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Influence of microplastics on the bioconcentration of organic contaminants in fish: Is the "Trojan horse" effect a matter of concern?☆

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ABSTRACT

Microplastics (MPs) have been shown to act as sorbent phases and thus carriers of organic chemicals in the aquatic environment. Therefore, concerns exist that MP ingestion increases the uptake and accumulation of organic chemicals by aquatic organisms. However, it is unclear if this pathway is relevant compared to other exposure pathways. Here we compared the bioconcentration capacity of two hydrophobic organic chemicals (i.e., chlorpyrifos and hexachlorobenzene) in a freshwater fish (*Danio rerio*) when exposed to chemicals through water only and in combination with contaminated polyethylene MPs. Additionally, a suite of biomarker analyses (acetylcholine esterase, glutathione S-transferase, alkaline phosphatase, catalase) was carried out to test whether MPs can enhance the toxic stress caused by chemicals. Two 14-day semi-static experiments (one for each chemical) were carried out with adult fish. Each experiment consisted of (1) a control treatment (no chemicals, no MPs); (2) a treatment in which fish were exposed to chlorpyrifos or hexachlorobenzene only through water; (3) a treatment in which fish were exposed to the chemicals through water and contaminated polyethylene MPs (100 mg MP/L). Two additional treatments were included for the biomarker analysis. These contained MPs at two different concentrations (5 and 100 mg MP/L) but no chemicals. The presence of contaminated MPs in contaminated water did not enhance but rather decreased the bioconcentration of both chemicals in fish compared to the treatment that contained contaminated water in absence of MPs. This was more pronounced for hexachlorobenzene, which is more hydrophobic than chlorpyrifos. Enzyme activity levels in fish were only significantly altered in the presence of MPs for alkaline phosphatase. This study indicates that MP presence in freshwater ecosystems is not expected to increase the risks associated with chemical bioconcentration in aquatic organisms and that other exposure pathways (i.e., uptake via respiration, skin permeability) may be of higher importance.

Author statement

Theresa Schell - Investigation; Methodology, Formal analysis; Writing - original draft. Andreu Rico – Conceptualization, Funding acquisition, Supervision, Writing - review & editing. Laura Cherta – Investigation, Methodology, Writing - review & editing. Raquel Dafouz - Investigation. Lenor Nozal – Methodology, Writing - review & editing. Roberto Giacchini – Investigation, Writing - review & editing. Marco Vighi – Conceptualization, Investigation, Project administration, Funding acquisition, Supervision, Writing - review & editing.

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1. Introduction

Microplastic (MP) ingestion has been reported for different freshwater organisms (Galafassi et al., 2021; Kumar et al., 2021), which may have detrimental physical consequences (Gray and Weinstein, 2017; Lei et al., 2018; Qiao et al., 2019b, 2019a). For instance, histopathological damage in intestines following MP ingestion has been reported in zebrafish (Lei et al., 2018; Qiao et al., 2019b) and European sea bass (Pedà et al., 2016). Furthermore, MPs may pose a risk to aquatic organisms due to the additional effects caused by associated chemicals. Plastics can contain several types of additives (e.g., plasticizers, flame retardants, and stabilizers), which are incorporated during plastic production to receive specific product properties (Hahladakis et al., 2018). The leaching of these additives may exert harmful effects in aquatic organisms (Ke et al., 2019; Oliviero et al., 2019). Moreover, due to MPs' big surface-to-volume ratio and their sorption affinity for hydrophobic organic contaminants, MPs can sorb other environmental contaminants (Teuten et al., 2007; Velzeboer et al., 2014). The sorption of chemicals to MPs has been confirmed in laboratory studies and for MPs recovered from environmental samples, which contained hydrophobic organic pollutants as well as different drugs and metals (Amelia et al., 2021; Syberg et al., 2020; Yu et al., 2019). Several studies have provided evidence for the transfer of organic contaminants from MPs into animals (Batel et al., 2018, 2016; Rochman et al., 2013). Consequently, MPs have been proposed as possible contaminant vectors, influencing the uptake of hydrophobic organic chemicals, a phenomenon also termed "Trojan Horse" effect. It has been suggested that the bioaccumulation of these contaminants in environmental media can be enhanced in the presence of MPs as high quantities of sorbed chemicals may be released into the organisms' gut following ingestion, thus favoring chemical uptake (Bakir et al., 2014; Coffin et al., 2019). Outcomes of a meta-analysis of existing data demonstrated that the presence of MPs significantly enhanced the bioaccumulation of other contaminants by 31% in aquatic organisms under laboratory conditions (Sun et al., 2022). However, in most laboratory studies, contaminated MPs are fed to organisms in a clean environment, while in the natural environment, other media also contribute to the organisms' contamination (such as water, sediment, and food) (Gerdes et al., 2019; Koelmans et al., 2016). Furthermore, modeling studies suggest that the role of MPs as contaminant vectors may have limited relevance compared to other uptake routes (Gouin et al., 2011; Lohmann, 2017; Mohamed Nor and Koelmans, 2019). Thus, current literature is rather inconclusive on the relevance of contaminated MP ingestion as a novel pathway that enhances the ecological risk of chemicals, and experimental proof using environmentally relevant exposure conditions is rare, especially for freshwater ecosystems (Hartmann et al., 2017; Sun et al., 2022).

This study aimed to assess the capacity of MPs to act as contaminant vectors for freshwater fish and to compare the bioconcentration capacity of two organic chemicals: chlorpyrifos (CPF) and hexachlorobenzene (HCB). CPF and HCB are both listed as priority substances under the European Water Framework Directive (European Commission, 2013), and were chosen in this study due to their environmental relevance and their different hydrophobicity (log K_{OW} 4.7 and 5.8, respectively). In our study, zebrafish (*Danio rerio*) were exposed to CPF and HCB through water only and in combination with contaminated MPs for 14 days. Our experiment was designed to understand to what extent MP contamination may enhance bioconcentration and exacerbate the impact of other contaminants on freshwater organisms. Additionally, the capacity of MPs to enhance or decrease the toxic stress caused by chemicals (i.e., neurotoxicity, general stress, and antioxidant defense) in zebrafish was assessed using a multi-biomarker approach.

2. Material and methods

2.1. Fish maintenance

Adult zebrafish with an average length of 5 cm and 663 ± 26 mg weight (mean ± SD; n = 6) were purchased from Pisciculture Superior S. L. and were maintained at 26 °C, with a photoperiod of 12/12 h (light/dark) during three weeks before the start of the experiment. They were fed daily with fish food (Tetramin, Tetra GmbH, Germany) and checked visually to assess their active behavior and ensure proper health conditions.

2.2. Test materials

Polyethylene MPs (irregularly shaped with a mean particle size of 150 μm, Sigma-Aldrich) were chosen due to their widespread occurrence in the environment (Schell et al., 2020) and their higher sorption capacity for organic chemicals compared to other plastic types observed in the laboratory as well as in field studies (Alimi et al., 2018; Syberg et al., 2020).

To obtain MPs with sorbed chemicals, 2 g/L polyethylene particles were added to Milli-Q water containing CPF or HCB (Analytical standards, Merck) at concentrations of 200 μg/L and 36 μg/L, respectively. Prior to spiking, each chemical was dissolved in methanol. MPs were previously coated with 0.5 ml of the non-ionic detergent Tween® 20 (Scharlau) per g of MP to allow an even dispersion in water. This was performed in glass bottles and the suspension was kept under constant stirring. The same concentration of chemicals and Tween® 20 was added to a water control containing only Milli-Q water and no MPs. The procedure was done twice for each chemical. Sorption of chemicals to MPs and equilibrium state were confirmed by chemical analysis of water at different time points (up to 168 h) before the addition of the contaminated MPs to the experiment. Further details on the chemical-MP partitioning process are provided in the Supplemental Information (SI; Section S1, Tables S1-S4).

2.3. Experimental design

This study consisted of two parts, a bioconcentration analysis and a biomarker analysis. For the bioconcentration analysis two 14-day semi-static experiments (one for each chemical) were carried out with adult zebrafish. Each experiment consisted of: (1) a control without addition of chemicals nor MPs (combined for both experiments; n = 6); (2) a treatment in which fish were exposed to CPF or HCB only through water (n = 6); (3) a treatment in which fish were exposed to the respective chemical through water and contaminated MPs (in this case, 100 mg MP/L were added (n = 6)). For the biomarker analysis, the same chemical treatments were used, and in addition, two MP controls containing clean MPs at two different concentrations (5 and 100 mg MP/L, n = 3, Table 1). The high MP concentration tested (100 mg/L or 57,000 MP/L) is around two orders of magnitude higher than maximum

Table 1
Nominal test concentrations of the different treatments used in the experiments.

Treatment	Chemical water concentration (μg/L)	Chemical concentration sorbed to MPs (μg/g)	MP concentration (mg/L)
Control	0	–	0
CPF	15.5	–	0
CPF + MPs	15.5	45	100
HCB	4.2	–	0
HCB + MPs	0.6	16	100
MP Low	0	0	5
MP	0	0	100

measured concentrations in freshwaters based on particle counts (Lu et al., 2021). However, the study was designed as proof-of-principle to test whether MPs could enhance bioconcentration compared to the uptake through water.

Two different scenarios were tested in our study: (1) the presence of contaminated MPs in contaminated water (with CPF as an example) and (2) the addition of highly contaminated MPs to water with a very low chemical background concentration (with HCB as an example). The nominal concentrations are shown in Table 1. Each test unit consisted of a 3 L beaker with dechlorinated tap water containing six fish. MPs were kept distributed throughout the water phase by aerating the beakers. The test medium was renewed on days 3, 7, and 10 after the start of the experiment. Fish were fed before the start of the experiment and before each medium renewal. Fish, water, and MP samples were taken at the start of the experiment and after each exposure period (i.e., at each medium renewal and the end of the experiment after 14 days). In each sampling event, one fish sample was taken per replicate and euthanized by rapid cooling (Wallace et al., 2018). The digestive tract was separated from the remaining fish tissue and samples were stored frozen until analysis. Water samples (4 ml for CPF and 10 ml for HCB approximately 30 min after spiking and 150 ml for both chemicals at the end of each exposure period) were taken from the test beakers before and after medium renewal using glass pipettes. To obtain the MP samples, 50 ml of stock suspensions (to measure initial chemical load) and 500 ml of test medium before medium renewal were taken using glass pipettes. These samples were filtered onto glass filter papers (Borosilicate filter GMFF, Scharlau) to separate the MPs. All samples were stored frozen until analysis. Water temperature, pH, conductivity, and dissolved oxygen were measured before and after each medium renewal with a multiparameter meter (Hanna HI91894).

2.4. Analytical method

A gas chromatograph (GC) system (Agilent 7890 A) coupled to a mass spectrometer (MS) with a triple quadrupole analyzer (Agilent 7000 GC/MS Triple Quad) was used to determine the concentration of CPF and HCB in water, MPs, and fish samples. Details on the GC-MS conditions are provided in the SI (Section 2.1, Table S5). Prior to the instrumental analysis, different extraction methods of CPF and HCB from the different matrices were applied. Water sample treatments were based on Stir Bar Sorptive Extraction (SBSE, also called *Twister* extraction). For MP and fish tissue, sample treatments were based on a solid-liquid extraction and an additional cleanup step for fish. Details on all sample treatments are outlined in the SI (Section S2.2). Isotopically labeled internal standards (ILIS), hexachlorobenzene-13C6 and chlorpyrifos-(diethyl-d10), were used to correct possible losses during sample treatment and/or instrumental deviations.

Validation of the three analytical methodologies (including sample treatment and instrumental analysis) was performed in order to provide quality assurance of the results (conditions of the validation study are shown in the SI, Section 2.3). The method was validated for linearity, accuracy, precision, and limits of detection (LOD) and quantification (LOQ), with satisfactory results. Mean recovery (n = 3) was between 90 and 103% for chemicals extracted from water and fish samples, and between 83 and 104% from MPs, with good precision expressed as relative standard deviation. Detailed results can be seen in Table S6.

During sample analysis, daily quality controls (spiked matrices as done in the validation study) were injected to ensure the reliability of the results.

2.5. MPs detection and counting

To assess MP ingestion by fish, the digestive tract of the organisms exposed to MPs was digested using 15% H_2O_2 for 48 h at 50 °C. The solution was then vacuum filtered onto filter papers (Borosilicate filter GMFF, Scharlau) and retained until visual assessment. MPs were counted using a stereo microscope (Olympus SZX7) with 40× magnification. Recovery tests (n = 3) were carried out by adding 25 polyethylene MPs to the digestive tract of two clean fish from the fish stock and 10 ml H_2O_2. This was kept for 48 h at 50 °C, followed by the same filtration and visual analysis procedure as described above. Recovery of MPs based on MP count showed satisfactory results (between 88 and 92%), with no visible changes of the MP particles observed.

2.6. Biomarker determination

The activity of four enzymes was measured in the current study: acetylcholinesterase (AChE), catalase (CAT), glutathione S-transferase (GST), and alkaline phosphatase (ALP). Seven treatments were considered for the biomarker analysis (Table 1). Fish samples were taken at the end of the experiment (day 14) from each treatment and immediately frozen in liquid nitrogen. Samples were stored at −80 °C until analysis. Control fish from the fish stock were also sampled at the start (day 0) of the experiment. Fish specimens were divided into head (for AchE analysis) and body (for the remaining enzymes). For each treatment, six specimens were analyzed, and two replicates of the spectrophotometric measurement were performed. The detailed analytical procedures are outlined in the SI (Section S3).

2.7. Data analyses

The CPF and HCB bioconcentration factors (BCFs) were calculated as the ratio of measured chemical concentrations in fish and water at the end of each exposure period (i.e., 3, 7, 10 and 14 days), for the treatments containing chemicals alone and for the treatments containing chemicals in the presence of MPs. Normal distribution and equality of variances of the calculated BCF values were tested using Shapiro–Wilk test and Bartlett's test, respectively. Significant differences between the calculated BCF in the water only treatment and the water and MPs treatment were assessed for each sampling day by preforming student t-tests using a significance level of 0.05.

For the analysis of biomarker responses, the measured enzymatic activities were log transformed when data did not meet the requirements for parametric testing. Student t-tests were used to compare the enzymatic activities of the control treatments on day 0 and day 14. One-way analysis of variance (ANOVA) followed by a Tukey post hoc multiple comparison tests was used to test differences in enzymatic activities levels induced by different MP concentrations compared to the control. To assess differences in enzymatic activities between the treatments containing only MP at the higher concentration, only chemicals, and chemicals with MP, a two-way ANOVA followed by a Tukey post hoc multiple comparison tests was performed. Statistical analyses, including the construction of graphs, were performed using the software R Version 4.1.1 (R Core Team, 2021) in RStudio (RStudio Team, 2021) or the R language-based software Jamovi (The jamovi project, 2021) and the required extension packages (Alboukadel, 2020; Fox and Weisberg, 2019; Wickham, 2016). Statistically significant differences were assumed when the calculated p-value was <0.05.

3. Results

3.1. Fish survival and MP ingestion

No mortality was observed due to exposure to chemicals, MPs, or the combination of both during the experiment. One fish died in the MPs only treatment containing the lower MP concentration (5 mg/L), possibly due to damage during medium renewal and manipulation. Ingestion of MPs by fish was confirmed but varied between fish and sampling times (Table 2). The number of ingested MPs in the CPF with MP treatment was relatively stable over time with an outlier on day 7. In the HCB with MP treatment, the concentration of MPs extracted from the digestive tract of the fish seemed to increase over time. Small amounts of

Table 2
Mean MP particle ingestion (min-max) displayed in number of MPs for different treatments at different time points (day 0 to day 14). Calculated by dividing the total number of MPs extracted from the digestive tracts of the fish of one sample by the number of fish. NA: time point was not assessed.

Treatment	Day 0	Day 3	Day 7	Day 10	Day 14
Control	0 (0–0)	0.2 (0–0.5)	0.3 (0–1)	0 (0–0)	2.5 (0.5–4)
CPF	NA	0.2 (0–0.5)	0 (0–0)	0 (0–0)	0.2 (0–0.5)
HCB	NA	0.2 (0–0.5)	0.2 (0–0.5)	0 (0–0)	0 (0–0)
CPF + MP	NA	163 (15–353)	962.5 (203–1720)[a]	167 (29–436)	80.3 (53–150)
HCB + MP	NA	10.8 (3.5–21.5)	18 (5–42.5)	66.7 (14–112)	417 (256–632)

[a] One sample was lost, the mean ingestion was calculated from two samples only.

contamination in control samples have been observed. However, this contamination likely occurred during sample processing and not due ingestion of MPs by fish.

3.2. Chemical analysis

The results of the measured chemical water concentrations in the stock suspension are provided in the SI (Table S8). The chemical concentrations on MPs before their addition to the test medium were relatively stable over time (Tables S9 and S10). The CPF concentration on MPs decreased strongly after the three to four days in the test medium (measured prior to medium renewal), probably caused by the establishment of a new equilibrium as a result of the lower CPF concentrations and the different physical chemical conditions in the test medium compared to the stock suspension. However, an increase in concentration was observed along with a temporal trend. The calculated MP-water partitioning coefficients (K_{PW}; Table S11) were higher than those calculated for the stock suspension, suggesting that CPF was strongly sorbed to the MPs. The HCB concentrations measured on MPs in the test medium after the three to four days were also lower, indicating a new partitioning equilibrium was established. Again, the K_{PW} were higher than those calculated for the stock suspension, suggesting that also HCB is strongly sorbed to the MPs (Table S11).

The measured CPF concentrations in the test medium are shown in Tables S12 and S13. Most of the chemical dissipated (on average 96% for the CPF only treatment and 93% for the CPF with MPs treatment) within the three to four days until the next medium renewal. Dissipation was probably a cause of microbial degradation, uptake by fish, sorption to MPs and the wall of the test vessels or organic suspended material. The measured HCB concentrations are shown in Tables S14 and S15. In both treatments (HCB only and HCB with MPs), the HCB water concentrations were lower than the nominal values. As for CPF, most of the chemical dissipated within three to four days until the next medium renewal (i.e., 93% for the HCB only treatment, and 73% for the HCB with MPs treatment).

The CPF concentration in fish tissue increased over time in the CPF only and the CPF with MP treatment (Fig. 1A). The CPF concentrations were almost identical in the two treatments (i.e., CPF only and the CPF with MP) after three and seven days relative to the start of the experiment. At days 10 and 14, relative to the start of the experiment, a lower CPF concentration was observed in fish in the CPF with MP treatment compared to the CPF only treatment. Although a steady state had not been reached within the 14 days of the experiment, the CPF concentration increase rate in fish tissue notably slowed down over time (Fig. 1A).

For HCB, the steady state in the body concentration was reached within the time of the experiment (Fig. 1B). The concentration in the HCB only treatment was higher compared to the HCB with MP treatment. This was expected based on the experimental design, as less HCB was added to the water containing MPs.

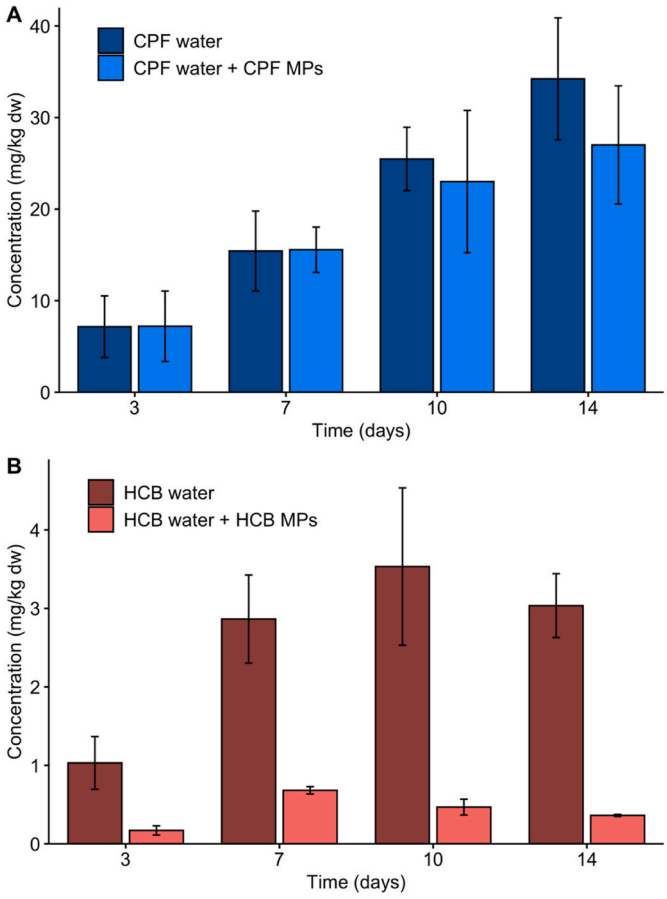

Fig. 1. Concentration of (A) chlorpyrifos and (B) hexachlorobenzene measured in fish tissue exposed only through water or through the combination of water and MPs over a period of 14 days. The values show the mean of the three measurements per treatment on sampling days 3–10 and the six measurements on sampling day 14 with their corresponding Standard Deviation.

3.3. Bioconcentration factors

In general, the BCF of CPF was higher in the CPF only treatment compared to the CPF with MP treatment, showing statistically significant differences on day 14 relative to the start of the experiment (Fig. 2A). The BCF for the HCB only treatment was also higher (about one order of magnitude) as compared to the HCB with MP treatment, showing statistically significant differences for the entire exposure period (Fig. 2B).

3.4. Biomarkers

The measurements of the enzymatic activities are shown in Fig. 3. No statistically significant differences between the control fish samples taken at the start of the experiment (day 0) and the control fish samples taken at the end of the experiment (day 14) were observed. The different chemical and MP treatments were only sampled at the end of the experiment; thus, the following enzymatic activity comparisons refer to statistical tests performed between the different chemical and MP treatments and the control samples taken at the end of the experiment.

The results of the ANOVA show that AChE was significantly inhibited by CPF, while there was no significant interaction between the AChE activity measured in the CPF only treatment and the CPF with MP treatment. No statistically significant differences were observed between the AChE activities of HCB only and the control, neither with those in the HCB with MP treatment (Fig. 3A).

The GST activity was not influenced by CPF nor by the treatment

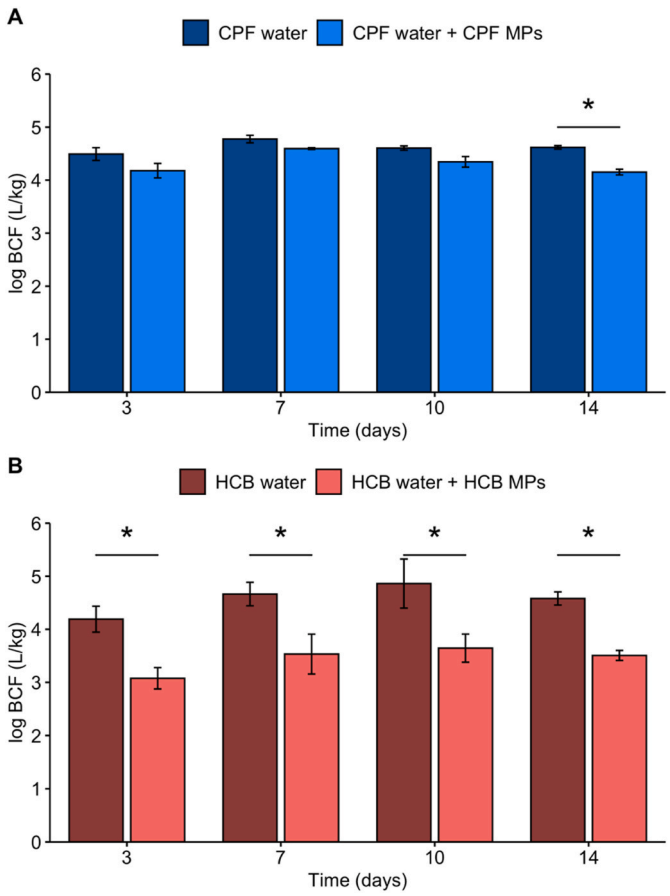

Fig. 2. Bioconcentration factors of (A) chlorpyrifos and (B) hexachlorobenzene for fish exposed only through water or through the combination of water and MPs over a period of 14 days. The values show the mean of the three measurements per treatment on sampling days 3–10 and the six measurements on sampling day 14 and the corresponding Standard Deviation. Asterisks indicate statistically significant differences ($p < 0.05$) between treatments in each sampling time.

containing CPF and MPs. The GST activity was significantly decreased by the HCB treatment, and the treatment containing HCB and MPs, however there were no significant differences between these two treatments (Fig. 3B).

The exposure to HCB and CPF alone, and to MPs alone, resulted in a slight (not significant) reduction of the ALP production, however the combination of the test chemicals with MPs increased the ALP production, becoming statistically significant for the HCB with MP treatment (Fig. 3C).

All treatments, except MP alone at the higher concentration, produced a statistically significant increase of CAT. For both chemicals, the presence of MPs induced a slight increase in the enzyme's activity, however the differences were not statistically different compared to the chemical only treatments (Fig. 3D).

4. Discussion

The presence of MPs in contaminated water did not enhance but rather decreased the bioconcentration of both tested chemicals in fish compared to the absence of MPs. Therefore, our results show that direct water uptake (via skin and gills) is a more relevant uptake route for fish compared to the ingestion of contaminated MPs, even though MPs were present at higher concentrations than those reported in the environment. Therefore, under environmentally relevant conditions, the MP portion taken up by fish would be lower, which further reduces the importance of MPs for the chemical uptake.

These findings are in agreement with other experimental studies which observed that the surrounding medium is more important for the uptake of chemicals compared to the uptake along with MPs. In these studies, MPs either did not affect the bioaccumulation of polychlorinated biphenyls (Besseling et al., 2017) or the bioconcentration 4-n-Nonylphenol and 4- Methylbenzylidene-camphor (Beiras et al., 2019), or decreased the bioconcentration of the triazole fungicide difenoconazole (Li et al., 2021) compared to chemical exposure only through the surrounding medium. Chemical uptake via gills and skin goes directly into the bloodstream, while ingested chemicals may be partly metabolized in the intestinal tract prior to uptake into blood (e.g., Pickford et al., 2003). Nonetheless, also food is likely a more relevant uptake route compared to MPs, particularly for chemicals with high biomagnification potential (i.e., persistent, not eliminated or reduced through metabolism by the organism). Higher transfer of contaminants from dried fish food compared to MPs has been shown in marine amphipods (Scopetani et al., 2018). During digestion, the substances that are accumulated in the food are released into the digestive tract while MPs are non-digestible and thus may maintain their sorption capacity (although possibly influenced by the digestive fluids, e.g., Bakir et al., 2014; Coffin et al., 2019) and therefore their chemical load.

Previously a "cleaning effect" following plastic ingestion has been proposed by experimental and modeling studies (Gerdes et al., 2019; Gouin et al., 2011; Mohamed Nor and Koelmans, 2019). Gouin et al. (2011) predicted that for chemicals with a log K_{OW} between 6.5 and 7.5, the chemical body burden can be reduced by >20%, if the diet is composed of 10% MP. The underlying principle is that the ingestion of clean MPs and their high sorption affinity for organic chemicals favor the transfer to plastic within the gastrointestinal tract and decreases the body burden (Gouin et al., 2011). This was also shown by the modeling study of Mohamed Nor and Koelmans (2019), where polychlorinated biphenyls were estimated to be transferred from contaminated food to clean polyethylene MPs in a simulated gut condition. However, these authors also observed that, in an environmentally relevant chemical and MP exposure scenario, the presence of plastic might not influence the chemical uptake (Mohamed Nor and Koelmans, 2019). This is supported by an experimental study, in which no higher elimination rates of polychlorinated biphenyls by rainbow trout were observed if clean polyethylene microspheres were ingested with contaminated food compared to the fish that did not ingest plastic (Rummel et al., 2016). Both sorption and desorption of chemicals to and from MPs in the organism's gut depend on the gut retention time; therefore, a short gut retention time may not allow sufficient time to transfer chemicals from or to biota (Chua et al., 2014; Mohamed Nor and Koelmans, 2019). In our study, we did not observe a temporal increase of the number of MP particles inside the fish gut in the treatment containing CPF with MPs, indicating that MPs were not accumulated and that the gut retention time of CPF was not prolonged. A slightly different pattern in ingested MPs was observed for the HCB with MP treatment, which was very low during the first two sampling days and increased slightly on day 10 and 14.

Besides the potential "cleaning effect" described above, it has been suggested that MPs can lower the bioconcentration/bioaccumulation potential of chemicals because they can alter the equilibrium between biota and the exposure media, reducing chemical bioavailability (Chua et al., 2014; Rehse et al., 2018; Teuten et al., 2007). From the available bioconcentration results, it was difficult to decide whether MPs facilitated a higher depuration from the organisms by sorbing chemicals in the digestive tract or if MPs sorbed a higher proportion of chemicals already from the water and made them therefore less available, or a combination of both. However, based on the measured concentration of chemicals on MPs and in the test medium, the K_{PW} calculated for both CPF and HCB was much higher in the test medium compared to the ones calculated in the stock suspensions (Table S11), suggesting reduced availability of the chemicals. Such an increase in the K_{PW}, may have been influenced by the different physico-chemical conditions (i.e., pH,

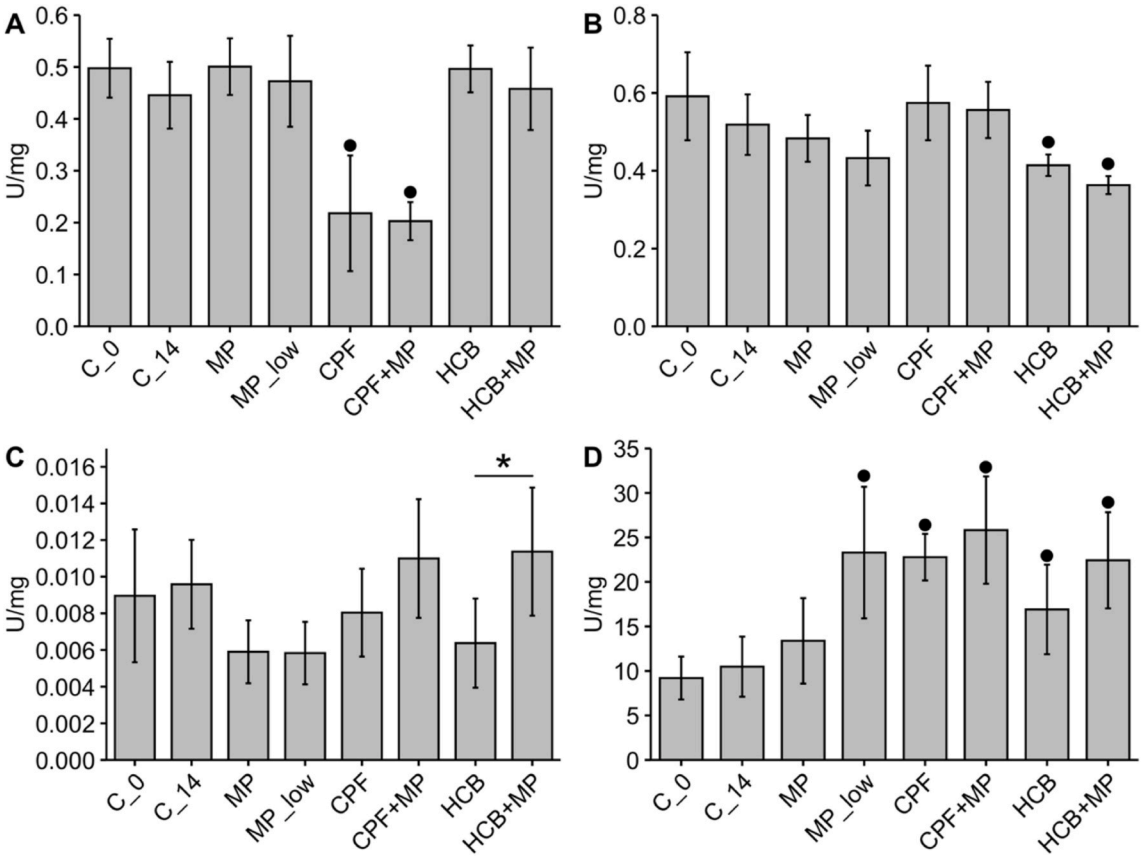

Fig. 3. Enzymatic activities of (A) acetylcholinesterase, (B) glutathione S-transferase, (C) alkaline phosphatase, and (D) catalase to the different treatments. The graphs show mean enzymatic units "U" per mg protein with the corresponding standard deviation. Dots indicate statistically significant ($p < 0.05$) differences between C_14 and all other treatments. The asterisk indicates statistically significant ($p < 0.05$; Tukey post hoc multiple comparison test) interaction between the treatments containing HCB and the treatment containing HCB and MP. C_0: control at the start of the experiment; C_14: control at the end of the experiment; MP_low: MP only treatment containing 5 mg MP/L; MP: MP only treatment containing 100 mg MP/L.

temperature, concentration of dissolved carbon) of the test medium as discussed in several studies (Seidensticker et al., 2019; Velzeboer et al., 2014; Wang et al., 2020; Wang and Wang, 2018). Moreover, the decrease in bioconcentration in presence of MPs was higher for HCB, which is the most hydrophobic chemical of the two tested. This supports the already established relationship between hydrophobicity and the sorption affinity of chemicals to MPs (Razanajatovo et al., 2018). HCB was less available to fish because it was bound strongly to MPs, indicated by the higher log K_{PW} (Table S11).

In our study, we evaluated two different environmental scenarios: the presence of contaminated MPs in contaminated water (with CPF as example), where a theoretical equilibrium between MPs and water was reached, and the addition of contaminated MPs into water with a low exposure concentration (with HCB as example). The later represents a scenario in which MP are transported from contaminated areas to remote environments. Our experiment showed that even in a scenario of low water exposure concentration (i.e., the HCB one), MPs did not increase the bioconcentration of chemicals in fish. Furthermore, as contaminated MPs were added to water with a very low background exposure concentration, desorption of HCB from MPs occurred and a new equilibrium between the MPs and water was reached, which would also happen under natural conditions.

Our study showed that the exposure to MPs alone, at two different concentrations, had no significant effects on the tested biomarkers, with exception for the CAT response to the lower MPs concentration. Most studies assessing effects of MPs on biomarker response were carried out using small MPs (≤20 μm). These showed that MPs can alter biomarker responses including oxidative stress, neurotoxicity, and inflammatory effects depending on the MP size, dose and the exposure time (Karami et al., 2017; Prokić et al., 2019). The here observed influence of CPF on AChE was expected as AChE is an indicator for neurotoxicity and its activity is usually decreased in the presence of organophosphate and carbamate insecticides (Amiard-Triquet et al., 2013). However, MPs (despite slightly reducing CPF internal concentrations) did not alter the fish response to CPF. CAT and GST are both indicators for oxidative stress. GST is involved in detoxification processes of xenobiotics (Nimmo, 1987) and although a decreased activity of this enzyme was observed in fish exposed to HCB alone, the presence of MP did not significantly affect the inhibitory effect of HCB. Similar to our results, no effect of MPs on the AChE and GST activity were found in juveniles of the common goby *Pomatoschistus microps* when exposed to pyrene (Oliveira et al. 2013). CAT belongs to the first line defense enzymes and protects cells from oxidative damage. Its activity was increased by both chemical treatments, indicating a stress condition. However, the presence of MPs did not induce significantly higher levels of CAT, suggesting that MPs had no influence on chemical stress. Finally, ALP is a plasma membrane-bound enzyme which is involved in phosphate hydrolysis. There was a trend of a slight reduction in ALP activity compared to the control by exposure to any of the tested chemicals, and by MPs alone at both tested concentrations. Fish exposed to HCB with MPs showed a significantly higher ALP activity compared to the fish exposed to HCB only. This trend was also observed for CPF. Food intake and food quality have previously been reported to cause changes in intestinal ALP (Lallès, 2020). MP ingestion may have led to food dilution (Amariei et al., 2022) and thus affected the ALP activity. However, in this study, the ALP activity was measured in the whole-body extract of the fish, therefore, the

alteration may also be attributed to ALP changes in other body parts (e.g., in blood or liver). Similar to our results, the ALP activity in the body tissue of juvenile Amazonian cichlids (*Symphysodon aequifasciatus*) was significantly reduced at 200 μg/L of MPs, which the authors attributed to deficits in digestive capabilities (Wen et al., 2018). A significant increase in ALP activity was measured in the blood of the common carp (*Cyprinus carpio*) after exposure to MPs alone (2 mg/L), the herbicide paraquat alone, and a combination of both (Nematdoost Haghi and Banaee, 2017). These authors attributed the increase of ALP in plasma to the damage of cell membranes in the liver, bile ducts, and damage to the mucous lining of the intestine and the renal tract.

5. Conclusions

Our study shows that the presence of MPs in water exposure media does not increase the bioconcentration of hydrophobic organic chemicals in fish. This indicates that MP ingestion could be considered a negligible chemical uptake route as compared to other exposure routes, supporting the findings shown by former experimental and modeling studies. No consistent trends on the influence of MPs on the fish enzymatic activities could be elucidated from this study. Therefore, we conclude that MP concentrations above current exposure levels are not likely to increase fish stress levels, neither increase hydrophobic organic chemical exposure and risks to fish in freshwater ecosystems.

Declaration of competing interest

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

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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.2022.119473.

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