Interactive Effects of Microplastics and Tetracycline on Bioaccumulation and Biochemical Status in Jian Carp (Cyprinus carpio var. Jian)

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Microplastics (MPs) and tetracycline (TC) are severe emerging pollutants in the aquatic environment. But there is a lack of research to investigate the interactive effects of MPs and TC in vivo. This study used Jian carp (Cyprinus carpio var. Jian) as the model organism to explore the bioaccumulation and biochemical status when exposed to MPs and TC, alone and combined. The accumulation of TC and MPs in intestine, variation of enzyme activities in intestine, and expression of immune-related genes in muscle were evaluated. Our results found the bioaccumulation of MPs was not affected by TC, but the presence of MPs would change the content of TC within 48 h. The superoxide dismutase (SOD) and lactate dehydrogenase (LDH) activity showed that TC-MP combined exposure could reduce oxidative damage to Jian carps compared to MP exposure alone. The integrated biomarker response (IBR) index showed that SOD activity was sensitive to TC-MP exposure. In addition, co-exposure to MPs and TC could alleviate the overexpression of interleukin 1 beta (IL-1β), interleukin 10 (IL-10), transforming growth factor beta (TGF-β), and toll like receptor 2 (TLR-2) induced by TC in muscles. TLR-2 gene has the potential to be the candidate gene reflecting the injury of TC exposure. In conclusion, it is inferred that co-exposure may reduce the toxicity of individual exposure in the living organisms. This study provides essential information for the risk assessment of pollution with MPs and TC, individually and combined, as well as a foundation to investigate the interactive effects of MPs and antibiotics on aquatic ecosystems.

Keywords: microplastics, tetracycline, Jian carp, bioaccumulation, antioxidant response

INTRODUCTION

Modern life seems to be inextricably linked to the use of plastics. Plastics have become an indispensable solution to meet the ever-changing needs of society. Global production nearly reached 370 million tons (Mts) in 2019 at an average annual rate of 3.1% (Plastics Europe, 2020). However, the extensive use of plastics has given rise to millions of plastic wastes being discarded into the environment (Cózar et al., 2014; Jambeck et al., 2015). Plastic pollution has been an urgent challenge nowadays, which cause severe damage to various ecosystems, both directly and
indirectly, especially as the main source of microplastics in aquatic ecosystems and organisms (de Sa et al., 2018; Fu and Wang, 2019; Jacob et al., 2020; Zhou et al., 2021). Microplastics (MPs), originating from both primary and secondary sources, are tiny plastic particles of less than 5 mm in size (Thompson et al., 2004; Cole et al., 2011). They are not only ubiquitously distributed in marine and freshwater ecosystems (Beaumont et al., 2019; Sarijan et al., 2021), atmosphere (Zhang et al., 2020a; Chen et al., 2020), and soil (He et al., 2018; Wang et al., 2020) but also found in the most remote habitats (Morgana et al., 2018). For example, 1,190 synthetic polymers <5 mm from sea ice cores and 125 synthetic polymers from surface water samples were detected in the Arctic Central Basin (Kanhai et al., 2020). In China, MPs are pervasive in surveyed freshwater environments, and the detected MPs mainly show smaller size (<1 mm), fibers, and transparency in freshwater ecosystems (Fu and Wang, 2019). Wright et al. (2020) found that the atmospheric deposition rates of MPs in atmosphere in central London were 771 ± 167 particles/m²/d, and fibers were the overwhelming majority (Wright et al., 2020). To sum up, MP pollution is drawing attention on a global scale.

MPs may be ingested by various organisms because of their small size and/or similar food shape, including fish (Jovanovic, 2017), shellfish (Ding et al., 2020), birds (Carlin et al., 2020), and mammals (Hernandez-Gonzalez et al., 2018). Adverse effects caused by MP ingestion on organisms have been extensively demonstrated. For example, the activities of SOD and catalase (CAT) in zebrafish were significantly increased by polystyrene MP exposure, indicating that oxidative stress could be caused by MPs (Lu et al., 2016). Further, if exposed to MPs at environmentally relevant concentrations, marine medaka (Oryzias melastigma) could delay gonad maturation, decrease fecundity, and negatively regulate female HPG axis in female fish (Wang et al., 2019). What is more, MPs can absorb many other pollutants, potentially altering their environmental fate and ecological impact, and producing multiple forms of toxicity after being ingested (Barboza et al., 2018; Trevisan et al., 2019; Zhou et al., 2020). Many studies have reported that owing to their strong hydrophobicity and large specific surface area, MPs can adsorb heavy metals (Breenecke et al., 2016; Mao et al., 2020), polycyclic aromatic hydrocarbons (PAHs) (Kleinteich et al., 2018; José and Jordao, 2020), polychlorinated biphenyls (PCBs) (Llorca et al., 2020), pharmacologically active compounds (PHACs) (Li et al., 2018), and so on. For example, the existence of MPs could influence the metabolism of roxithromycin (ROX) in red tilapia (Oreochromis niloticus), but co-exposure mitigated oxidative damage in fish livers (Zhang et al., 2019). Trevisan et al. (2019) indicated that nanoplastics promoted the sorption of PAHs from the exposure medium, increased the agglomeration rate of nanoplastics, and decreased the bioavailability and bioaccumulation of PAHs. Although research on combined MPs and other contaminants is in progress, reports about the interactive effects between MPs and pharmacologically active compounds (PhACs) is still insufficient, due to the diversity of biological types and the lack of standard dose of pollutants.

Tetracycline (TC) is one of the major categories in PhACs. As an ionizable and polar antibiotic, TC plays an important role preventing and treating diseases in livestock farming, although most of it cannot be absorbed by livestock, thus draining into the environment (Fu et al., 2021; Scaria et al., 2021). Our research team previously found that the contents of tetracyclines were significantly higher than that of sulfonamides in Guangdong coastal areas, ranging from 0.26 to 81.54 ng/L (Xu et al., 2019). Recent studies confirmed that MPs could adsorb TC on the surface mainly through an ion exchange mechanism (Zhao et al., 2021), owing to the surface properties of MPs and chemical characteristics of the aqueous solution (Wan et al., 2019; Yu et al., 2020). The depletion of the bees’ gut microbiota using TC dramatically increased the lethality of MPs (Wang et al., 2021). MPs compound with TC caused gastric cancer cell damage under 24 h exposure (Yan et al., 2020). Oral exposure to MPs and TC resulted in significant bioaccumulation of TC in Enchytraeus crypticus, increased the anti-resistance gene (ARG) diversity and abundances, and significantly perturbed the balance of microbiome (Ma et al., 2020). However, the potential toxicological impact and ecological risk of the combination of MPs and TC, especially on vertebrates, still need further study to be understood.

The present study aims to evaluate interactive effects of MPs and TC on bioaccumulation and biochemical status in fish. In this study, the common freshwater carp (Cyprinus carpio var. Jian) was used as the model organism. Jian carp is the first artificially bred aquatic species in China and has been an important economic fish species cultured nationwide (Gu et al., 2015). The effects of fluorescent polystyrene microplastics (PS-MPs; average diameter: 5 μm; concentration: 700 μg/L) and TC (concentration: 1 μg/L) were investigated. Selected materials and concentrations were based on the results of our previous experiment (unpublished data). The accumulations of TC and MPs in intestine, variation of enzyme activities in intestine, and expression of immune-related genes in muscle were evaluated. Our results provide important information for the risk assessment of pollution caused by MPs and PHACs on fish, as well as form a foundation to investigate the interactive impacts of MPs and antibiotics on aquatic ecosystems.

MATERIALS AND METHODS

Chemicals

The green fluorescent polystyrene microspheres (PS-MPs; inspire: 488 nm, launch: 518 nm) with the average size of 5 μm were bought from Da’E Scientific Co., Ltd. (Tianjin, China). Fluorosphere dyes were contained in PS-MPs, rather than adhere to the surface. Accordingly, the dyes of the potential effects on experimental were negligible (Zhang et al., 2019). PS-MPs were stored in the dark at 4°C and treated with ultrasound before application. Tetracycline (TC; analytical grade; purity >98%) was bought from Heowns Biochemical Technology Co., Ltd. (Tianjin, China) and stored in the dark at 4°C. The chemical stock solution was prepared in ultrapure water with concentration of 0.389 mg/ml.
**Animals and Exposure Test**

Healthy Jian carp were bought from an aquafarm (Shunde, Guangdong, China) and acclimated in 50 L glass aquariums in College of Marine science, South China Agricultural University for 2 weeks. Fishes were fed twice per day with 3–6% commercial feeds of their body weight until 3 days before the start of the test. During the experiment, MPs and TC were added according to the experiment design in corresponding tanks. We set up the following four experimental groups: the control group (dechlorinated circulating water), TC exposure group (1 μg/L), TC-MP exposure group (700 μg/L PS-MPs + 1 μg/L TC), and MP exposure group (700 μg/L). The selected concentrations were suitable for the detection of the variation of physiological and biochemical indexes in fish and the minimum value for instrument. In addition, our research team previously investigated the contents of tetracyclines and MPs in the field (Xu et al., 2019; Zhang et al., 2020b). The chosen concentrations were based on our field investigation results and laboratory simulation verification (Zhang et al., 2021). Aeration was set in each experimental group to prevent the uneven distribution of MPs and TC. After being starved, 120 carp (3.57 ± 0.25 cm in body length, 0.96 ± 0.25 g in wet weight) were randomly placed in four 50 L glass tanks containing 30 L test solutions (temperature 25.0 ± 2.0°C; pH 7.5 ± 0.3; dissolved oxygen >6.0 mg/L). During the test, water was not changed.

After 48 and 96 h exposure, four fishes were taken from each group and rinsed to remove surficial body particles. Three replicates were performed for each treatment group. The weight and length of sampled fish were measured and recorded, then the fishes were sacrificed to recover the intestine, liver, and muscle. The above processes were carried out while the fish were anesthetized, and animal welfare was considered. The sampled tissues of Jian carp were stored at −80°C for further studies. The enteric samples were used for enzyme activity assay and bioaccumulation measurements of MPs and TC. The sarcous samples were used for the detection of quantitative polymerase chain reaction (qPCR). Due to the light weight of the liver, hepatic samples were synthesized to detect the content of TC in each group.

**Determination of PS-MP Concentration**

The concentration of PS-MPs in intestinal samples was analyzed according to Van Cauwenberghhe and Janssen (2014) and Van Cauwenberghhe et al. (2015) with some slight modifications. To be specific, intestine samples were weighted and digested in 1 ml KOH (10%, v:v) at 60°C for 24 h. After complete digestion, 10 μl digestion solution was added in the hemocytometer BX-K-25 (Shanghai Qiujing Biochemical Reagent Instrument Co., Ltd.) and the number of MPs was counted under a polarizing microscope (Model Eclipse E200; Nikon, Inc., Japan) equipped with the MShot Image Analysis System 1.1.4. MP concentration was expressed as the number of MPs per gram of intestinal wet weight (particles/g).

**Determination of TC Concentration**

On the basis of Ding et al. (2016), a slight modification was made to improve the method of sample preparation and extraction. Specifically, each intestine and liver sample was homogenized with 1.5 ml methanol in the high-throughput tissue grinder (Shanghai Jingxin Industrial Development Co., Ltd.) at 4°C. After centrifuging at 4,000 rpm for 15 min at 4°C, the supernatant was transferred to a clean centrifuge tube for analysis of TC concentration using an ultra-high-performance liquid chromatography-tandem mass spectrometer (UPLC/MS/MS, Uplc1290-6470A, Agilent, United States). The TC concentration was expressed as ng/g in wet weight.

**Biochemical Analysis**

In the application of biomarkers, glutathione (GSH) content, SOD, and LDH enzyme activity in the intestine were applied to evaluate oxidative damage at the protein level. The enteric samples in each treatment group were homogenized with ice-cold 0.9% saline solution (1 g: 9 ml) with a high-throughput tissue grinder (Shanghai Jingxin Industrial Development Co., Ltd.). After centrifuging at 3,000 g for 10 min at 4°C, the supernate was transferred to the clean centrifuge tube for the analysis of biomarkers by using microplate test kits following the manufacturer’s instructions (Nanjing Jiangcheng Bioengineering Institute, China). All the above indexes were detected by a microplate reader (Synergy™ HTX Multi-Mode Microplate Reader, Biotek, VT., United States) and analyzed by the Gen5 software (Gen5 CH5 3.03., Biotek, VT., United States). Three replicates were performed.

**Target Gene Expression Analysis**

The expression levels of genes related to IL-1β, IL-10, TGF-β, and TLR-2 in muscle were applied to evaluate immune stress at the gene level. The experimental methods of RNA extraction and cDNA synthesis are presented in Supplementary Text S1. The cDNA was stored at −80°C until further analysis. The expression levels of the genes were quantified via RT-PCR assay. Details of the RT-PCR materials and program are presented in Supplementary Text S2. With 18s as the internal standard gene, the selected gene was amplified with specific primers. Specific primer sequences are listed in Table 1 (Meng et al., 2021).

**Statistical Analysis**

All data were quantified as mean ± standard deviation (SD). The statistical significance between the control group and the

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**Table 1** List of gene primers used for qPCR.

| Fish | Genes | Sequence, forward/reverse (5’–3’) |
|------|-------|---------------------------------|
| Jian carp | 18S | F: CTGAGAAAACGCTCCATTTTC<br>R: GGCCTGAAAGAGAACCCTGTATTG |
| | IL-1β | F: GAGTGAAGCTGACAAACAAAA<br>R: GTGGGAGCTGACTGCAGATTAAT |
| | IL-10 | F: CTCCGTTCTGCACTACAGAGAAA<br>R: TCATGACGTCAGACAGGATAAG |
| | TGF-β | F: AGGTTCAGATGTGTTCAAG<br>R: GCCACTCTTTTGTGAGGGATA |
| | TLR-2 | F: GTGCTCCCTGAGATGTGTATCT<br>R: TGGAGTGTGCACACATAATAG |
experimental group was analyzed by one-way ANOVA with Tukey’s post-hoc test using the SPSS 17.0 (SPSS Inc., United States). Differences were considered significant at $p < 0.05$ and highly significant at $p < 0.01$. IBR was the integration of all measured biomarker responses into one general "stress index" (Beliaeff and Burgeot, 2002). The specific method of IBR calculation (Broeg and Lehtonen, 2006) can be found in Supplementary Text S3. The relative gene expression heat map was produced by GraphPad Prism 8 (GraphPad Software, San Diego, CA, United States) software.

RESULTS AND DISCUSSION

Bioaccumulation of PS-MPs

During the exposure period, no acute toxicity, such as mortality or abnormalities of the treated fish, was observed. Besides, no MPs were observed in fish intestines of control group or TC exposure group. The average concentration of MPs in water was about $10^6$ particles/L. In the intestine of Jian carp, the average concentration of MPs in TC-MP exposure group in 48 and 96 h was $2.29 \times 10^3 \pm 5.86 \times 10^2$ and $5.93 \times 10^3 \pm 2.88 \times 10^2$ particles/g, respectively. The concentration in MP exposure group was $2.51 \times 10^4 \pm 4.82 \times 10^3$ and $4.55 \times 10^4 \pm 8.92 \times 10^3$ particles/g in 48 and 96 h, respectively (Figure 1A). The concentration of MPs in the treatment groups exposed for 96 h was 2–3 times higher than that of the treatment groups exposed for 48 h ($p < 0.05$). The above results indicated that MPs in intestine gradually increased with time at both groups with MPs, and TC had barely effect on the accumulation of TC in intestine. MPs are synthetic hydrophobic polymer with high molecular weight, which are difficult to be metabolized or degraded by organisms (Rist et al., 2017). MPs easily accumulate in the gastrointestinal tract of organisms, and the accumulation increases over time until an equilibrium state is reached (Zhang et al., 2019). Huang et al. (2021) also found that the accumulation pattern of MPs in fish did not vary with the presence of antibiotic. The accumulation of MPs in the intestine is most likely related to their physical properties (e.g., size and shape, difficulty in degradation, etc.) (Hirt and Body-Malapel, 2020; Huang et al., 2021).

Bioaccumulation of TC

The accumulation of TC in intestine and liver of Jian Carp was detected at two exposure times (48 and 96 h). The average concentrations of TC in the intestines were $206.00 \pm 54.44$, $21.37 \pm 7.56$, $30.50 \pm 3.65$, and $16.91 \pm 7.99$ ng/g, corresponding to TC-48 h, TC-96 h, TC + MPs-48 h, and TC + MPs-96 h treatments, respectively (Figure 1B). The presence of MPs extremely reduced the enteric accumulation of TC in the short-term ($p < 0.01$). It is reported that the absorption of TC in fish is mainly through gill adsorption and oral administration (Zhang et al., 2019). The basic structure of TC consists of a hydronaphthacene nucleus containing four hexacyclic fused rings, which facilitates the passage of TC through biological membranes into the organism (Dong et al., 2012). Xu et al. (2020) reported that drug absorption occurs mainly in the foregut and midgut, but may also occur to some extent in the hindgut. Normally, TC is attached to the intestine for transmembrane transport, but the presence of MPs may alter the distribution of TC in vivo. MPs have adsorption properties for antibiotics due to the presence of porous polymer, spherical protrusions and micropores on the surface, and internal cross-section (Hirt and Body-Malapel, 2020). The establishment of hydrogen bonds and adsorption of MPs may be related to the fact that TC have multipolar functional groups, such as ketone, hydroxyl, and amino groups (Shen et al., 2018). Feng et al. (2020) revealed that the surface charge of MPs that adsorb TC affected the toxicity of TC to cells. In brief, MPs can act as carriers of TC into fish and may change the fate and toxicological effect of TC.

We also analyzed the concentration of TC in fish liver based on limited samples with tiny weight. The concentration of TC in liver of Jian carp was $41.85, 97.02, 29.63$, and $20.61$ ng/g, corresponding to TC-48 h, TC-96 h, TC + MPs-48 h, and TC + MPs-96 h treatments, respectively (Supplementary Figure S1). According to the results of TC concentrations between intestine and liver, we assume that TC in intestine might be transferred to the liver in some way (such as intestinal absorption and penetration) after 48 h exposure. Liver is an important storage organ, and its main function in fish is detoxification. The accumulation of TC facilitates detoxification through some defense mechanisms (Nunes et al., 2015). We found that the
distribution of TC in vivo was influenced by the presence of MPs within 48 h. During the same exposure time, TC exposure group showed high concentration of TC in the intestine, while TC + MP exposure group showed high concentration of TC in the liver. MPs acted as adsorption carriers and reduced the bioaccumulation of chemical contaminants (Tourinho et al., 2019). We speculated that MPs transported TC to other tissues in the metabolic manner. However, our results only indicated the presence or absence of MPs, corresponding to a high accumulation of TC in the intestine or liver in the short term, and whether there is a substance transfer mechanism between them still needs to be investigated in depth.

Antioxidant Responses

It is well known that SOD is considered as the first-line defense against oxidative stress, which contributes to the conversion of reactive oxygen species (ROS) to harmless metabolites (Xie et al., 2016; Neamat-Allah et al., 2019). LDH, which is essential for cellular respiration, is released into the blood by damaged or stressed tissues. Gholamhosseini et al. (2020) found that when exposed to infection or stressed conditions, lower LDH activity might be beneficial to fish. The generation of endogenous antioxidants like SOD and GSH is recognized to neutralize toxic free radicals, as to maintain redox hemostasis and normal cell function (Abdel Mageid et al., 2019; Abdel-Daim et al., 2019). The rising level of GSH is possibly the primary mean of preventing generation of lipid hydroperoxide (Carmo et al., 2019).

SOD and LDH activities, as well as GSH content in the intestines of Jian carp in each group are presented in Figure 2. After 96 h of exposure, the MP group showed significant upregulation in SOD and LDH activity (p < 0.05), indicating that MPs could induce enteric oxidative stress in Jian carp. In contrast to the MP exposure group, the TC-MP and TC exposure groups showed no significant variations. Multiple evidence suggested that imbalance between ROS production and antioxidant defense would lead to oxidative damages, which related to the poisoning of aquatic organisms (Kaya et al., 2015; Giordo et al., 2020). Yonar (2012) found that oxytetracycline could lead to a significant reduction of SOD activity in a rainbow trout study. Nunes et al. (2015) showed that TC caused different toxic phenomena, including oxidative stress and neurotoxicity. Although antibiotics might cause oxidative stress by reducing the antioxidant capacity, our results did not find significant oxidative stress indicators in TC exposure group. It implies that TC is not the main factor causing oxidative stress in 96 h. Combined with the results above and Figure 1, we speculated that the presence of MPs alone caused oxidative stress in aquatic organisms. Our results revealed that the combination of TC and MPs alleviated the oxidative stress in the intestine of Jian carp and protected the fish from oxidative damage to some extent. A potential explanation is that TC could be adsorbed by MPs and then weaken the toxicological effects of MPs. Zhang et al. (2019) also revealed that after 14 d of exposure in red tilapia, co-exposure to MPs and ROX mitigated oxidative damage in fish livers. Therefore, the studied compounds might have a synergic effect in vivo.

The IBR index scores the response of multiple biomarkers and summarizes them into a single value for assessing the toxicological impact of contaminants. Huang et al. (2021) compared the contamination stress of red tilapia by the interaction of selected pharmaceuticals with MPs using the IBR index. It is recognized that the IBR index serves as a straightforward and valid tool, which clearly describes the health status of the organism. In this study, IBR was used to visually compare the stress among various treatments of Jian carps by integrating the multi-biomarker responses (SOD, LDH, and GSH) for 96 h of exposure (Figure 3A). The IBR values of SOD in TC-MP combined exposure group were higher than those of MP exposure group, indicating that SOD activity was more sensitive to the co-exposure. All the studied biomarkers of fish were significantly altered due to exposure to MPs, but SOD activity was the most fluctuant biomarker in TC-MP group. The estimated value of SOD remained high level throughout the study, which was a feedback to cope with oxidative stress exerted by MPs efficiently. In summary, seeking for the sensitive biomarker of pollutants should be further studied, and the effort will help
to estimate the interaction between MPs and other PhACs on aquatic organisms.

**Immune-Related Gene Expression**

Cytokines are considered to be an important regulator in the fish immune system (Zou and Secombes, 2016). The regulation of inflammatory response is the integration of stimulating and inhibiting signaling pathways in the immune system, which is a complex response to a variety of stimuli like pathogens and/or tissue damage (Yang et al., 2014). Therefore, the expression level of IL-1β, IL-10, TGF-β, and TLR-2 can reflect the inflammation of the organisms. In this study, sarcous mRNA expression profiles of the above genes in Jian carp after exposure of 96 h are shown in Figure 4. The relative expression levels of all detected genes only in TC exposure group were significantly upregulated ($p < 0.01$), while genes in TC-MP exposure group and MP exposure group were not.

The indicators of antibiotic toxicity are morphological and genetic changes. Studies on the genotoxicity caused by antibiotics in fish are generally concerned with the transcriptional regulation of antioxidant enzymes in muscle (Yang et al., 2020). Based on the changes of these indexes in fish tissues induced by antibiotics, it is speculated that antibiotics inhibit the survival, development, and hatchery rate of fish mainly by disrupting the intracellular redox balance and inducing oxidative stress. For example, oxytetracycline regulated the expression of IL-1β and TNF-α in the intestinal tract and liver of Nile tilapia (Limbu et al., 2018). Yu et al. (2019) revealed that chlortetracycline (CTC) or oxytetracycline (OTC) significantly impaired the antioxidant capacity in zebra fish larvae. Our study found that TC induced upregulation of immune-related genes after 96 h exposure in the muscle of Jian carp. Interestingly, the presence of MPs reduced such stress to some extent, possibly by changing the migration, accumulation, and metabolism of TC in fish. We speculated that 5 μm PS-MPs might adhere TC and prevent it from functioning in muscles. Zhang et al. (2019) also found that the presence of MPs affected metabolism of ROX in tilapia according to the variability of cytochrome P450 (CYP) enzyme activities in fish livers.

The heat map visually displayed the sensitive degree of relative expression of the target genes (Figure 3B). Results showed that TLR-2 gene had the potential to be the candidate gene reflecting the injury of TC exposure. TLR-2, as a pattern recognition receptor (PRRS), directly involves in the recognition of specific pathogen-associated molecular patterns (PAMPs) and activates proinflammatory cytokines in fish, which is important in initiating immune immobilization (Noor-Ul et al., 2020). Laboratory studies into the mechanism of combined antibiotic and MP exposure in fish are still in the early stages. More researches are needed to meet the challenge in future environmental toxicity studies.
CONCLUSION
This study investigated the interactive effects of microplastics and tetracycline on the bioaccumulation and biochemical status in Jian carp. Results revealed that accumulation of MPs was not affected by TC, but the presence of MPs changed the content of TC in vivo within 48 h. Exposure to MPs alone increased SOD and LDH activity in intestine of fish, while co-exposure to TC-MPs mitigated the oxidative damage. The upregulation of IL-1β, IL-10, TGF-β, and TLR-2 induced by TC in muscles was alleviated in the presence of MPs. We speculated that 5 μm PS-MPs might adhere TC and prevent it from functioning in muscles. Overall, this study suggests that the fate and impact of TC and MPs are affected by each other in vivo. Co-exposure may have the synergic effect in Jian carp. Adsorption and desorption rates between TC and MPs need to be focused in the future study. MP physical properties (e.g., size, shape, materials, concentration, exposure time, etc.) and fish feature should be clarified clearly. More studies about biological interaction of MPs and antibiotics are needed to perfect the evidence.

DATA AVAILABILITY STATEMENT
The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

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ETHICS STATEMENT
The animal study was reviewed and approved by National Institute of Health Guide for the Care and Use of Laboratory Animals of China.

AUTHOR CONTRIBUTIONS
All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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