution is an increasing environmental issue in the marine environment due to its ubiquitous presence and its role as a vector for organic pollutants such as tributyltin (TBT). Adsorption of TBT onto microplastics (MP) can increase its availability to filter-feeding organisms by prolonging its retention time in the water column. We investigated whether polyethylene (PE) MP spheres (10–63 μm) could act as vectors for TBT transfer in the mussel *Mytilus galloprovincialis* by exposing it to PE MP (50 mg L^{-1}) and TBT (0.001 mg L^{-1}) separately and in co-exposure for 21 days. Efficient adsorption on PE MP was confirmed, and higher concentration of TBT was observed in the PE MP + TBT group compared to TBT only exposed group, indicating a vector effect. This led to higher toxicity in mussels, as the combined stress conditions in the PE MP + TBT group resulted in a significant decrease in the catalase activity after 14 days of the exposure. Higher ETS activity was observed in both exposed groups after seven days of exposure, followed by lower ETS activity at 14 and 21 days, indicating depletion of energy reserves. Pronounced responses in catalase and electron transport system (ETS) activity were observed in the hepatopancreas compared to the gill tissue. DNA damage was present in all exposed groups and increased over time.

1. Introduction

Tributyltin (TBT) is considered the most hazardous anthropogenic chemical released in large quantities into the environment over the past 50 years (Beyer et al., 2022). Despite the ban on TBT use in antifouling paints for ships, new TBT inputs are still reported worldwide (Furdek Turk et al., 2020; Mikac et al., 2022; Mil-Homens et al., 2023) due to illegal manufacture and sale in the United States, Caribbean and South and Central America (Uc-Peraza et al., 2022). In addition to its historical use, TBT is employed as an additive in the production of plastics mainly polyvinyl chloride (PVC) for thermo-stabilisation and as an anti-yellowing agent (Fauser et al., 2022). The inputs in marine waters due to leaching from sewage treatment plants are low, compared to the leaching of up to 600 g per day of organotin from freshly painted ships with the antifouling paints containing TBT (Kucklick and Ellisor, 2019). Sediments act as a main long-term reservoir, as TBT once adsorbed onto sediments, can persist in the environment for more than 40 years making contaminated sediments a long-term secondary source of water contamination unless active measures are taken to remediate the sediments. Hotspots in Europe include the Norwegian coastline (Beyer et al., 2022) and Adriatic coastal areas, with concentrations in the Croatian transitional seawaters reaching up to 2546 ng TBT g^{-1} d.w. in sediments and 655 ng TBT g^{-1} d.w. in mussels (Furdek Turk et al., 2024). Sediment-bound TBT can be remobilised back into seawater following resuspension during storms and dredging in ports (Roberts, 2012).

Pollution with microplastics (MPs) and nanoplastics (NPs) is a global issue, as pollutants adsorb onto plastic surfaces and transport other contaminants (Sendra et al., 2021). The most common MP in the marine

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environment is polyethylene (PE) (de Haan et al., 2019; Zhao et al., 2025), which is widely used as food packaging material and has a density between 0.88 and 0.97 g cm^{-3} (ISO, Plastics, 2020). The increasing pollution of the marine environment with MPs and NPs provides a substrate for the adsorption of legacy and emerging pollutants, and this adsorption can alter their fate in the environment. Due to its physicochemical properties, TBT can adsorb onto the surface of polypropylene (PP), polyethylene (PE), polystyrene (PS), and poly (methyl methacrylate) (PMMA) particles. Studies of the adsorption and photodegradation of TBT on different MPs have highlighted the importance of light absorption and free radical generation on microplastic surfaces, which depends on the microplastic polymers. PE and PS are rubber-like plastics with greater porosity, which may facilitate the diffusion of sorbates into the inner surfaces due to lower UV absorption (Chen et al., 2020). Recent studies have shown, that MPs and NPs can act as vectors for organotin compounds with NPs showing higher adsorption capacities and a potential for increasing pollutant bioavailability in marine environments (Chen et al., 2020; Raymond et al., 2024). PS NPs (40, 480 and 760 nm) have been used to study adsorption of TBT in the presence of the competitive salts under a range of salinity gradient. The adsorption of TBT on NPs and MPs can prolong its residence time in the water column due to low density of plastic particles, increasing its bioavailability to biota (Raymond et al., 2024). An extensive search of public repositories for data on adverse outcomes of MPs and NPs with adsorbed TBT and their ecological relevance yielded no results except for a recent studies on the NPs adsorption and degradation process of TBT on different plastic polymers (Chen et al., 2020; Raymond et al., 2024).

The effects of exposure to TBT alone are well documented and include reduction of electron transport system (ETS) activity (Nesci et al., 2011), impairment of gene regulation (Toporova and Balaguer, 2020; Capitão et al., 2021), and endocrine disruption (Parmentier et al., 2019), resulting in impaired reproduction and growth (Harrison et al., 2020). Microplastics alone, have been shown to induce oxidative stress, apoptosis, histopathological changes, and disruption of energy metabolism in mussels (Shang et al., 2021; Mai et al., 2023). Despite the well-known detrimental effects of TBT on biota, little is known about the behaviour of TBT and MPs together in marine organisms.

The aim of this study was to investigate the harmful effects of TBT adsorbed onto PE microspheres (10 to 63 μm) in a controlled experiment on the Mediterranean mussel (*Mytilus galloprovincialis*). Mussels were exposed to nominally equal concentrations of pollutants: TBT-Cl + PE MP (50 mg L^{-1} PE MP + TBT-Cl; 0.02 mg TBT per gram of PE MP), 50 mg PE MP, and 0.001 mg TBT-Cl per litre. The exposure experiment with mussels lasted 21 days, with sampling for biomarker analysis at time points 0, 7, 14, and 21 days. The effects of PE MPs and TBT were assessed by measuring electron transport system activity (ETS), as determined by the 2-(*p*-iodophenyl)-3-(nitrophenyl)-5-phenyltetrazolium chloride (INT) reduction capacity assay, oxidative stress was evaluated by catalase activity in gill and hepatopancreatic cells, and genotoxic effects on haemocytes. We hypothesise that PE MPs with adsorbed TBT induce measurable biological responses in mussels after ingestion, with TBT as the primary driver of toxicity. MPs can act as vectors and modulators due to the shielding effect, that can affect TBT bioavailability and biological responses in mussels.

2. Materials and methods

2.1. TBT adsorption on PE MP

0.2 mL of 4.8 mg TBT-Cl mL^{-1} methanol (96% purity, Cat. No. T50202, Sigma-Aldrich) was diluted in methanol to a working concentration of 0.9 mg TBT-Cl per mL methanol and used to adsorb onto 20 g PE MP particles size from 10 to 63 μm (Cospheric, CPMS-0.96 10–63 μm, 10 g, REF: 210817) in a clean borosilicate glass container, ensuring the microspheres were fully immersed. The mixture was covered, and mixed for 24 h to facilitate adsorption, then dried for 48 h to evaporate the remaining methanol and ensure that only adsorbed TBT-Cl remained on the microplastic. The PE microspheres with adsorbed TBT-Cl were stored at 4 °C until further use.

2.2. TBT quantification on microplastic

PE MP spiked with TBT-Cl and pure PE MP were quantified for TBT content using the extraction method for organotin compounds (OTC) from sediments (Ščančar et al., 2007). Briefly, 0.25 g of MP was mixed with 20 mL of glacial acetic acid and shaken for 15 h. After centrifugation, an aliquot of the supernatant was transferred to 20 mL of acetic buffer solution. A 2% solution of sodium tetraethyl borate ($NaBEt_4$) was then added as the ethylating agent. TBT was extracted into isooctane, and the purified organic phase was analysed using GC-ICP-MS (Agilent 6890 GC and Agilent Technologies 7700x ICP-MS). Blank samples were prepared using the same procedure. Quantification was performed in three replicates by the standard addition method, with tripropyltin (TPrT) added as an internal standard before extraction. The measured concentration of adsorbed TBT was 0.02 mg per gram of PE MP. Calculated adsorption efficiency as the proportion of the initially added TBT that was adsorbed onto PE MP was 50 to 60%.

2.3. Chemical analysis of TBT in mussels

TBT was extracted from the mussel tissue following the procedure described in Bajt et al. (2024). Approximately 4 g of fresh mussel tissue by adding 20 mL of 0.1 M HCl in methanol. The mixture was stirred on an elliptical shaker followed by ultrasonic-assisted extraction for 2 h at 50 °C. The resulting suspension was centrifuged at 4500 rpm for 10 min. For derivatization of the extracted OTC 2% $NaBEt_4$ was used and the ethylated species were extracted into isooctane. After separation of the aqueous and organic phases, the latter was added to the GC-ICP-MS (Agilent 6890 GC and Agilent Technologies 7700x ICP-MS). The same procedure, but without the mussel sample, was used to determine blank values. OTC was quantified using the standard addition calibration method with TPrT as an internal standard added to the samples prior to extraction.

2.4. Exposure of mussels to PE MP and TBT-Cl

Mediterranean mussels were collected in May 2024 from the mussel farm located at a pristine area in the Bay of Piran, Slovenia. The epibiota was removed from the mussel shells, and mussels in size from 5 to 7 cm with intact byssus threads, were placed in aquaria with a natural seawater flow for 21 days for acclimatisation. During the experiment, the mussels were kept in aquaria with 0.5 L of filtered natural seawater (0.038 mm plankton sieve) per animal at a constant temperature of 20 °C in a semi-static system with a 12/12-hour day/night regime. Mussels were randomly divided into four test groups: (1) control, (2) PE MP, (3) TBT-Cl, and (4) PE MP + TBT-Cl. The final exposure concentrations were 50 mg L^{-1} PE MP, 0.001 mg L^{-1} TBT-Cl, and 50 mg L^{-1} PE MP + TBT-Cl (0.02 mg TBT per g of PE MP). A higher PE MP concentration than typically observed in the water column (Walton et al., 2025) was used to simulate extreme scenarios of sediment resuspension under environmental conditions in the shallow waters of the Northern Adriatic, where storms, strong winds and cargo vessels in ports cause sediment mixing. TBT concentration used was environmentally relevant to reflect realistic exposure levels (Zapata-Restrepio et al., 2023). Mussels were fed daily with the commercial feed Aquaforest Phyto Mix (5 μL L^{-1} seawater), and the seawater was changed twice a week. No mussel mortality was observed during the exposure. Ammonia, oxygen concentration and pH were measured daily in the aquaria to ensure suitable conditions. The pollutants were added to the aquaria immediately after the seawater change to maintain a consistent pollutant concentration during the experiment. Water circulation within the aquaria was

maintained using submersed pumps, ensuring homogeneous distribution of pollutants. Mussels were sampled for biomarker analysis at time points 0, 7, 14, and 21 days. The following endpoints were measured: electron transport system (ETS) activity using the INT reduction capacity assay, genotoxicity using the comet assay, and oxidative stress response via catalase (EC 1.11.1.6) activity.

2.5. Electron transport system activity

Electron transport system activity was measured by the INT reduction capacity assay using the spectrophotometric method (Erk et al., 2011; Mijošek Pavin et al., 2026). The method is widely used for measuring mitochondrial capacity in *Mytilus* species under a variety of exposure scenarios (Bielen et al., 2016; Janardhanan et al., 2025; Wu et al., 2025; Giannessi et al., 2026). Ten individuals per test group were randomly sampled at each time point (0, 7, 14 and 21 days of exposure). Gills and hepatopancreas were excised and separately placed in a homogenisation buffer (0.1 M Na_2HPO_4, 0.1 M KH_2PO_4, 0.05 M Tris Base, 75 μM $MgSO_4$, 1.5 mg mL^{-1} PVP, 0.2% Triton-X (pH 8.4) at a ratio of 1:8 (tissue wet mass: buffer) and homogenised using a Potter-Elvehjem homogeniser with 10 strokes, followed by sonication for 20 s (Ultrasonic Homogeniser 4710, Cole–Parmer, USA). The homogenates were centrifuged at 10,000 rpm for 5 min at 4 °C and the supernatant was stored at −80 °C until further analysis.

INT reduction capacity was measured in three replicates of 30 μL homogenate incubated with 150 μL substrate solution (0.1 M Na_2HPO_4, 0.1 M KH_2PO_4, 0.05 M Tris Base, 0.2% Triton-X, 1.7 mM NADH (Sigma Aldrich, CAT No. N8129), 0.25 mM NADPH (EMD Millipore Corp., CAT No. 481973), pH 8.4) and 50 μL INT solution (2.5 mM 2-(*p*-iodophenyl)-3-(nitrophenyl)-5-phenyltetrazolium chloride (Sigma, CAT No. 58030). The blank was prepared in the same way as the tissue replicates with homogenisation buffer added instead of homogenate. Formazan production was measured continuously every 25 s for 5 min at 490 nm (Spark Tecan, Austria). The measured oxygen consumption was converted to energy equivalents using the oxygen equivalents for an average lipid, protein and carbohydrate mixture (484 kJ mol^{-1} O_2) (Gnaiger, 1983) and ETS activity was calculated (Verslycke et al., 2004).

2.6. Catalase activity

Catalase activity was measured in protein extracts from the hepatopancreas and gills using spectrophotometric method (Aebi, 1984). The reaction mixture for hepatopancreas samples consisted of 20 μL of supernatant and 1980 μL of 50 mM potassium phosphate buffer (pH 7,0) and 40 μL of supernatant and 1960 μL of 50 mM potassium phosphate buffer with 10.2 mmol L^{-1} H_2O_2 (pH 7,0). A blank reaction was performed by measuring 2000 μL of 50 mM potassium phosphate buffer and homogenisation buffer instead of tissue homogenate. The absorbance was measured at 240 nm in a 2 mL quartz cuvette for 2 min at 25 °C. Specific CAT activity was expressed as μmoles of degraded hydrogen peroxide per min^{-1} mg^{-1} $protein^{-1}$ (ε_{240} = 43.6 L^{-1} mol^{-1} cm^{-1} for H_2O_2).

Total protein content was determined using the Pierce™ BCA Protein Assay (Thermo Scientific). Ten microliters of homogenate (tissue wet mass:buffer ratio 1:3) were mixed with 90 μL of Pierce reagents A and B (v:v ratio 50:1) in a well. The mixture was incubated for 30 min at 37 °C in the dark and absorbance was measured at 550 nm. Samples were measured in duplicate, and the calibration curve was prepared using BSA standards and protein content was calculated from the calibration curve.

2.7. Comet assay

Haemolymph was collected from 10 animals in each experimental group after 7, 14, and 21 days of exposure for the comet assay. Haemolymph withdrawal was made by a 5 mL syringe fitted with a 21G hypodermic needle. To prevent haemocyte clogging, the syringes contained 200 μL of physiological saline (0.5 M NaCl, 12.5 mM KCl, 5.5 mM EDTA, 20 mM HEPES, pH 7.4). The cell suspensions were then used for cell viability scoring and genotoxicity assessments.

Haemocyte viability in each mussel was assessed using differential staining (acridine orange and ethidium bromide; 100 μg of each dye per mL). Viability was determined from observing 100 haemocytes per sample, green-stained cells were classified as viable, while orange-stained cells were considered nonviable (Kolarević et al., 2016).

The comet assay was used to assess genotoxic potential following exposure to TBT-Cl, PE MP and PE MP + TBT-Cl in the experimental groups at the same time points. A high-throughput alkaline comet assay using minigels was performed, based on a modified protocol of Singh et al. (1988). Microscope slides were precoated with 1% normal melting point agarose (NMP). The second layer consisted of 10 μL of a cell suspension in 1% low melting point agarose (LMP). The cell suspension was prepared from 30 μL of haemocytes in physiological saline and 70 μL of LMP. Five specimens on triplicate were loaded onto each slide, resulting in 15 minigels per slide. After gel solidification, the slides were placed in cold lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, pH 10) for 24 h at 4 °C. The following day, slides were transferred to an electrophoretic chamber filled with electrophoretic buffer (300 mM NaOH, 1 mM EDTA, pH 13) and denaturation was carried out for 20 min, followed by electrophoresis for 20 min at 0.75 V per cm and 300 mA. After electrophoresis, the slides were immersed in cold neutralising buffer (0.4 M Tris, pH 7.5) for 15 min, then fixed in cold ethanol for 10 min and dried in the dark overnight. Staining was performed using 20 μL of GelGreen (Biotium) at a concentration of 1 μL mL^{-1}. The slides were examined under a fluorescence microscope (Leica DM4 B, Austria) at 400× magnification, with an excitation filter at 450 to 490 nm and a barrier filter of 510 nm. In each minigel, 50 nuclei were scored using Comet IV software (Instem, USA), and the percentage of DNA in the tail (TI %) was used as an indicator of DNA damage.

2.8. Statistical analyses

The data obtained in the experiments were statistically analysed using R (version 4.3.3, R Core Team, 2024) software. The Shapiro-Wilk test was used to assess the normality of the data distribution for each treatment and each combination of time points. As the normality assumption was not met in most groups, non-parametric tests were used for subsequent analyses. Differences between treatments within each time point and between timepoints within treatments were tested using the Kruskal–Wallis test, pairwise comparisons were performed using the Wilcoxon rank sum test with Bonferroni correction for multiple comparisons when statistically significant differences were found ($p < 0.05$). For viability and tail intensity Dunn-type rank comparisons with *p*-values adjusted by the Bonferroni correction for multiple tests were used to assess statistical differences between groups. Statistical significance was indicated as follows $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, and $^{****}p \leq 0.0001$.

3. Results

3.1. Chemical analysis of TBT in mussels

TBT and DBT (dibutyltin, a degradation product of TBT) were measured in mussel tissue during experimental exposure to TBT-Cl, PE MP and PE MP + TBT-Cl on sampling days 7, 14 and 21 using GC-ICP-MS. The nominal concentration of added TBT-Cl in the aquaria was 0.001 mg L^{-1}. Mussel tissue from the control and PE MP group did not contain detectable concentrations of TBT or DBT (see Fig. 1). Measured concentrations of TBT/DBT in the group exposed only to TBT-Cl ranged from 25.7 μg kg^{-1} d.w. TBT/13.3 μg kg^{-1} d.w. DBT at day 7 to 83.3 μg kg^{-1} d.w. TBT / 43.1 μg kg^{-1} d.w. DBT at day 21. TBT concentrations were 13 (day 14; 1073,5 μg kg^{-1} d.w. TBT) to 25-fold (day 7; 644,3 μg

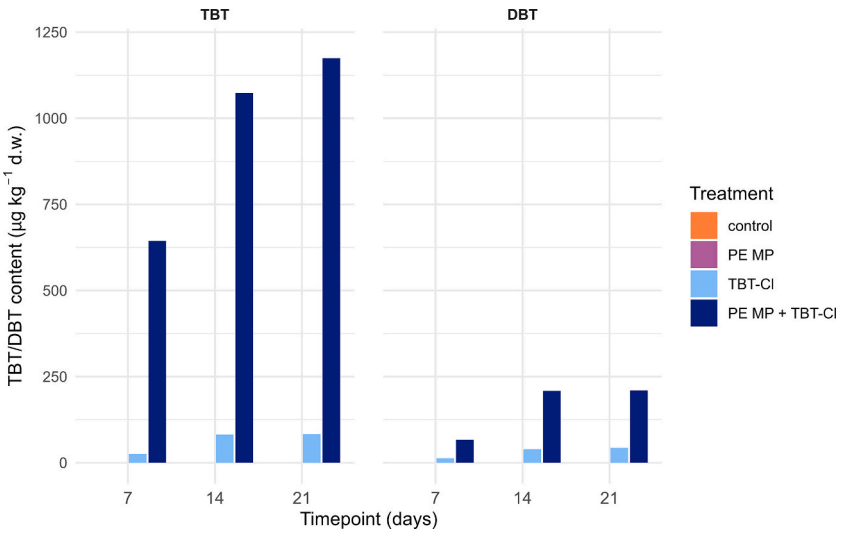

Fig. 1. TBT and DBT concentrations in mussels in aquaria of experimental groups: control (orange), PE MP (50 mg L^{-1}, violet), TBT-Cl (0.001 mg L^{-1}, light blue) and PE MP + TBT-Cl (50 mg L^{-1}; 0.02 mg TBT-Cl g^{-1} PE MP, dark blue) on days 7, 14 and 21. Control and PE MP groups did not contain detectable concentrations of TBT or DBT. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

kg-1 d.w. TBT) higher in PE MP with adsorbed TBT treated group compared to the aqueous TBT treatment. Difference in DBT concentrations in mussel tissue was less pronounced compared to TBT, with DBT levels in the PE MP + TBT group approximately five fold higher compared to TBT only treated group. TBT/DBT concentration in this group was in range of 644.3 µg kg^{-1} d.w. TBT/ 66.8 µg kg^{-1} d.w. DBT at day 7 to 1174.4 µg kg^{-1} d.w. TBT/209.8 µg kg^{-1} d.w. DBT at day 21.

3.2. Catalase activity

Fig. 2A shows the specific activity of catalase expressed in µmol min^{-1} mg^{-1} protein in the gills in response to different treatments (control, PE MP, TBT-Cl and PE MP + TBT-Cl) over four time points (0, 7, 14 and 21 days). The specific catalase activity in the gills ranged from 0.0710 to 0.513 µmol degraded H_2O_2 min^{-1} mg^{-1} protein. On day 7, a significant increase in catalase activity was observed in the TBT-Cl group (0.225 ± 0.107 µmol min^{-1} mg^{-1} protein) compared to the control (0.186 ± 0.03 µmol min^{-1} mg^{-1} protein). No significant differences in catalase activity were observed between the different treatments after 14 and 21 days of exposure nor within the exposure groups between different time points. On day 21, noticeable individual differences within the control (0.262 ± 0.138 µmol min^{-1} mg^{-1} protein) and PE MP

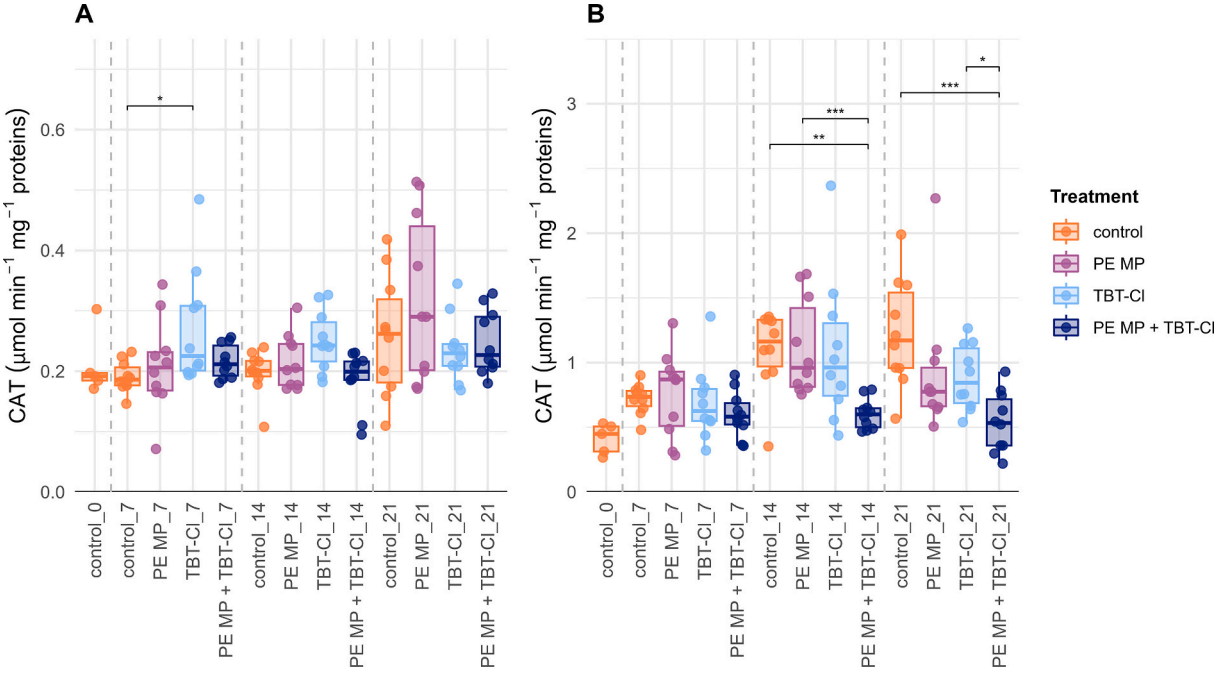

Fig. 2. A. Catalase activity in gills of experimental groups: control (orange), PE MP (50 mg L^{-1}, violet), TBT-Cl (0.001 mg L^{-1}, light blue) and PE MP + TBT-Cl (50 mg L^{-1}; 0.02 mg TBT-Cl g^{-1} PE MP, dark blue) on days 0, 7, 14 and 21. The sample size was $n = 10$ for all groups, except for the control at time point 0, where $n = 5$. B. Catalase activity in hepatopancreas of experimental groups: control (orange), PE MP (50 mg L^{-1}, violet), TBT-Cl (0.001 mg L^{-1}, light blue) and PE MP + TBT-Cl (50 mg L^{-1}; 0.02 mg TBT-Cl g^{-1} PE MP, dark blue) on days 0, 7, 14 and 21. The sample size was n = 10 for all groups, except for the control at time point 0, where n = 5. Statistical significance is indicated as follows *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, and ****$p \leq 0.0001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

group (0.29 ± 0.238 μmol min^{-1} mg^{-1} protein) were observed.

Fig. 2B shows the specific catalase activity (μmol degraded H_2O_2 min^{-1} mg^{-1} protein) in the hepatopancreas in response to different treatments (control, PE MP, TBT-Cl and PE MP + TBT-Cl) over the four time points (0, 7, 14 and 21 days). Catalase activity in the hepatopancreas control group ranged from 0.266 to 1.989 μmol min^{-1} mg^{-1} protein and was generally about ten times higher than catalase activity in the gills. No significant differences were observed between the groups after 7 days of exposure. On day 14, a significant decrease in catalase activity was observed in the PE MP + TBT-Cl group (0.600 ± 0.146 μmol min^{-1} mg^{-1} protein) compared to the control (1.163 ± 0.360 μmol min^{-1} mg^{-1} protein) and PE MP (0.960 ± 0.609 μmol min^{-1} mg^{-1} protein) groups in the hepatopancreas. Greater within-group variability was observed at the 21-day exposure time point compared to the previous time points. After 21 days of exposure catalase activity in the PE MP + TBT-Cl group (0.533 ± 0.358 μmol min^{-1} mg^{-1} protein) remained significantly lower compared to the control (1.172 ± 0.583 μmol min^{-1} mg^{-1} protein) and TBT-Cl only exposed group (0.844 ± 0.423 μmol min^{-1} mg^{-1} protein). No significant differences were observed within exposure groups over time.

3.3. Electron transport system activity

Fig. 3A shows the ETS activity (mJ mg^{-1} w.w. h^{-1}) in the gills in response to the different treatments (control, PE MP, TBT-Cl and PE MP + TBT-Cl) over four time points (0, 7, 14 and 21 days). ETS activity in the control group ranged from 1.394 to 26.717 mJ mg^{-1} w.w. h^{-1}. No significant differences in ETS activity were observed between the groups or over time in gill tissue. The variability of individual responses increased on day 14 in all groups, possibly due to energy allocation and different adjustments in metabolic strategies. As the observed variability is higher in the control group compared to the treated groups, it is likely of a natural origin and due to individual mussel differences, such as feeding activity or filtration rate. Unequal contaminant ingestion could be an additional reason in exposed groups, but since the observed variability was highest in the control group, it is unlikely the cause. A non-significant increase in ETS activity was observed after 14 days of exposure and on day 21, values decreased again. ETS activity in the TBT-Cl group ranged from 2.788 to 48.323 mJ mg^{-1} w.w. h^{-1} and in the MP PE + TBT group ranged from 4.646 to 37.171 mJ mg^{-1} w.w. h^{-1}, both higher than in the control and PE MP groups, suggesting an effect on energy metabolism by added TBT, but no significant differences were observed between the groups.

In the hepatopancreas (see Fig. 3B), ETS activity ranged from 4.414 to 27.878 mJ mg^{-1} w.w. h^{-1}, with the greatest variability in the control group. After 7 days of exposure ETS activity was significantly increased in TBT-Cl (16.843 ± 4.008 mJ mg^{-1} w.w. h^{-1}) and the PE MP + TBT-Cl (20.444 ± 3.717 mJ mg^{-1} w.w. h^{-1}) groups compared to the control (9.525 ± 3.891 mJ mg^{-1} w.w. h^{-1}) and PE MP (8.712 ± 6.447 mJ mg^{-1} w.w. h^{-1}) groups. On day 14, significantly higher ETS activity was observed in the PE MP + TBT-Cl group compared to the PE MP group. High variability in the control group (18.237 ± 8.015 mJ mg^{-1} w.w. h^{-1}) was observed in ETS hepatopancreas activity at the same timepoint, resulting in significantly higher ETS activity compared to the control groups at time points 0 (11.848 ± 3.020 mJ mg^{-1} w.w. h^{-1}), 7 (9.525 ± 3.891 mJ mg^{-1} w.w. h^{-1}) and 21 (9.757 ± 3.659 mJ mg^{-1} w.w. h^{-1}). After 14 days of exposure, the PE MP + TBT-Cl group (16.030 ± 2.846 mJ mg^{-1} w.w. h^{-1}) had significantly higher ETS activity than the PE MP group (10.919 ± 4.182 mJ mg^{-1} w.w. h^{-1}), while there was no significant difference between the two TBT-Cl treated groups. After 21 days of exposure, the TBT-Cl treated group (16.030 ± 2.265 mJ mg^{-1} w.w. h^{-1}) had a significantly higher ETS activity compared to the control group at the same time point. The PE MP (13.242 ± 5.634 mJ mg^{-1} w.w. h^{-1}) and PE MP + TBT-Cl (12.545 ± 6.156 mJ mg^{-1} w.w. h^{-1}) groups showed higher variability, but no significant differences were observed between the treated groups or compared to the control group for these two groups. No statistically significant differences were found between the pollutant exposed groups over time.

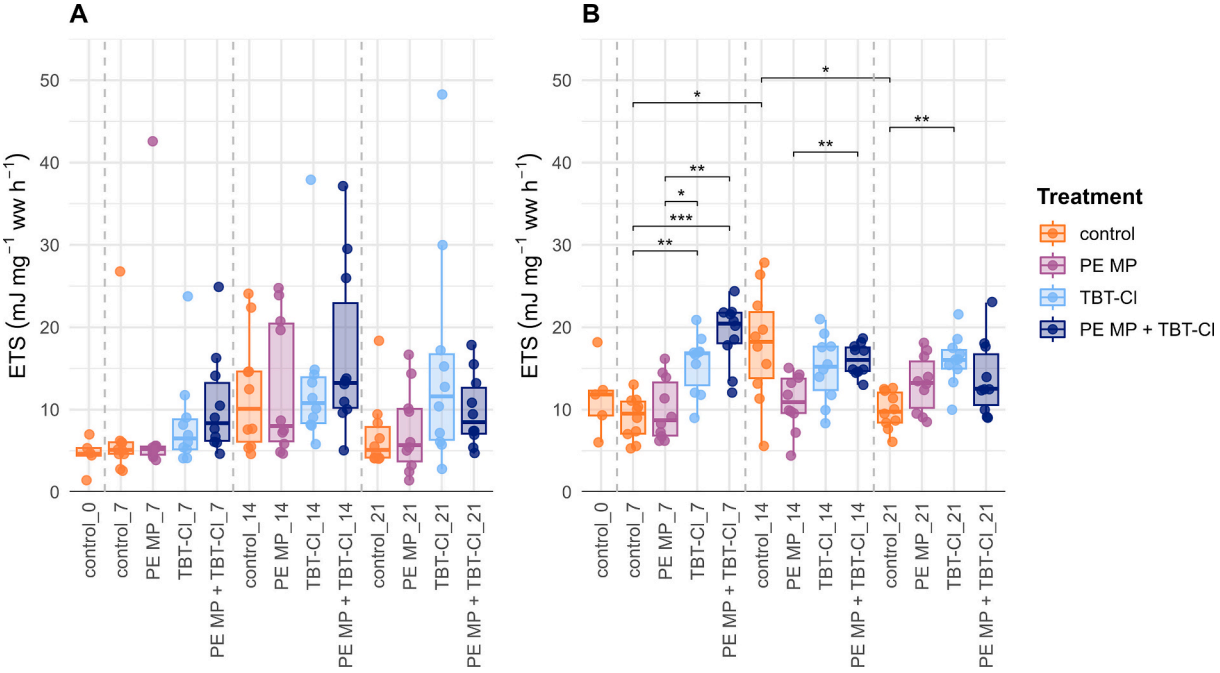

Fig. 3. A. ETS activity (mJ mg^{-1} ww h^{-1}) in gills of experimental groups PE MP (50 mg L^{-1}, violet), TBT-Cl (0.001 mg L^{-1}, light blue) and PE MP + TBT-Cl (50 mg L^{-1}; 0.02 mg TBT-Cl g^{-1} PE MP, dark blue) on days 0, 7, 14 and 21. The sample size was n = 10 for all groups, except for the control at time point 0, where n = 5. B. ETS activity (mJ mg^{-1} ww h^{-1}) in hepatopancreas of experimental groups: control (orange), PE MP (50 mg L^{-1}, violet), TBT-Cl (0.001 mg L^{-1}, light blue) and PE MP + TBT-Cl (50 mg L^{-1}; 0.02 mg TBT-Cl g^{-1} PE MP, dark blue) on days 0, 7, 14 and 21. The sample size was n = 10 for all groups, except for the control at time point 0, where n = 5. Statistical significance is indicated as follows *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, and ****p ≤ 0.0001. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. Comet assay

DNA integrity was analysed by comet assay in haemolymph cells and expressed as tail intensity (TI%). Fig. 4 shows TI% in mussels exposed to control conditions, PE MP, aqueous TBT-Cl, and PE MP + TBT-Cl after 7, 14, and 21 days of exposure. After 7 days TI% was significantly higher in the TBT-Cl exposed group (9.526 ± 27.8%) compared to all other groups. A statistically lower TI% was observed in the PE MP + TBT-Cl group (2.184 ± 8.106%) compared to all other groups. On day 14, TI% increased in both the TBT-Cl (11.566 ± 19.425%) and PE MP + TBT-Cl (11.733 ± 20.74) groups compared to the control (6.465 ± 12.69%) and PE MP (7.438 ± 19.302%) groups. On day 21, all three experimental groups, PE MP (12.514 ± 17.948%), TBT-Cl (13.924 ± 19.771%) and PE MP + TBT-Cl (11.939 ± 18.019%) showed significantly higher TI% compared to the control group. TI% was lowest on day 7 in the PE MP + TBT-Cl group and highest in aqueous TBT-Cl (which mussels cannot avoid).

Within the PE MP group, significant differences were observed between the time points after 7 days (5.721 ± 15.372%) and 21 days (12.514 ± 17.948%) and between 14 days (7.438 ± 19.302%) and 21 days of exposure. In the TBT-Cl exposed group, TI% was significantly higher after 21 days of exposure in comparison to day 7. In the PE MP + TBT-Cl group TI% was significantly higher after 14 and 21 days of exposure compared to the 7th day of exposure, but there was no significant difference between days 14 and 21. The TI% in the control group showed no significant change throughout the experiment.

Cell viability of haemocytes remained above 90% in all four experimental groups after 7 and 14 days of exposure (Fig. 5). After 21 days, viability in the control group remained above 90%, while the median decreased in the PE MP (88.5 ± 12%), TBT-Cl (83.5 ± 12.25%) and PE MP + TBT-Cl (86.5 ± 7%) groups, with viability significantly lower in the TBT-Cl group compared to the control.

Cell viability in the TBT-Cl group increased significantly after 14 days (99.5 ± 1.75%) compared to day 7 (91 ± 5.5%), then decreased significantly on day 21 (83.5 ± 12.25%). A similar pattern was observed in the PE MP + TBT-Cl group, while in the PE MP group only a significant decrease in haemocyte viability from day 14 (100 ± 2.75%) to day 21 (86.5 ± 7%) was observed.

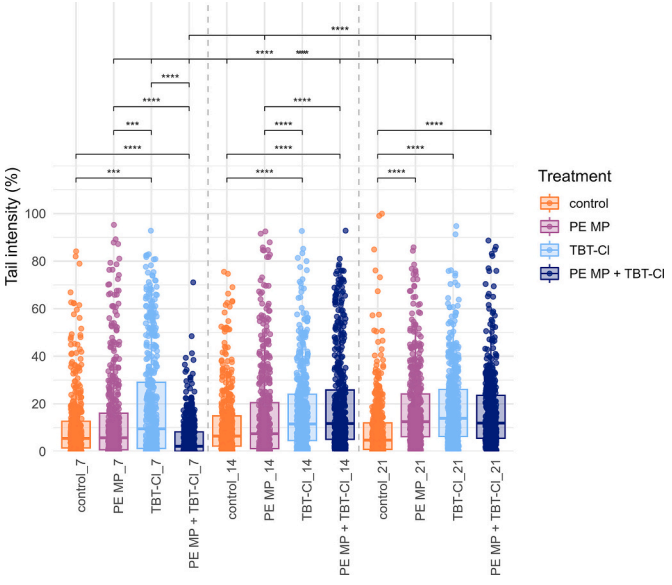

Fig. 4. The levels of DNA damage (expressed as TI%) in mussels across four experimental groups: control (orange), PE MP (50 mg L^{-1}, violet), BT-Cl (0.001 mg L^{-1}, light blue) and PE MP + TBT-Cl (50 mg L^{-1}; 0.02 mg TBT-Cl g^{-1} PE MP, dark blue) on days 7, 14 and 21. The sample size was n = 10 for all groups. Statistical significance is indicated as follows *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, and ****p ≤ 0.0001. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

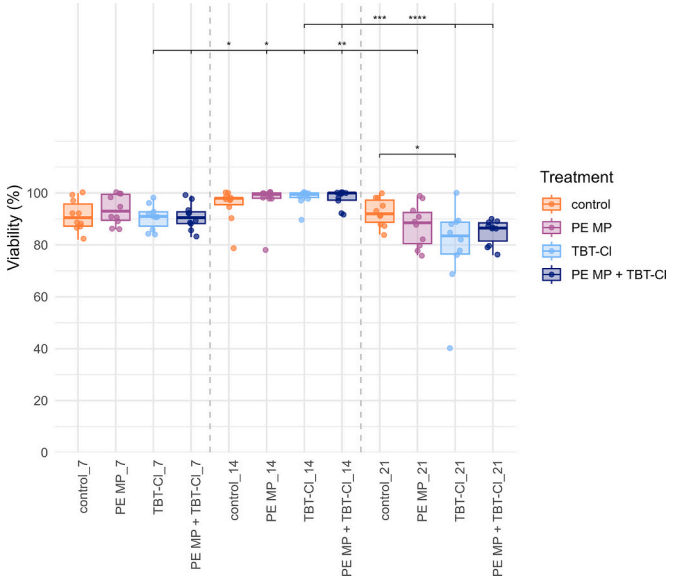

Fig. 5. Cell viability in four experimental groups: control (orange), PE MP (50 mg L^{-1}, violet), TBT-Cl (0.001 mg L^{-1}, light blue) and PE MP + TBT-Cl (50 mg L^{-1}; 0.02 mg TBT-Cl g^{-1} PE MP, dark blue) on days 7, 14 and 21. The sample size was n = 10 for all groups. Statistical significance is indicated as follows *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, and ****p ≤ 0.0001. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

The detrimental effects on *M. galloprovincialis* were investigated following exposure to nominally equal concentrations of PE MP (50 mg L^{-1} PE MP), TBT-Cl (0.001 mg L^{-1} TBT) and PE MP spiked with 0.02 mg TBT-Cl per gram PE MP (in total 50 mg L^{-1}). This approach enabled a direct comparison between aqueous and particle-bound TBT, demonstrating that microplastics can act as effective vectors, increasing TBT bioavailability and toxicity in mussels. To our knowledge, this is the first study to use TBT-Cl adsorbed on PE MPs to demonstrate the consequences of a realistic marine scenario for mussels. The results provide new insights into the interactions between PE MP and TBT in the marine environment, highlighting the threat posed by TBT adsorbed on PE MP that remains in the water column rather than depositing in the sediment. The outcomes measured in this study were antioxidative stress response (catalase activity) and energy metabolism (ETS activity) in gills and hepatopancreas, while DNA integrity and cell viability were assessed in mussel haemocytes. This multi-biomarker approach provides a holistic view of the cellular stress responses induced by PE MP, TBT-Cl, and TBT-Cl adsorbed on PE MP. These biomarkers represent interconnected physiological processes: antioxidant defence capacity, mitochondrial energy metabolism, and genomic integrity and together allow a mechanistic interpretation of pollutant-induced toxicity. The results confirm: 1.) high efficiency of TBT adsorption on PE MP; 2.) higher concentrations of TBT in mussels exposed to PE MP with adsorbed TBT-Cl; 3.) the detrimental effects on mussels indicated activation of the antioxidant defence system and changes in mitochondrial respiration capacity after exposure to PE MP, TBT-Cl and PE MP + TBT-Cl.

4.1. PE MP as a vector for TBT

We demonstrated efficient TBT adsorption on PE MP (adsorption efficiency of 53% m. m.) and ingestion of these particles by

M. galloprovincialis, resulting in significantly higher accumulation of PE MP + TBT-Cl (27 times more) compared to the exposure to dissolved TBT-Cl alone, despite equal nominal concentrations in the aquaria. The concentration (0.02 mg) of TBT per gram of PE was quantified before the experiment, and the concentration of TBT in mussels was measured on days 7, 14, and 21. The only known study on TBT adsorption on PS-NP indicated that the highest adsorption (49.5–85.6% (m/m)) was observed with the smallest plastic particles used in the study and within the brackish water matrix (40.0–85.6% (m/m)). This suggests that adsorption capacity increases as particle size and salinity decrease, highlighting that NPs have greater potential to act as a vector for TBT transport than microplastics, and that adsorption is limited by the presence of competing salts. This also suggests that PS-NPs have a similar potential for TBT adsorption as naturally occurring sediment particles (Raymond, 2025).

The selected size of PE MP in our study corresponds to particles known to be ingested by mussels and several studies providing evidence of microplastics ingestion in highly polluted marine environments from resuspended sediments and the water column (Tlili et al., 2020; Wakkaf et al., 2020). Gut retention time of ingested microplastics in *M. galloprovincialis* depends on particle size, with smaller particles <10 μm retained for up to 50 h and larger particles for more than 80 h, providing sufficient time for desorption of adsorbed contaminants and interaction with epithelial tissues (Kinjo et al., 2019). The effect of MP size on mussel's responses (oxidative stress, lipid peroxidation, immune system responses and cellular effects) as well as mechanistic relationships between particle size and these responses, were recently investigated (Nardi et al., 2024).

Mussels exposed to 50 mg L^{-1} PE MP + TBT-Cl (0.02 mg TBT adsorbed per gram PE MP) had higher measured concentrations of TBT/DBT in tissue compared to mussels exposed to 0.001 mg L^{-1} TBT-Cl only. This may result from the accumulation of PE MP + TBT-Cl in mussel's bodies due to the high concentration of PE MP items and the consequent desorption of TBT from PE MP. This is similar to the condition when heavily polluted sediment is highly disturbed in shallow waters and resuspended in the water column. TBT can be released from PE MP upon contact with the epithelium in the gills (during particle sorting) or in the intestine, but there are no studies on the desorption of TBT from PE MP, and the kinetics of this process have not been yet elucidated. Desorption processes from MPs are influenced by extrinsic and intrinsic conditions (Hartmann et al., 2017). This mechanism has been studied mostly in fish (Al-Emran and Nayem, 2025), but the efficiency and kinetics of TBT desorption from plastic particles and its uptake across epithelial barriers remain poorly understood in mussels. Although microplastics carrying tributyltin hydride (TBTH) have been shown to cause only slightly increased toxicity in *Gammarus fossarum* compared to the dissolved TBTH, these results support the possibility that microplastics act as a vector for TBT in aquatic systems. Tributyltin hydride adsorbed to microplastic particles had no effect on uptake, survival, feeding or locomotory behaviour during the three weeks of exposure. Dissolved TBTH, however, was already very toxic after few days of exposure ($LC_{50\text{-}96h}$ < 1 ng L^{-1}) (Gerhardt et al., 2020).

To our knowledge only one study has focused on the distribution of TBT in *M. edulis* adsorbed on PS NP and aqueous TBT, revealing that TBT accumulated in *M. edulis* within 24-h exposure period. The gills and digestive gland were the main sites of TBT accumulation, while the posterior and the anterior adductor muscles contained less TBT. There is also a notable absence of TBT in the carbon-rich adductor muscles, suggesting that TBT accumulates preferentially in the fattier tissues (Raymond, 2025). This study also highlights a potential shielding effect of nanoplastic, thereby challenging the hypothesis regarding the danger of adsorbed pollutants (Raymond et al., 2024; Raymond, 2025). The nature of the interaction between TBT and NP confirms that TBT can be remobilised from natural sediments, depending on the chemical characteristics of the plastic polymer. Adsorbed TBT on MP/NP can have a longer retention time in the water column due to the significantly lower density of MP/NP particles, thereby increasing the availability of particle-bound contaminants to organisms (Raymond et al., 2024).

4.2. Oxidative stress response

Many studies investigate cellular responses such as inflammation, oxidative stress, DNA integrity damage, blockage of electron transfer, etc., following exposure to PE MP, with mechanistic relationships elaborated in some cases (Abidli et al., 2021; Mai et al., 2023; Nardi et al., 2024) Similarly, exposure to the legacy pollutant TBT has been intensively studied due to its extreme toxicity (Nesci et al., 2011; Martinović et al., 2016; Beyer et al., 2022), but the effects of TBT adsorbed to MPs in mussels and the ecological relevance of this interaction have not been yet elucidated. Oxidative stress is a central cellular response, induced by environmental stressors or pollutants in mussels (Lushchak, 2011). Measuring catalase activity, provides insight into the capacity of the antioxidant system and allows evaluation of an increase in ROS production and the efficiency of their removal in the gills and hepatopancreas exposed to PE MP, TBT-Cl and PE MP + TBT-Cl. Catalase activity revealed pronounced tissue-specific responses after the exposure, with activities approximately one order of magnitude higher in the hepatopancreas than in the gills. PE MP exposure did not elicit significant changes in catalase activity in the gills, suggesting that pro-oxidative damage did not induce major perturbations or antioxidant responses in gill tissue. A moderate increase in catalase activity after 21 days of exposure may indicate weaker induction of oxidative stress or accumulation of damage after prolonged exposure, possibly due to epithelial damage. Physical damage to gill epithelia caused by microplastic fibres and spheres has been reported previously and contributes to oxidative stress responses (Mai et al., 2023). In the hepatopancreas the catalase response in the PE MP + TBT-Cl group was most prominent after 14 days, while after 21 days catalase activity dropped compared with the control group (see Fig. 2B). Exposure to PE MP + TBT-Cl resulted in significantly lower catalase activity in the hepatopancreas compared with exposure to a single stressor, especially after 14 and 21 days, which may suggest an enzymatic depletion or disruption of the induction pathways of the antioxidant defence system under combined stress conditions. Overall, mussels exposed to TBT-Cl alone showed a stronger induction of catalase activity compared with those exposed to PE MP, which showed a weaker response, while combined exposure may show a dampened antioxidant defence at later stages or a possible shielding effect of MPs. Similar results are retrieved with PP exposure on mussels with consequences on decreased feeding rates and lower condition index of the mussels, especially at the high dosage of 50 mg L^{-1}, without alterations occurring in metabolic enzymes, while CAT activities decreased after four days of acute exposure, but significantly increased after the chronic exposure of 28 days (Daniel et al., 2024).

An increase in catalase activity in the gills of *M. galloprovincialis* was demonstrated following exposure to NP-PS and MP-PS at a low concentration of 1.5 ng L^{-1} of MP/NP particles (Capolupo et al., 2021). A two-week exposure to 100 μg L^{-1} and 1000 μg L^{-1} of PE MP (40 to 48 μm) inactivated the antioxidative system (catalase and glutathion *S*-transferase) in the digestive gland of *M. galloprovincialis*, along with a reduced filtration rate (Abidli et al., 2021). A significant increase in catalase activity was observed in the gills (at 100 μg L^{-1} of MP mixture) and the digestive gland (at 0.008 μg L^{-1} and 10 μg L^{-1} of MP mixture) after 10 days of exposure to a combination of PE and PP plastic fragments (<400 μm) in *Mytilus* mussels (Revel et al., 2019). In general, lower concentrations of MPs induce catalase activity, while higher concentrations (>100 μg L^{-1} MP) are inhibitory and the relationship between MP concentration and the oxidative defence system has not yet been fully elucidated. Prolonged exposure to MPs may cause damage to antioxidant response system. It has been reported that a 7-day exposure to 2 and 6 μm MPs decreased antioxidant enzyme activities and intensified lipid peroxidation in the digestive glands of *Mytilus* spp. (Paul-Pont et al., 2016; Wang et al., 2024). Inhibition of catalase activity after

extended exposure to TBT has been reported in zebrafish, where sustained mitochondrial damage and excessive ROS production impair antioxidant enzyme function (Zhang et al., 2017).

The literature on combined exposure is limited, as the mechanism of TBT adsorption to MPs and NPs has only recently been addressed, with descriptions of adsorption and photodegradation of TBT on MPs (Chen et al., 2020) and adsorption of TBT on PS NPs (Raymond et al., 2024). Only a few studies have examined the consequences of adsorbed TBT on MPs or NPs in invertebrates. In particular, the concentration of TBT in *M. edulis* is lower when TBT is adsorbed on PS NPs, with a proposed mechanism being the shielding effect of the NPs, as TBT is desorbed slowly from the NPs in the mussel body (Raymond, 2025). To our knowledge, there is no study describing such a phenomenon in mussels with MPs and adsorbed TBT. Simultaneous exposure to TBT and PS MPs has been shown to increase reactive oxygen species, decrease reproduction, and alter lifespan in the rotifer *Brachionus koreanus*, but the toxicity of TBT (10 μg L^{-1} TBT) was attenuated by PS MP (1 and 10 μg L^{-1}), and most notably, by high nutritional status (Yoon et al., 2021). Reviews of studies on co-exposure to MPs and pollutants are contradictory; some report increased detrimental effects, while others report less severe effects (Kolarević et al., 2023; Nardi et al., 2024; Mijošek Pavin et al., 2026). Including additional biomarkers of oxidative stress response, such as measuring enzymatic activities of SOD and GPx in future studies would provide a more comprehensive assessment of oxidative stress conditions. We believe that detailed analysis and comparison of experimental conditions are needed to evaluate the concentrations of MPs and pollutants.

4.3. Energy metabolism (ETS activity)

The INT reduction capacity assay was used to estimate the activity of the electron transport system (ETS) in mussels exposed to PE MP, TBT-Cl and PE MP + TBT-Cl, as a biomarker for assessing metabolic capacity. This biomarker integrates the maximum potential for aerobic energy production and reflects the energetic costs associated with stress responses and detoxification. We observed that tissue-specific responses in ETS activity were maintained, with greater sensitivity in the hepatopancreas than in the gills. The same pattern of greater sensitivity in the hepatopancreas compared to the gills was also observed when assessing catalase activity, confirming its primary role in metabolic and detoxification processes (Erk et al., 2011). ETS activity in the gills showed no statistically significant changes between treatments, although there was a tendency towards increased values in the TBT groups. As with catalase activity, ETS activity demonstrated higher sensitivity of the hepatopancreas to pollutant disturbances, including those affecting energy metabolism, consistent with its role in digestion, detoxification and metabolism.

Exposure to PE MP with adsorbed TBT-Cl resulted in significantly higher ETS activity in the hepatopancreas compared to all other groups after 7 days, indicating a rapid increase in metabolic demands under these combined conditions. Elevated activity during these early exposure phases may represent compensatory regulation of mitochondrial respiration to sustain antioxidative defence mechanisms and maintain cellular homeostasis. After 14 days, ETS activity in the combined treatment remained elevated, but declined by day 21. This temporal pattern suggests that prolonged exposure to the combination of PE MP and TBT-Cl can lead to energy depletion.

Exposure to TBT-Cl alone resulted in similar ETS activities over time, but with significantly higher values after 14 and 21 days compared to the control group in the hepatopancreas. This persistent increase may reflect long-term metabolic disturbance and is consistent with the documented inhibitory action of TBT on oligomycin-sensitive Mg-ATPase activity in isolated mitochondria of *M. galloprovincialis* (Nesci et al., 2011). Inhibition of ATP synthesis can lead to ATP depletion in cells, forcing them to increase ETS activity to meet high energetic demands. In contrast to the co-exposure treatment, ETS activity in mussels exposed to only TBT-Cl remained elevated compared to the control, indicating a lasting effect of TBT-Cl on mitochondrial energy metabolism. Therefore, comparison of the two TBT-Cl containing treatments showed distinct dynamics. The combination of PE and TBT induced the highest activity in early exposure (days 7 and 14), but this declined by day 21, matching the TBT-Cl only group in both gills and hepatopancreas. This pattern may suggest that PE MPs enhanced early metabolic demands through additive stress responses to the mixture but did not amplify long-term disturbance beyond that caused by TBT-Cl alone. These results indicate that TBT-Cl is the main driver of impairment of energy metabolism, while MPs additionally modulate the timing and magnitude of early responses. This is especially relevant given the continued presence of TBT in the environment (Beyer et al., 2022; Mil-Homens et al., 2023; Uc-Peraza et al., 2022) and strong affinity for MPs surfaces (Chen et al., 2020; Raymond et al., 2024). In addition, TBT is still used as an additive in plastic production, particularly in polyvinyl chloride (PVC), where it functions as a thermal stabiliser and anti-yellowing agent, providing a direct link between plastic materials and ongoing TBT emissions (Fauser et al., 2022).

Our results show additive early effects of PE MP and TBT-Cl on ETS activity, which may therefore reflect both direct TBT toxicity and vector-mediated interactions between MPs and adsorbed contaminants. Exposure to PE MP alone induced weaker responses in ETS activity than treatments containing TBT-Cl treatments. The only modest increase compared to the control mussels after 21 days likely reflects metabolic adjustment to physical stress, epithelial irritation, or mild oxidative stress after prolonged exposure, rather than direct mitochondrial toxicity. Similar results were reported recently, showing that polypropylene MPs (1 mg L^{-1}, 40 μm) also did not affect ETS activity in mussels after 14-days exposure (Mijošek Pavin et al., 2026). However, exposure to high concentrations of small PS MP (2 μm microplastics, 10^{-4} or 10^{-6} particles L^{-1}) significantly increased ETS activity in mussel *M. coruscus*, indicating increased mitochondrial metabolism and cellular ATP demand (Shang et al., 2021).

Our results support the conclusion that TBT-Cl adsorbed on PE MP interferes with energy metabolism more strongly and persistently than PE MP alone. In the combined exposure group, additive effects were observed at early time points, indicating a higher energy demand when mussels are exposed to multiple stressors. Considering the continued presence of TBT in marine environments and its ability to adsorb onto microplastics (Chen et al., 2020; Raymond et al., 2024), such additive early effects on metabolic capacity represent an ecologically relevant stress scenario for filter-feeding organisms, including mussels.

4.4. Genotoxic effects

The extent of DNA damage after exposure to PE MP, TBT-Cl and PE MP + TBT-Cl was assessed using the comet assay in haemocytes. In the control group, the level of DNA damage remained low, with a median of TI% up to 20%, corresponding to low background DNA damage according to established scoring criteria (Collins et al., 2023). This stability indicates that the experimental conditions did not induce genotoxic stress in unexposed mussels.

In the PE MP group, DNA damage increased gradually over time and became significantly higher than in the control after 14 and 21 days but remained at a moderate level. These findings are consistent with previous studies reporting low to moderate DNA damage in haemocytes of *M. galloprovincialis* exposed to milled polyethylene at environmentally relevant concentrations (10 and 100 μg L^{-1}), suggesting that microplastics primarily induce genotoxicity indirectly through prolonged cellular stress rather than direct interaction with DNA (Revel et al., 2019). However, a variety of responses regarding DNA damage can be found in the literature, depending on the polymers, their sizes, concentrations, shapes or the duration of the experiment (Kolarević et al., 2023; cf. Mijošek Pavin et al., 2026).

Exposure to TBT-Cl (0.001 mg L^{-1}) induced DNA damage that was

significantly higher from day 7 and continued to increase until day 21. This rapid response is consistent with the high sensitivity of the comet assay, which can detect DNA strand breaks before changes in other physiological biomarkers become evident (Bolognesi et al., 2019). TBT has been shown to induce DNA strand breaks at environmental concentrations (0.5 μg L^{-1}, 1 μg L^{-1} and 5 μg L^{-1} TBT) in bivalves leading to apoptosis through cell cycle arrest (Hagger et al., 2005; Martinović et al., 2016). DNA damage is induced via an oxidative stress cascade, leading to lysosomal destabilisation and interference with DNA repair in aquatic organisms. Damage to mitochondrial membranes and sulfhydryl (–SH) groups impairs electron transport, resulting in excessive production of reactive oxygen and nitrogen species (ROS/RNS), which in turn cause DNA strand breaks, base modifications, and fragmentation detectable by the comet assay (Châtel et al., 2015). In addition, TBT interferes with DNA repair processes and activates stress-related signaling pathways, including MAPK cascades and caspase-3-mediated apoptosis, further contributing to genomic instability (Châtel et al., 2015; Dash and Rahman, 2023). These mechanisms explain the rapid and persistent DNA damage observed in TBT-Cl exposed mussels and confirm TBT-Cl as a strong genotoxic agent in *Mytilus* species.

In the PE MP + TBT-Cl group, DNA integrity was significantly higher on day 7 compared to the TBT-Cl only group. Microplastics may modulate TBT-Cl genotoxicity and bioavailability through behavioural and physiological defences, as well as altered contaminant dynamics, resulting in lower net DNA damage in *Mytilus* during simultaneous exposure. Used MP particles were too large to enter haemocytes, and most of the TBT remained bound to MPs, making it insufficiently bioavailable to cause pronounced genotoxic effects. This could explain the lower DNA damage observed in mussels exposed to PE MPs with adsorbed TBT, despite the higher TBT concentration detected in the mussel tissue of this group (due to accumulation of PE particles with TBT in pallial fluid) compared to the group exposed to aqueous TBT. Another possible mechanism for reduced DNA damage in mussels exposed simultaneously to microplastics and TBT-Cl may involve an adaptive cellular response that enhances antioxidant defences and DNA repair processes. Exposure to microplastics can induce mild oxidative stress, which triggers upregulation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (see Fig. 2), and glutathione peroxidase. This preconditioning effect may help mussels better manage the oxidative stress induced by TBT-Cl during co-exposure, reducing overall DNA damage compared to TBT-Cl exposure alone. After 14 and 21 days of exposure, DNA damage in the PE MP + TBT-Cl group was at the same level as in the TBT-Cl group. This indicates that, over time, the genotoxic effects of TBT-Cl override any modulation by microplastics, highlighting TBT-Cl as the dominant driver of long-term genotoxicity, whether in single or combined treatment. TBT-Cl can interfere with DNA repair processes and induce apoptosis in *Mytilus* tissues. Studies show significant increases in DNA strand breaks and chromosomal damage in mussel gills and haemocytes after TBT-Cl exposure at environmentally relevant concentrations (Hagger et al., 2005; Dash and Rahman, 2023). These genotoxic effects contribute to cellular dysfunction and potential impacts on mussel health and reproduction.

Taken together, the genotoxicity results demonstrate distinct temporal patterns for the two tested contaminants. PE MPs induced only delayed and moderate DNA damage, while TBT-Cl caused rapid, persistent, and pronounced DNA damage through both direct genotoxicity and oxidative stress-mediated pathways. Combined exposure led to a transient attenuation of DNA damage at early stages, followed by cumulative genotoxic effects driven primarily by TBT-Cl. These findings highlight the importance of considering time-dependent responses and mixture effects when assessing the genotoxic risks of microplastics and associated contaminants in marine organisms.

5. Conclusions

Our study provides one of the first insights into microplastics as effective vectors for the transfer of TBT into *M. galloprovincialis*, alongside assessments of oxidative stress through catalase and ETS activity measurements, and DNA integrity. To our knowledge, this is the first study to examine the effects of TBT-Cl adsorbed on PE microplastics on *M. galloprovincialis* and to demonstrate that PE microplastics can act as vectors for TBT, increasing its bioavailability and toxicity. The results confirm the high efficiency of TBT-Cl adsorption on PE MP, higher concentrations of TBT in mussels fed PE MP with adsorbed TBT-Cl, and the detrimental effects on mussels, indicated by activation of the antioxidant defence system and changes in mitochondrial respiration capacity after exposure to PE MP, TBT-Cl, and PE MP + TBT-Cl. We observed high accumulation of TBT in mussel tissue in the PE MP + TBT-Cl group compared to the group exposed to TBT-Cl alone. Activation and subsequent depletion of the antioxidant defence system were observed. In the hepatopancreas, the catalase response in the PE MP + TBT-Cl group was most prominent after 14 days, but later catalase activity dropped compared with the control group. Exposure to PE MP + TBT-Cl resulted in significantly lower catalase activity in the hepatopancreas compared with exposure to a single stressor, especially after 14 and 21 days, suggesting enzymatic depletion or disruption of the induction pathways of the antioxidant defence system under combined stress conditions. Increased ETS activity at the early timepoint was recorded in the PE MP + TBT-Cl group, indicating additive early effects of PE MP with adsorbed TBT-Cl. This suggests that PE MP can modify the trajectory and intensity of TBT-induced stress, while DNA damage was most pronounced in the TBT-Cl only group, demonstrating that TBT is the dominant driver of toxicity in combination with PE MP. In light of increasing MPs and NPs pollution in the marine environment, it is necessary to evaluate the effects of adsorbed pollutants and conduct ecotoxicological assessments, particularly regarding the harmful potential of complex interactions such as vector-mediated transport and the shielding effects of MPs which may modulate contaminant bioavailability and toxicity. In conclusion, we emphasise the ecological relevance of pollutant mixtures in marine systems, as MPs and organotin compounds continue to co-occur. Future studies using environmentally relevant concentrations of MPs are needed to demonstrate significant impacts on organisms such as mussels, given that this study showed possible vector-borne entry of TBT into the organism. As multiple pollutants occur simultaneously in the environment, further research should focus on the long-term exposure of organisms to environmentally relevant concentrations of pollutant mixtures.

CRediT authorship contribution statement

Tanja Kobal: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Tea Zuliani:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Stoimir Kolarević:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Margareta Kračun-Kolarević:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Tatjana Simčič:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Tatjana Mijošek Pavin:** Writing – review & editing, Validation, Methodology, Formal analysis. **Andreja Ramšak:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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Declaration of competing interest

There are no known competing financial interests or personal relationships that could have influenced the work of the authors reported in this paper.

Data availability

Data are stored and are publicly available at Zenodo (https://doi.org/10.5281/zenodo.18598962) after publication. Additional data can be obtained from the corresponding author upon request.

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