Environmental Science and Pollution Research (2022) 29:19273–19282
https://doi.org/10.1007/s11356-021-17198-8

RESEARCH ARTICLE

Combined toxicity of polystyrene microplastics and sulfamethoxazole on zebrafish embryos

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Received: 22 August 2021 / Accepted: 21 October 2021 / Published online: 29 October 2021

Abstract

Despite extensive investigation on the toxicity of microplastics (MPs), an emerging global concern, little is known about the combined toxicity of MPs and co-occurring pollutants in aquatic environments. In this study, the combined toxicity of polystyrene MPs and sulfamethoxazole (SMZ) antibiotics was explored in zebrafish embryos in terms of the developmental, physiological, and endocrine toxicities. Exposure to PS and SMZ induced mortality (rate: 25.0 ± 7.5%) and malformation (rate: 20~35%) at multiple regions and stages of zebrafish development. Physiological toxicity was also induced as shown by the significant decrease in fetal movement (by 31.1~37.0%) and swimming frequency (by 26.9~36.8%) and the increase in heartbeat rate (by 19.0~20.9%). Finally, PS and SMZ exposure also induced extensive endocrine toxicities in zebrafish as confirmed by increases in various biomarkers including vitellogenin, 17β-estradiol, testosterone, and triiodothyronine. The combination index showed that antagonistic effects were present between PS and SMZ toxicity, which slightly decreased their combined toxicity. This study aims to further understand the combined toxicity of MPs and co-occurring pollutants in aquatic environments.

Keywords Zebrafish · Microplastics · Sulfamethoxazole · Toxicity · Antagonistic effect

Introduction

As an emerging global concern, microplastics (MPs), defined as plastics with diameter < 5 mm, have been detected in aquatic environments worldwide. Though oceans are dominant sinks of most MPs with up to ~ 10^4 particles/m^3 detected (Desforges et al. 2014; Song et al. 2018), freshwater is also an important reservoir in which MPs are transported from continents to oceans. MP's abundance in rivers is up to 1 particle/m^2 (Mani et al. 2015) or 5 particles/m^3 based on different sampling and calculation methods (Scherer et al. 2020), with polystyrene (PS), polypropylene (PP), and polyethylene (PE) MPs being the most prevalent (Pan et al. 2020). In China, MPs are also frequently detected in both freshwater and seas (Xu et al. 2020). For example, the abundance of MPs is over 30 particles/m^2 in the Three Gorges Area (Zhang et al. 2017). This widespread prevalence of MPs in aquatic environments presents an urgent need to understand their toxicological effects.

MP's exposure induces toxicity in most aquatic organisms from phytoplankton and microorganisms to aquatic animals. For example, MPs affect photosynthesis and metabolism when adsorbed onto phytoplankton (Mao et al. 2018). For microorganisms, MPs may induce SOS response, elevate expression of hazardous genes (e.g., antibiotic resistance genes), and contribute to the spread of pathogens (Viršek et al. 2017; Wu et al. 2019). In aquatic animals, MP's ingestion frequently causes inflammation by being enriched in the stomach, gut, and liver and can move up food chains through bioaccumulation (Collard et al. 2017; Lu et al. 2018; Shi et al. 2021). Among aquatic organisms, zebrafish is of particular concern due to its genetic similarity with humans as well as being a model organism for toxicology (Bhagat et al. 2020). MP exposure induces multiple toxicities in zebrafish including developmental toxicity, reproductive toxicity, neurotoxicity and locomotor toxicity, immunotoxicity,

Responsible Editor: Bruno Nunes

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genotoxicity, and intestinal and metabolome damage (Bhagat et al. 2020). However, studies using biomarkers, which are usually more sensitive to toxicants than other indicators (Yin et al. 2018; Zhong et al. 2014), remain scarce.

The toxicity of MPs can be even stronger by adsorbing various pollutants in aquatic environments. MPs have been widely reported to be vectors of persistent organic matter and heavy metals (Chen et al. 2019; Dong et al. 2020b; Li et al. 2020a). Amongst all pollutants, MPs carrying antibiotics are of particular concern because of the global crisis of antibiotic resistance as well as widespread co-occurrence between them (Han et al. 2021; Zhou et al. 2020). The adsorption of antibiotics by MPs is frequently reported in various aquatic environments, with the adsorption capacity of up to 0.6 mg antibiotics/g MPs (Li et al. 2018a, b, c). Sulfonamide antibiotics are of particular concern due to their high prevalence in aquatic environments (Cui et al. 2020; Luo et al. 2011) and potential of being adsorbed by MPs (Li et al. 2018a, b, c; Wang et al. 2020b). Though the toxicity of antibiotics in aquatic organisms including zebrafish has been frequently investigated (Zhou et al. 2018), the combined toxicity of MPs and antibiotics has yet to be explored. Specifically, the interactive effect of MPs and antibiotics, which can be synergistic or antagonistic, needs to be understood better like other chemicals that have been reported (Gu et al. 2015; Na et al. 2021).

In this study, individual and combined toxicities of MPs and antibiotics in zebrafish were explored in terms of the developmental, physiological, and endocrine toxicities. Zebrafish embryos were chosen as the representative life stage of zebrafish given its high sensitivity to toxicants (van Aerle et al. 2013). Changes in endocrine-relevant biomarkers including vitellogenin (VTG), 17β-estradiol (E_2), and testosterone (T) and development-relevant biomarkers triiodothyronine (T3) were examined post exposure to explore the toxicity in the endocrine system of zebrafish. Polystyrene (PS) and sulfamethoxazole (SMZ) were chosen as the representative MP and antibiotic due to their widespread occurrence in aquatic environments (Dong et al. 2020a; Xie et al. 2020). This study will further the understanding of the combined toxicity of MPs and co-occurring pollutants in aquatic environments.

Materials and methods

Materials

Wild-type zebrafish (Tübingen line) embryos at 6 hpf (hours post fertilization) were purchased from Nanjing EzeRinka Biotech Company (China). Sulfamethoxazoles (BR grade) were purchased from Aladdin Industrial Corporation (China). Polystyrene microplastics were purchased from Tianjin BaseLine ChromTech Research Centre (China). The polystyrene is sphere-shaped with an average size of 327.3 ± 72.1 nm as determined by scanning electron microscope (Zeiss Sigma 300, Germany) and zeta particle size and zeta potential analyzer (Brookhaven Instruments Corporation, U.S.A) (Online Resource Figure S1 and S2). The density of the polystyrene sphere is at 1.06 g/cm^3.

Exposure test

Zebrafish embryos were cultivated in Petri dishes (90-mm diameter) spiked with the embryonic culture medium based on OECD TG 203 before the exposure. Since 12 hpf, zebrafish embryos were exposed to PS and SMZ individually at a series of common concentrations adopted for toxicity tests (i.e., 10 μg/L for PS, 1 μg/L, 10 μg/L, and 100 μg/L for SMZ as nominal concentrations) (Li et al. 2020b, 2021; Lian et al. 2014; Schirinzi et al. 2019; Wang et al. 2019; Xie et al. 2019). The combined exposure of PS and SMZ at corresponding regimes (i.e., 10 μg/L PS + 1 μg/L SMZ, 10 μg/L PS +10 μg/L SMZ, 10 μg/L PS + 100 μg/L SMZ, all were nominal concentrations) was conducted to examine the combined toxicity of these two substances. The group without any exposure was also conducted as the blank control. One hundred fifty zebrafish embryos were used in each well for biomarkers' measurements, while 10 zebrafish embryos were used in each well for other parameters' measurements (i.e., acute toxicity, teratogenicity, and physiological toxicity). Exposure tests were conducted in triplicates.

SMZ was quantified by high-performance liquid chromatography (HPLC) to make sure the working concentrations during exposure are close to nominal ones as detailed in Online Resource Text S1 and Table S1. During exposure, 3/4 of the culture medium was replaced every day. To maintain a stable growth environment, the temperature was fixed at 28 ± 0.5 °C and a simulative light/dark cycle (14 h/10 h) was adopted though the experiment. Dead embryos were removed as soon as possible during the exposure to prevent bacterial growth in the exposure medium.

Measurement of acute toxicity and teratogenicity

The number of dead zebrafish embryos was recorded at 48 hpf and 72 hpf in each test. The death of the embryos was defined as the following apical endpoints, including the coagulation of the embryo, non-detachment of the tail, lack of somite formation, and lack of heartbeat (He et al. 2021). The mortality rate was calculated by the ratio between the number of dead zebrafish embryos and the number of total zebrafish embryos in each test. The morphological malformation was observed at 48 hpf and 72 hpf by the biological inverted microscope and stereo microscope (Jiangnan JSZ6S, China). The malformation rate was calculated by the

ratio between the number of malformed zebrafish and the number of total Zebrafish embryos in each test.

Measurement of physiological toxicity

Four parameters related to the physiological toxicity in zebrafish including the spontaneous movement at 24 hpf, the heartbeat rate at 48 hpf, the body length at 72 hpf, and the swimming frequency at 144 hpf were determined. For the spontaneous movement at 24 hpf and the heartbeat rate at 48 hpf, five embryos were selected from each well and recorded in 1 min by biological inverted microscope, then the spontaneous movement and swimming frequency were counted from the video as also described previously (He et al. 2021; Li et al. 2018c). For the body length at 72 hpf, photos of larval were acquired by stereo microscopy (Jiangnan JSZ6S, China) and the body length was then measured by the Jifei software (Jiangnan JSZ6S, China) as also described previously (Abe et al. 2021; Zhao et al. 2021). For the swimming frequency at 144 hpf, a 1-min video of larval was acquired by stereo microscopy (Jiangnan JSZ6S, China) for each group, then the swimming frequency was counted from the video as described previously (Abe et al. 2021; Zhao et al. 2021).

Measurement of biomarkers

Vitellogenin (VTG), 17β-estradiol (E2), and testosterone (T) were measured to evaluate the toxicity in the reproductive endocrine system at the early development stage of zebrafish. Thyroid hormone triiodothyronine (T3) was measured to evaluate the toxicity in the thyroid system at the early development stage of zebrafish. Post exposure, zebrafish embryos were washed for three times by deionized water and the embryonic culture medium, respectively. Zebrafish embryos were then homogenized in 0.6% NaCl by a hand-held cell crusher (MP FastPrep-1, USA) for 10 s with 5 cycles. Suspensions were centrifuged at 8000 rpm for 10 min at 4 °C and supernatants were collected. Biomarkers were then measured using kits (i.e., VTG Elisa Kit for VTG (Shanghai Jining Shiye, China), 17β-estradiol Elisa Kit for E2 (Shanghai Jining Shiye, China), testosterone Elisa Kit (Shanghai Jining Shiye, China) for T and thyroid hormone triiodothyronine Elisa Kit (Shanghai Zhenke Biological Technology, China) for T3) following the producers' manual.

Data analysis

Data was presented as means ± standard deviation (SD). The homogeneity of the data and the normality of variances were checked by the Levene's test and the Shapiro-Wilk's test, respectively. A one-way analysis of variance (ANOVA) was employed to determine the significant differences at 0.05 level. When $p < 0.05$, Tukey's honest significance test was used to determine the significant difference among treatment groups. For each assay, significant differences among groups are presented with different letters (i.e., a, b, c, and d).

The combined toxicity of PS and SMZ in zebrafish was assessed using the isobologram equation (BLISS CI 1939; Chou 2006). This involved calculation of combination index (CI) as follows:

$$CI = \left(E_p + E_s - E_p \times E_s\right)/E_{p+s}$$

where E_p, E_s, and E_{p+s} represent the proportions changing (e.g., decrease or increase) of a parameter of zebrafish post exposure to PS, SMZ, and PS and SMZ relative to that without exposure. For example, the heartbeat rate of zebrafish post exposure to SMZ (1 μg/L), PS (10 μg/L), and PS (10 μg/L) and SMZ (1 μg/L) were 72.4 beats/60 s, 77.6 beats/60 s, and 80.7 beats/60 s, while the heartbeat rate of zebrafish without exposure was 67.8 beats/60 s, so E_p=(77.6-67.8)/67.8=0.14, E_s=(72.4-67.8)/67.8=0.07, E_{p+s}=(80.7-67.8)/67.8=0.19, and CI=(0.14+0.07-0.14×0.07)/0.19=1.05.

Results and discussion

Exposure to PS and SMZ induced mortality and malformation in zebrafish

Developmental toxicity in zebrafish from exposure to PS and SMZ was examined in terms of mortality and malformation. PS or SMZ (1 ug/L) resulted in around 10% zebrafish mortality. Higher SMZ concentrations significantly ($p < 0.05$) increased the mortality to 15.0 ± 7.1% (10 ug/L) and 13.3 ± 5.8% (100 ug/L), respectively (Figure 1A). Combined exposure of PS and SMZ further increased mortality (except for the group exposed to "10 ug/L SMZ and 10 ug/L PS"). Specifically, mortality of zebrafish increased to 25.0 ± 7.5% after exposure to 100 ug/L SMZ and 10 ug/L PS together. The level of mortality rate in this study is comparable or even higher than well-known toxicants such as nanoparticles (Asharani et al. 2008) and heavy metals (Yin et al. 2018), implying strong toxicity of MPs and adsorbed antibiotics.

Furthermore, exposure to PS or SMZ induced malformation of live zebrafish (Figure 1B). Of the zebrafish, 13.3~25% were malformed post exposure to SMZ and even higher level (20~35%) of malformation was induced post exposure to PS and SMZ together (Figure 1B). Exposure to PS or SMZ individually only induced the malformation at a single part or a certain stage of development of zebrafish, while combined exposure frequently induced malformation at multiple regions and stages such as the

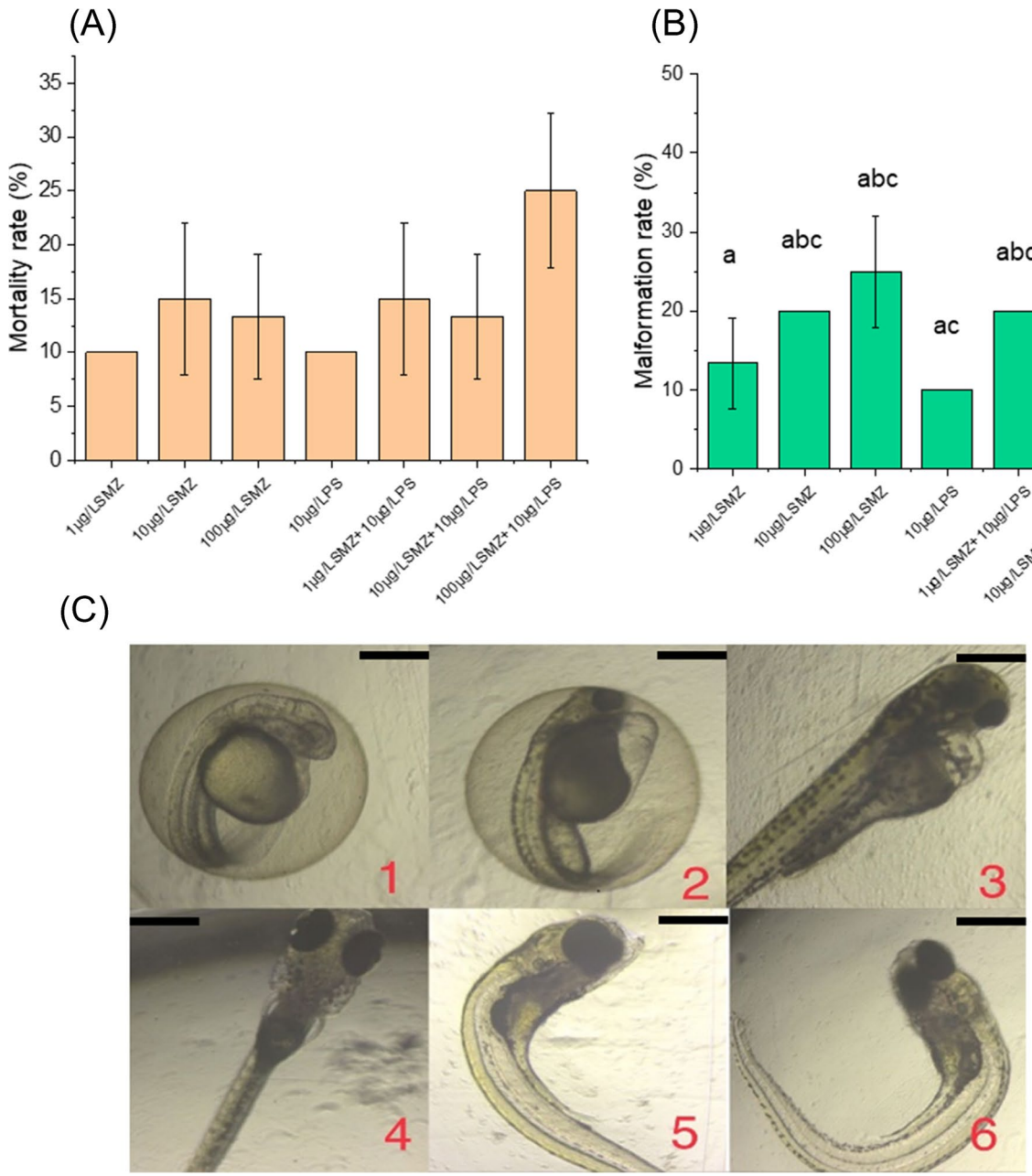

Figure 1 Exposure to PS and SMZ at various regimes enhanced the mortality rate (**A**) and malformation rate (**B**) of zebrafish. **C** Exposure to PS and SMZ together induced multiple malformations of zebrafish embryos and juveniles. 1, normal embryo; 2, embryonic pericardial edema; 3, pericardial edema and hemagglutination of juvenile at 96 hpf; 4, normal juvenile; 5, spine curvature of juvenile at 96 hpf; 6, spinal curvature and tail deformity of juvenile at 96 hpf. Mean values that do not share the same superscript were significantly different ($p < 0.05$). Scale bars represent 1 mm

pericardial edema of the embryo, and the spinal curvature and tail deformity spine of the juvenile (Figure 1C). These results are similar to those induced by other well-known/widespread chemicals. For example, exposure to sodium benzoate induced the gut abnormality, malformation of pronephros, edema in the pericardial sac, and deformed hatching gland of zebrafish (Tsay et al. 2007).

Exposure to PS and SMZ induced physiological toxicity in zebrafish

To evaluate the physiological toxicity of PS and SMZ on the development of zebrafish, four representative endpoints including the spontaneous movement (fetal movement) at 24 hpf, the heartbeat rate at 48 hpf, the body length at 72

hpf, and swimming frequency at 144 hpf were examined. Spontaneous movement at 24 hpf is the most widely adopted indicator to reflect the early development of zebrafish without the participation of nervous system (Yang et al. 2016b). The heartbeat rate at 48 hpf is a typical indicator of the cardiovascular system of zebrafish (Tshering et al. 2021). The body length at 72 hpf is a typical indicator to examine the whole developmental behavior of zebrafish (Johnson et al. 2007). And the swimming frequency of 144 hpf is an indicator to show the development of nervous system of zebrafish (Gabriel et al. 2011).

PS exposure slowed down fetal movement of zebrafish to 86.0% of the control group (Figure 2A). SMZ exposure also caused the significant decrease ($F_{7, 69} = 24.6$, $p < 0.05$) of fetal movement positively correlated to the SMZ concentration which corroborates with previous studies. For example, zebrafish fetal movement decreased significantly ($p < 0.05$) post exposure to 2000 ug/L SMZ (Yan et al. 2018) or 50 mg/L PE (Malafaia et al. 2020). In this study, combined exposure further increased physiological toxicity by 31.1~37.0% ($F_{7, 69} = 24.6$, $p < 0.05$). After exposure to 100 ug/L SMZ and 10 ug/L PS, fetal movement decreased to 2.83 ± 0.21 bends/30 s, which was 62.9% of the control group. Significant increase ($p < 0.05$) in the heartbeat rate was also observed post exposure to PS and SMZ individually (Figure 2B). A further increase in the heartbeat rate (by 19.0%~20.9%) was found after combined exposure to PS and SMZ (Figure 2B), implying higher toxicity than single exposure. Increase in zebrafish heart beat rate induced by exposure to SMZ is also reported previously (Yan et al. 2018), but the effect of MPs on the heart beat rate is not consistent in previous studies (Cheng et al. 2021; Malafaia et al. 2020), possibly due to different dose and type of MPs.

Body length is a direct indicator of zebrafish larvae growth (Yang et al. 2018). In this study, exposure to PS and SMZ individually did not cause significant variation ($p > 0.05$) of body length of zebrafish (Figure 3A), implying the insensitivity of this indicator to toxicants, as was also observed previously (Zhao et al. 2020). Only the combined exposure of PS and SMZ at the highest concentration (i.e., 100 ug/L SMZ and 10 ug/L PS) induced a significant change (increase) in body length (Figure 3A). Exposure to only SMZ did not cause a significant decrease ($p > 0.05$) (Figure 3B) to swimming frequency at 144 hpf, an indicator of locomotor and neuro-system development (Gabriel et al. 2011). In contrast, exposure to PS induced a significant decrease ($F_{7, 69} = 6.2$, $p < 0.05$) in the swimming frequency, which was 83.0% of the control group. Combined exposure to PS and SMZ together further increased toxicity ($p < 0.05$, except the group exposed to 1ug/L SMZ + 10 ug/L PS) as the swimming frequency decreased by 26.9~36.8% relative to the control group (Figure 3B). The decreased swimming frequency, also observed previously (Dimitriadi et al. 2021), may be related to the reduced frequency of cardiac ventricular contraction.

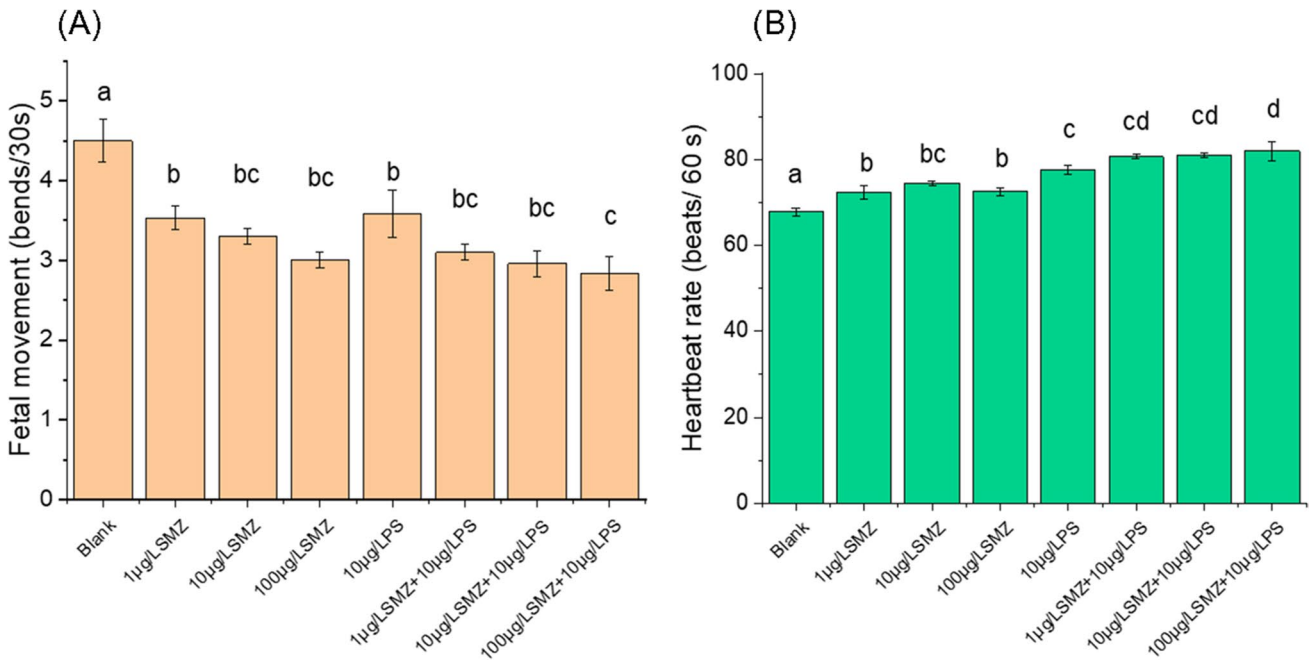

Figure 2 Exposure to PS and SMZ resulted in decrease of fetal movement (**A**) and increase of heartbeat rate of zebrafish (**B**). Mean values that do not share the same superscript were significantly different ($p < 0.05$)

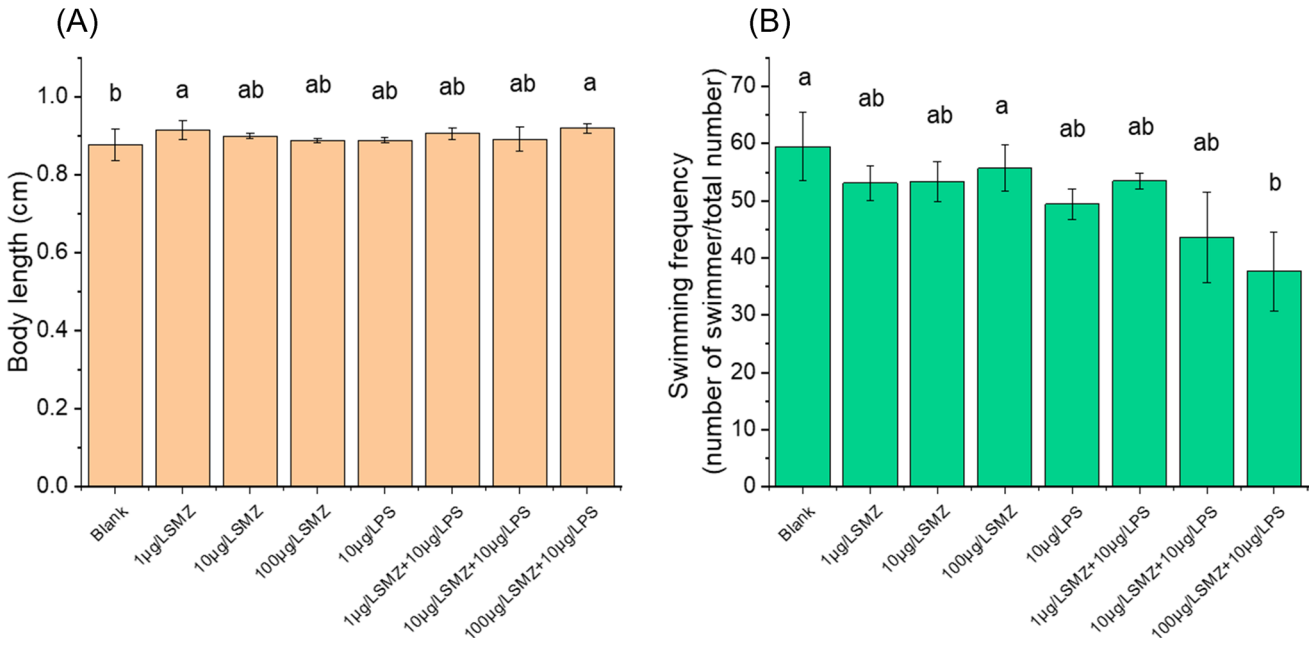

Figure 3 Effects of exposure to PS and SMZ at various regimes on the body length (**A**) and the swimming frequency (**B**) of zebrafish juvenile. Mean values that do not share the same superscript were significantly different ($p < 0.05$)

Exposure to PS and SMZ induced endocrine toxicity in zebrafish

The impact of PS and SMZ on zebrafish reproductive endocrine system during the early development stage was examined using three biomarkers: vitellogenin (VTG), 17β-estradiol (E2), and testosterone (T). VTG is a major precursor of egg yolk and is synthesized in the liver of females in all oviparous vertebrates (Yang et al. 2016a). In this study, exposure to SMZ did not result in the significant increase ($p > 0.05$) of VTG abundance even at a very high concentration (100 ug/L SMZ) (Figure 4A). However, exposure to PS significantly ($F_{7, 23} = 6.2$, $p<0.05$) increased VTG abundance ($p < 0.05$). Accordingly, exposure to PS and SMZ together showed similar VTG production with that exposure to PS alone. A dose-dependent mode of VTG production was often reported in previous studies since no significant VTG production was observed unless at an especially high dose of toxicants (Yang et al. 2016a). Similar trends were observed for the biomarker T, an indicator of hormone induction (Saad et al. 2017) which did not change after exposure to SMZ individually, but significantly ($F_{7, 23} = 8.0$, $p<0.05$) increased post exposure to PS or PS and SMZ together ($p < 0.05$, Figure 4B).

The biomarker E2 is an indicator of estrogen production and decreased significantly ($F_{7, 23} = 4.2$, $p < 0.05$) post exposure to SMZ but increased significantly post exposure to PS ($F_{7, 23} = 4.2$, $p < 0.05$). Post exposure to PS and SMZ together, no significant changes ($p > 0.05$) were found compared to the control group (Figure 4C). Triiodothyronine (T3) is normally synthesized and secreted by the thyroid endocrine system and commonly used as a biomarker to reflect physiological processes including growth, development, and metabolism (Wang et al. 2013). Disturbance of thyroid function can lead to unfavorable outcomes in zebrafish embryo such as developmental defects (Wang et al. 2020a). Post exposure to SMZ, T3 increased with the concentration of SMZ (Figure 4D). Exposure to PS also significantly ($p < 0.05$) increased the abundance of T3, resulting in a further increase post exposure to PS and SMZ together, which may result from the accumulation of SMZ on the surface of PS. However, this result is different from a previous study which reported a significant decrease in the T3 level post exposure to hydroxyanisole and MPs together (Zhao et al. 2020).

PS and SMZ showed antagonistic effects on toxicity in zebrafish

The combined effect of two toxicants on organisms is often classified into synergistic, antagonistic, or additive effect (Chou 2006). However, no consistent results for two toxicants on zebrafish could be summarized in previous studies since all combined effects have been reported previously. For example, Ding et al. reported that microcystin and glyphosate was additive in their toxicity in the gut of zebrafish (Ding et al. 2021). Wang et al. reported synergy between fenpropathrin and paclobutrazol toxicities on zebrafish at the

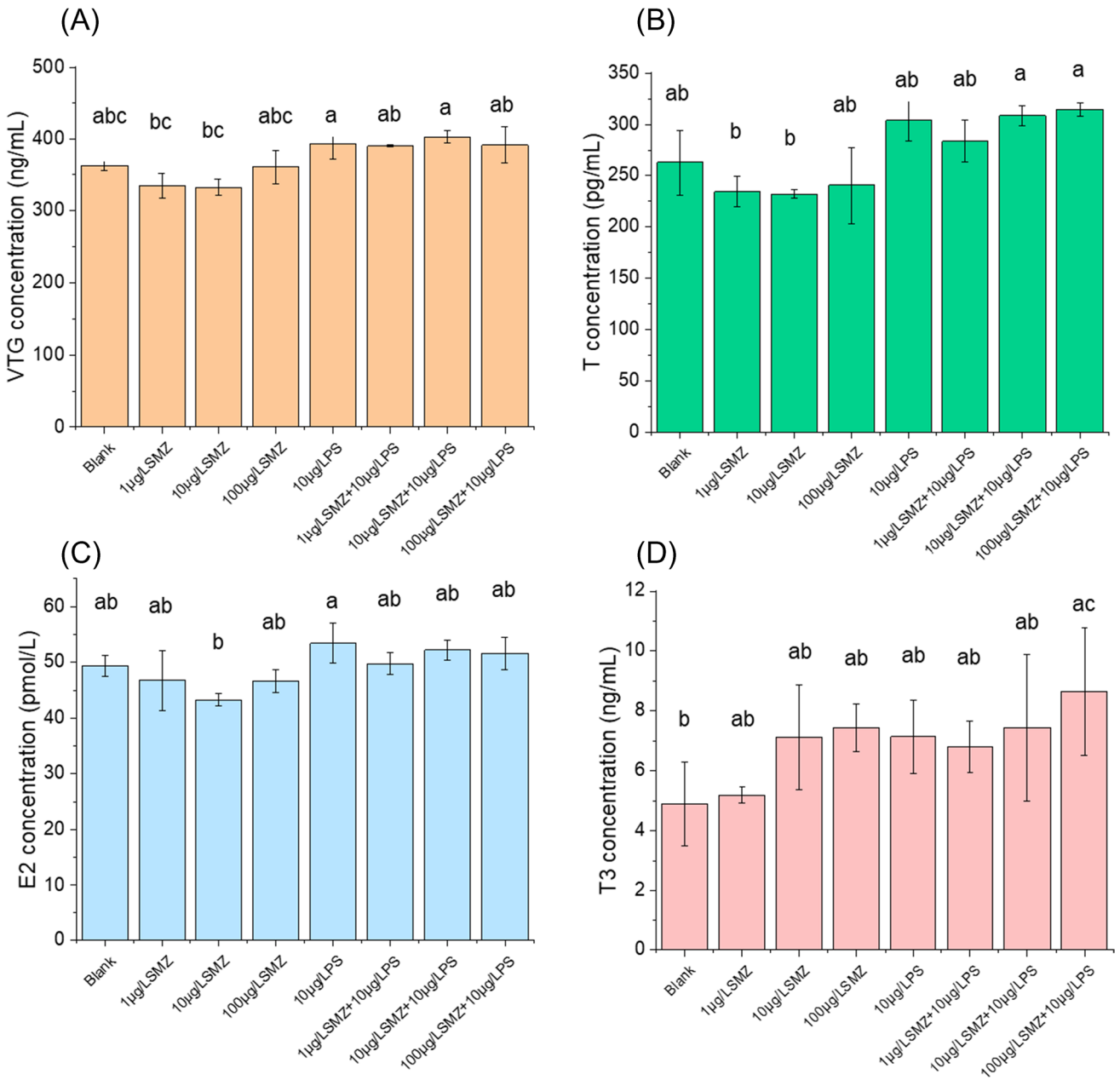

Figure 4 Effects of exposure to PS and SMZ at various regimes on the endocrine system in terms of various biomarkers of zebrafish including vitellogenin (VTG) (**A**), 17β-Estradiol (E2) (**B**), testosterone (T) (**C**), and triiodothyronine (T3) (**D**). Mean values that do not share the same superscript were significantly different ($p < 0.05$)

early life stage (Wang et al. 2020c). Exposure to Cd(II) and Cu(II) or MPs and phenanthrene was also found to have synergistic effects on zebrafish mortality (Pilehvar et al. 2020; Xu et al. 2021). However, antagonistic effects on toxicity was also frequently reported from zebrafish exposure to two toxicants such as chlorpyrifos and Ni(II) (Kienle et al. 2009), methyl parathion and Zn (II)(Ling et al. 2011), and toxic metals and deltamethrin (Jijie et al. 2020). The major reason for the inconsistent results may stem from the influence of various factors, such as the type and state of toxicants, the interaction between toxicants and the adopted indicator of the organism. Another reason may be the lack of the quantitative assessment in the combined effect of toxicants in many studies.

In this study, the combined toxicity of PS and SMZ in zebrafish was examined based on the combination index (CI), which are categorized as synergistic, additive, and antagonistic effect when CI < 1, = 1 and > 1, respectively. We found that though the combination of PS and SMZ generally exhibited a stronger toxicity than PS or SMZ did

Table 1 CI values for the combined effect of PS and SMZ on zebrafish in terms of various parameters in this study

Parameter	CI value		
	PS 10 ug/L and SMZ 1 ug/L	PS 10 ug/L and SMZ 10 ug/L	PS 10 ug/L and SMZ 100 ug/L
Mortality	1.27	1.77	0.88
Malformation	1.10	0.80	1.08
Movement	1.21	1.22	1.27
Heartbeat rate	1.05	1.18	0.98
Body length	1.72	2.34	0.56
Swimming frequency	2.55	0.95	0.61
VTG	2.00	1.43	1.09
E2	15.6	3.38	2.88
T	3.07	1.46	1.15
T3	1.27	1.36	0.97

individually (Figures 1, 2, 3, 4), antagonistic effects were present for these two substances since CI was bigger than 1 for most toxicity relevant parameters in zebrafish (Table 1). It should be noted that the antagonistic effect only slightly decreased the toxicity of PS and SMZ given that CI was just bigger than 1 for most parameters. A possible explanation for the antagonistic effect is that the toxicity of SMZ in the water is alleviated after being adsorbed on the surface of PS due to the lower mass transfer potential than that in water (Li et al. 2018d). Similar results were also reported previously. For example, though glyphosate showed a strong toxicity on the growth of *M. aeruginosa*, it was significantly alleviated by MPs (nPS-NH_2) because many glyphosate was adsorbed on MPs (Zhang et al. 2018).

Conclusions

This study addressed the stronger combined toxicity of PS and SMZ at environmentally comparable concentrations than either PS and SMZ alone on zebrafish. This was assessed in terms of developmental toxicity, physiological toxicity, and endocrine toxicity and showed the severe risk of the combined toxicity of MPs adsorbing pollutants. This presents an urgent need to reduce the discharge of plastics globally. The combination index shows that an antagonistic effect slightly alleviates the combined toxicity of PS and SMZ, which may be manipulated (amplified) in the future by optimizing the influencing factors to minimize the health risk of MPs carrying pollutants. Further investigation on the mechanism of the antagonistic interactions, such as the state of pollutants and the interaction between MPs and carrying pollutants, is suggested in future studies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-021-17198-8.

Acknowledgments We thank Dr. Pengxiao Zuo for his generous help in the writing improvement.

Author contribution Jiarui Lu conducted the developmental toxicity measurement. Jie Wu conducted the physiological toxicity and endocrine toxicity measurement. Gong Lulin conducted the exposure test and physiological toxicity measurement. Yuan Cheng did the combination index calculation and prepared the data visualization. Qingbin Yuan was a major contributor in writing the manuscript. Yide He reviewed the manuscript and provided the funding.

Funding This study was funded by the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (grant number 19KJA460006), Natural Science Foundation of Jiangsu Province of China (grant number BK20201367), and National Natural Science Foundation of China (grant number 42177348).

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate The Laboratory Animal-Guideline for ethical review of animal welfare (GB/T35892-2018) was strictly followed during the study, and authorized by Animal Care and Use Committee in School of Environmental Science and Engineering of Nanjing Tech University.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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