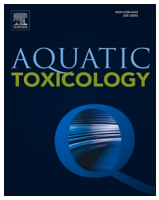
Aquatic Toxicology 263 (2023) 106697

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aqtox



Bioaccumulation of polystyrene microplastics and changes in antioxidant and AChE pattern in a freshwater snail (*Filopaludina bengalensis*) from river Ganga

Shreya Roy [a,b], Dhruba Jyoti Sarkar [a], Nabanita Chakraborty [a], Kausik Mondal [b], Basanta Kumar Das [a,*]

[a] *Aquatic Environmental Biotechnology and Nanotechnology Division, ICAR-Central Inland Fisheries Research Institute, Barrackpore, Kolkata 700120, India*
[b] *Department of Zoology, Kalyani University, Kalyani, West Bengal 741235, India*

ARTICLE INFO

Keywords:
Microplastics
Bioaccumulation
Filopaludina bengalensis
Toxicity

ABSTRACT

Microplastic pollution is a leading global problem affecting terrestrial and marine aquatic ecosystems. Due to the stagnant nature of microplastics, the toxic effect of microplastics is more pronounced to benthic organisms than the surface feeder. Hence, the present study effort was to study the microplastic bioaccumulation pattern and changes in the enzymatic and nonenzymatic antioxidant and AChE pattern of freshwater snail *Filopaludina bengalensis,* which were subjected to 0.5 ppm to 5 ppm levels of polystyrene microsphere (~ 30 μm) for 27 days. The study showed that microplastics were easily accumulated in the test organism in a dose and time-dependent manner, amounting to 82 ± 6.02 particles /individuals at a 5 ppm dose on the 27th day. However, no mortality was observed at the test microplastic dosages. The enzymatic antioxidant profile (SOD and catalase) showed limited variability and remained stable with increased duration and microplastic dose. However, the nonenzymatic antioxidant profile showed distinct variability with the complete seizing of the DPPH activity on the 27th day at 5 ppm microplastic dose and a gradual decrease of ABTS and FRAP activity at all the dose ranges. Even the AChE activity decreased with higher exposure concentrations. The present study for the first time shows the direct impact of microplastics on a freshwater snail widely available in the Indian subcontinent, indicating the role of microplastic pollution will create havoc in the Ganga river eco-biosystem in the long run.

1. Introduction

Microplastic (MPs) pollution is recently considered the leading environmental problem due to its omnipresent nature and potentially harmful effect on aquatic biota. MP pollution has been studied well in marine ecosystems for more than 50 years (Scherer et al., 2020; Bergmann et al., 2015), while recently, researchers are focusing on the impact of microplastics pollution in the freshwater aquatic ecosystem and their effect on different biotic components (Wagner and Lambert, 2018; Sarkar et al., 2021a). MPs pollution is caused by synthetic polymers of different size ranges (< 5 mm) with varied morphology (fibres, pellets, beads, foam fragments or small fragments), which are being introduced into the environment through various anthropogenic activities (Sarkar et al., 2019, 2022). Due to their small size, MPs accumulate easily in aquatic organisms through ingestion (Tanaka et al., 2015; Besseling et al., 2017). MPs contamination through trophic transfer was reported to invoke physiological changes in higher vertebrates like fish (Carbery et al., 2018). Neurotoxicity, reproduction and endocrine disruption, histopathological alterations, reduction of body weight and oxidative stress were all highly evident in fish species (Santos et al., 2020). Aquatic vertebrates and invertebrates are highly prone to the toxins leached from the plastic materials absorbed by the marine environment (Nakashima et al., 2016). Toxic compounds originating from MPs eventually migrate through the biological species into humans, creating a chemical hazard in the food chain (Wright and Kelly, 2017).

Not only in fish but MPs contaminations are also reported to occur in macrobenthic organisms through trophic transfer or direct ingestion (Carbery et al., 2018). MPs tend to accumulate in the soft tissues of the macroinvertebrates and are transferred to the next trophic level through the food chain (Farrell and Nelson, 2013). Hence, the bioaccumulation pattern of MPs was extensively studied in various strata of the trophic pyramid, including plankton, benthos, macrobenthic organisms and

* Corresponding author.
E-mail address: basantakumard@gmail.com (B.K. Das).

https://doi.org/10.1016/j.aquatox.2023.106697
Received 15 June 2023; Received in revised form 2 September 2023; Accepted 13 September 2023
Available online 15 September 2023

fishes, especially in the coastal and marine ecosystem. Prior research on marine gastropods, *Crypidula onyx*, revealed decreased growth of juveniles and their unsettling behavior at higher concentrations of MPs (60,000 to 140,000 particles/L) (Lo and Chan, 2018). However, their impact assessment on freshwater benthic organisms could be more extensive. According to a previous study MPs exposure did not cause any harmful toxic effects on the growth and reproduction of freshwater mud snails like *Potamopyrgus antipodarum* (Imhof and Laforsch, 2016). A significant filtration rate increase was observed in freshwater gastropods like *Cipangopaludina cathayensis* after exposure to polystyrene MPs of size 0.7 μm (Qu et al., 2020). Investigations on freshwater snails (*Lymnaea stagnalis*) showed accumulation of nylon particles in the sediments, but yet there was no record of any physiological alterations (Horton et al., 2020), while recent studies showed adaptive tolerance behavior of *L. stagnalis* towards polystyrene MPs (Weber et al., 2021). Microplastic beads are found to be trapped in the tissues and organs of marine mussels commonly, *Mytulis edulis,* studied in Belgium and might have a possible chance of bioaccumulation in the higher trophic level of organism (Von Moos et al., 2012). MPs toxicity leads to physiological alterations leading to ROS activity, the uptake causes histological changes in the digestive system with inflammatory responses and lysosomal membrane and peroxisomal proliferation (Avio et al., 2015). The enzymatic antioxidant profile SOD is an essential antioxidant enzyme that protects cells from oxidative stress by scavenging superoxide radicals. Conversely, catalase catalyses the decomposition of hydrogen peroxide into water and oxygen, further reducing oxidative stress. Acetylcholinesterase (AChE) is an important neurotransmitting enzyme that catalyses the breaking down of acetylecholin and measures the oxidative stress line SOD and CAT. On exposure to toxicity, the activity of the AChE decreasing causing hamper in the ROS activity (Kukkola et al., 2021). According to a previous study, Blue Mussels (*Mytulis edulis*), were reported to collect a large number of polystyrene MPs (0 – 80 μm) in their circulatory system translocated from different organs (Bouwmeester et al., 2015). Later studies on *M. edulis* showed that the MP particles were also retrieved from the intestine of the species (Li et al., 2018). A survey on the Iranian coast of the Persian Gulf revealed that MPs of size 10 and 5000 μm accumulated in five species of marine molluscs (*Cerithidea cingulata, Thais mutabilis, Amiantis purpuratus, Amiantis umbonella* and *Pinctada radiata*) with different feeding strata. The morphological characteristics of the isolated MPs from the organisms comprised of polyethylene terephthalate (PET), nylon (PA) and polyethylene (PE) denoting filmed particles (14%) followed by pellets (2%), and other types were less observed (Naji et al., 2018).

There has been some report on microplastics occurrence and toxic effect on the benthic organism from the Indian subcontinent (Naidu et al., 2018; Goswami et al., 2021; Jeyavani et al., 2022). However, until now, there are nil reports on the effect of microplastics on the macrobenthic organism of one of the largest freshwater resources of the Indian subcontinent, i.e. the river Ganga. Ganga is the longest river in India, passing through vast stretches of land and is a receiver of different kinds of municipal and industrial waste (Gowd et al., 2010). Plastic pollutants were reported to be higher in the mid and lower stretches of the Ganga, where densely populated cities and different industries (dairy factories, paint factories, rayon industries, and tannery units) are present (Vass et al., 2009). An empirical model estimated that the Ganga river catchment ranked second globally by the annual discharge of 0.12 million tons of plastic garbage into the ocean (Lebreton et al., 2017). Our research team first reported evidence of microplastic occurrence in river Ganga sediments (Sarkar et al., 2019), where the major contributing microplastics were polyethene terephthalate followed by polyethene. However, there is scarce data on the effect of microplastic pollution on the various benthic organisms in the river Ganga (Sarkar et al., 2022b). Recently studies have been performed regarding MP pollution in river Ganga, but knowledge of its adverse effects on benthic organisms is inadequate. It has been reported that riverine sediments from Ganga are contaminated with mesoplastics of size >5 mm and microplastics of size < 5 mm (Sarkar et al., 2019).

F. bengalensis is abundantly found in the South East Asian countries (India) and Africa (Gu et al., 2019). The origin of this species was reported first from West Bengal, India, after the marine water retreated from Bengal basin. Through multiple calibrated phylogenetic studies, it was finally concluded that this species gradually dispersed from Bengal basin to the other inland ecosystems of the world through terrestrial dispersion (Sil et al., 2019). *F. bengalensis* is a viviparid organism and mostly breed during winter. The juvenile species are found with complete shells within the soft tissue sac of the adult females (Lai et al., 2012). These organisms have well established gills-bearing prosobranchs used for well adequate dissolved oxygenated streams, and can also survive in anoxic condition. They are usually sedentary animals, detrivourus and filter feeders; capturing the suspended particles by gills or foot (Brown et al., 1989) and was reportedly tolerant of trace metal and pesticide pollution as well as are highly edible among the riparian community (Dhara et al., 2022). Hence, to elucidate present scenario of the Ganga River, a common freshwater Gangetic gastropod, *Filopaludina bengalensis*, has been used as a model organism to deduce the impact of polystyrene microplastics on riverine benthic biota. Therefore, the study will demonstrate the consequences of MP toxicity on the target gastropod species with possible impact on environmental sustainability and human nutrition.

2. Materials and methods

2.1. Materials

Polystyrene (PS) microsphere was synthesised as per previous methods with modification (Gorsd et al., 2012; Liu and Hu, 2020). Briefly, polystyrene beads (2 g) are dissolved in dichloromethane (30 mL) followed by dropwise addition to aqueous surfactant solution (water, 300 mL and Tween 80, 7 mL) while stirring (300 rpm) and heating at 60 °C. After 3 hrs of stirring the developed PS microsphere were collected over a membrane filter (0.5 μm) under a vacuum. The synthesised polystyrene microsphere was characterised by FT-IR (Spectrum 100 FTIR Spectrometer, Perkin Elmer). Percent absorbance was recorded at a range of 650–4000 cm^{-1} with a resolution of 1 cm^{-1} (Sarkar et al., 2019).

2.2. Test organism

The test organism, *Filopaludina bengalensis* was collected from the Gangetic River belt at Balagarh, Hooghly district, West Bengal, India. These species are common freshwater snails belonging to the family: Viviparidae. The colonization of these viviparid gastropods is scattered all over the world, from East and Southeast Asia to Africa and Australia (Sengupta et al., 2009). These organisms scavenge on the algae, aquatic plants and decayed organic matter at the bottom of the waterbody (Dillon et al., 2006; Sarkar, 2022). They have distinct gills for respiratory and filtration purposes with the proper digestive tract. *F. bengalensis* has an average length and weight of (1.775 0.69 cm) and (1.52 1.28 g) respectively. They are a rich source of protein for the tribal community and are taken as food in many parts of North Bengal (Sarkar et al., 2021) and are potential bioindicators of aquatic systems (Oehlmann et al., 2003). Therefore, these species were considered as model organism for the accumulation of MPs in the soft tissues of the gastropod.

2.3. Experimental design

Before the experiment, the glass aquarium tanks (10 L) were filled with tap water and the initial physicochemical parameters viz; Temperature (25.5 ± 2.2 °C), Dissolved Oxygen (5.2 ± 0.7 mg L^{-1}), pH (7.9 ± 0.23), Free CO_2 (7.3 ± 0.21 mg L^{-1}), Alkalinity (196.4 ± 6.31 mg L^{-1}) and Hardness (164.3 ± 5.49 mg L^{-1}) were recorded (APHA, 2017) at ICAR-CIFRI wet laboratory. The gastropods were then released in the

tanks and kept for acclimatization for two weeks. The experiment was designed with five treatments used in triplicates, with the volume of each tank measuring 10 L as mentioned previously and the experiment was continued with the same glass aquarium. During the experiment period, the tanks were exposed to 12 h with light (LED: T5 15 W 6400 K, 80μmol m-2s-1) and 12 h dark regime. The concentrations of synthetically produced polystyrene MP beads used in the experiment were control (0 ppm), 0.5 ppm, 1 ppm, 2 ppm and 5 ppm respectively. A continuous supply of aeration in the tanks was provided. The PS microsphere of different concentrations were introduced on the 1st day of the experiment except for control for even mixing of the microsphere throughout the water column. Hereafter, 30 individuals of *F. bengalensis* were released into each tank. The experiment was conducted for 27 days, and sampling was conducted on the 6th day, 12th day and 27th day to analyze the accumulation of PS microsphere and physiological alterations in the organisms. The animals were not fed with external feeds during the experiment, to avoid interference during the biochemical assay. The viability of the organisms was checked for 10 secs by analysing motions using forceps (Dhara et al., 2022).

The samples were collected for PS microsphere count ingested by the species and measured the biochemical assays.

2.4. Microplastic extraction and observation

The gastropods were randomly selected from each tank and anesthetised in clove oil (CAS No. 8000–34–8). Three gastropods were taken from each replication (n = 9) and crushed in glass beakers for PS microsphere analysis. The treated samples were digested in 30% hydrogen peroxide (H_2O_2) to degrade the organic mass (Gbogbo et al., 2020). Then, the residues were filtered through 0.5 μm filter paper and collected in 2 ml Eppendorf tubes. Following previously described procedures, the extracted MPs were stained with the hydrophobic fluorescent dye Nile Red, and examined under a fluorescence microscope. (Shim et al., 2016; Sarkar et al., 2021b). The photomicrographs (excitation and emission wavelength: 534–558 nm and > 590 nm, respectively) were taken under an epifluorescence microscope (Carl Zeiss, Axioscope. A1 microscope, Oberkochen, Germany) coupled with Axiocam ICc5 CCD colour camera and Sony ICX 655 sensor (Tokyo, Japan) with the spectral sensitivity of 400–700 nm at 40X zoom. A 540–590 nm filter was used to view the beads mounted on a slide. To check preexisting microplastics contamination in the collected gastropods

2.5. Preparation of sample for antioxidant assay

Three gastropods ($n = 3$) were collected from each tank to be used for antioxidant analysis. The gastropods were picked randomly and anesthetised in clove oil (CAS No. 8000–34–8). The shells of *F. bengalensis* were broken, and 1.0 g of the molluscan mass was taken to be preserved in 2% sucrose solution. The samples were then macerated in the solution and centrifuged at 3300 rpm at 4 °C for 10 min. Aliquots of the sample supernatant were pipetted and stored at −20 °C for further testing.

2.6. Enzymatic antioxidant assay

2.6.1. Super oxide dismustase (SOD)

According to a previous method, SOD was estimated at pH 10.2 based on the ability to inhibit the antioxidant of epinephrine (Misra and Fredovich, 1972). A tube holding 50 μl of the sample was filled with 1.5 ml of 0.1 M carbonate-bicarbonate buffer containing 0.57 mg of EDTA (pH 10.2) and 0.5 ml of 3 mM epinephrine before being vortexed. At 30 secs, the optical density was measured for 2 mins at 480 nm, using enzyme linked immune sorbent assay (ELISA) cum spectrophotometer reader (BioTekEpoch™ 2 Take-3 plate reader). The quantity of protein required to inhibit 50% anti-oxidation of epinephrine is one unit of SOD activity.

2.6.2. Catalase (CAT)

Catalase activity was measured through a standard protocol method (Takahara et al., 1960). The reaction mixture consisted of a 50 μl sample and 2.45 ml phosphate buffer (50 nM, pH 7). Adding 1.0 ml of H_2O_2 (0.3% in phosphate buffer), solution initiated the former reaction. Using ELISA cum spectro reader (BioTekEpoch™ 2 Take-3 plate reader), the reaction absorbance was computed for 3 min at 15 s intervals at 240 nm. The blank was run simultaneously by taking 1.0 ml of distilled water instead of H_2O_2 solution. The enzyme activity was expressed as micromoles H_2O_2 decomposed min^{-1} mg $protein^{-1}$ ml^{-1}.

$$k_t = 1/t\log_{10}\ x_0/x$$

where, x_0 is the initial peroxide concentration expressed as millilitres of permanganate and x is the concentration at time (t).

2.7. Non-enzymatic antioxidant assay

Using the commercial-free radicals DPPH, ABTS, and FRAP, the radical scavenging capability of the samples was used to assess their antioxidant potential. The results were compared with standard antioxidants and expressed as mean ± SD.

2.7.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay

The previously reported process was modified to assess the ability to scavenge DPPH radicals (González-Palma et al., 2016). The sample reaction mixture contained 280 μl of freshly prepared DPPH solution (0.1 mM), 10 μl ethanol and 10 μl sample. DPPH solution and 20 μl ethanol were used to prepare the control. The absorbance was measured at 517 nm after 30 min of dark incubation. The results were compared to butylated hydroxytoluene (BHT) and represented as a percentage of DPPH radical scavenging (Sigma Aldrich).

$$DPPH\ radical\ scavenging\ activity(\%) = \{Ac - At\,/\,Ac\} \times 100$$

Where, Ac = the absorbance of the blank reaction
At = the absorbance in presence of the sample of the extracts.

2.7.2. 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonicacid) (ABTS+) antioxidant assay

According to Arnao et al. (2001), the ABTS antioxidant assay was performed to test the antioxidants in the samples of *F. bengalensis*. The test reaction mixture consisted of 2.4 mM potassium per sulfate (Sigma Aldrich) and 7 mM ABTS solution (Sigma Aldrich). 10 μl of methanol, 280 μl of ABTS solution, and 10 μl of a supernatant sample of *F. bengalensis*. The control contained 280 μl of ABTS solution and 20 μl of methanol. The absorbance was measured at 734 nm to observe the production of ABTS•+ from ABTS in the presence of antioxidants. Results were compared to ascorbic acid and expressed as percent ABTS free radical scavenging activity:

$$ABTS\ radical\ scavenging\ activity(\%) = \{Ac - At\,/\,Ac\} \times 100$$

Where, Ac = the absorbance of the blank reaction
At = the absorbance in the presence of the sample of the extracts.

2.7.3. Ferric reducing antioxidant power (FRAP)

The Fe^{3+} (ferric) to Fe^{2+} (ferrous) reduction by antioxidants in the sample is the basis of the ferric reducing antioxidant potential (FRAP) (Sigma Aldrich) technique. The test was carried out by the previous method (Risso et al., 2021). 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (10 mM) and the sodium acetate buffer (300 mmol/L) were dissolved in 37% HCl. In 10 ml of millipore water, 20 mM iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) was dissolved along with 10 μl of supernatant sample. The Fe^{2++} TPTZ absorbance was read at 593 nm.

$$Absorbance = Absorbance\ of\ the\ FeSO4.\ 7H2O\ recorded - Blank\ absorbance$$

Sample absorbance = sample absorbance recorded − Sample blank absorbance

2.8. Acetylcholinesterase (AChE)

The estimation of AChE was conducted according to the previous method (Dietz et al., 1977; Das and Mukherjee., 2003), in which the substrate utilised was propionyl thiocholine iodine solution (PTCI) (20 mmol) to 1 ml of sample, and the reaction was stopped by adding a quinidine reagent (14 mmol). The optical density of AChE was measured through ELISA cum spectrophotometer reading comprising a 96-well plate at 410 nm.

$U\ mL^{-1} = 14.71\ X\ \Delta\ A$

(U ml^{-1} = µmol min^{-1} ml^{-1} at 37 °C, and Δ A is the increased absorbance corrected for blank).

Δ A = corrected OD (Sample OD — Blank OD)

14.71 = common factor

2.9. Statistical analysis

The interrelationship between the accumulation of MPs in the soft tissues of the gastropods and the stress-related antioxidants and neurological enzymes was established with the help of Karl Pearson's correlation through multivariate software IBM-SPSS Statistics 22. Homogeineity test was checked by Levene's Test at significance level of $p< 0.05$ using IBM-SPSS Statistics 22 software. Oneway analysis of variance (ANOVA) followed by Duncan Multiple Range Test was performed to observe the significant differences at a level of $p<0.05$ within the days of the experiments through IBM-SPSS Statistics 22. Shapiro-Wilk normality test was performed through PAST (Paleontological Statistics, 4.03) software to check the accuracy of the data.

3. Results

3.1. Characterisation of polystyrene microsphere

To understand the morphology, the optical images of the synthesised polystyrene microsphere were taken (Fig. 1A) and the size distribution of the same image was obtained after processing using Image J software (ImageJ 1.51j8, NIH, USA). The histogram analysis revealed that the microsphere's mean diameter was 30–35 µm, with a more rightward skewed distribution of particle size (Fig. 1C). Further, the FT-IR spectra of the microsphere were analysed which showed a major characteristic band at 696 cm^{-1} (aromatic C—H out of plane bending), 1452 cm^{-1} (CH_2 bending), 1492 cm^{-1} (aromatic C = C stretching vibration), 2917 cm^{-1} (Aliphatic C—H stretching), 3025 cm^{-1} (aromatic C—H stretching) which are characteristic to polystyrene polymer (Omidi et al., 2017; Jung et al., 2018).

3.2. Microplastic accumulation

The abundance of PS microsphere in the soft tissues of *F. bengalensis* was counted under the microscope after exposure to the concentrations of 0.5 ppm, 1 ppm, 2 ppm and 5 ppm on the 6th day, 12th day and 27th day of the experiment (Fig. 2A). The number of extracted PS microsphere per individual on a different day of exposure is given in Fig. 1B. On the 6th day of sampling, the maximum number of PS microsphere in the gastropod was 14 ± 2.08 particles /individuals at 5 ppm concentration. In contrast, PS microspheres were absent at 0.5 ppm. The accumulation patterns on the 12th day showed an increasing trend of 77 ± 3.60 particles /individuals at 5 ppm concentration, whereas 2 ppm concentration showed 51 ± 4.04 particles /individuals. In the lowest concentration of 0.5 ppm, the accumulation of PS microsphere was 3 ± 1 particles /individuals. The maximum ingestion of the PS microsphere was observed on the 27th day, in which all the concentrations showed accumulation of PS microsphere from 11 ± 2 particles /individuals at

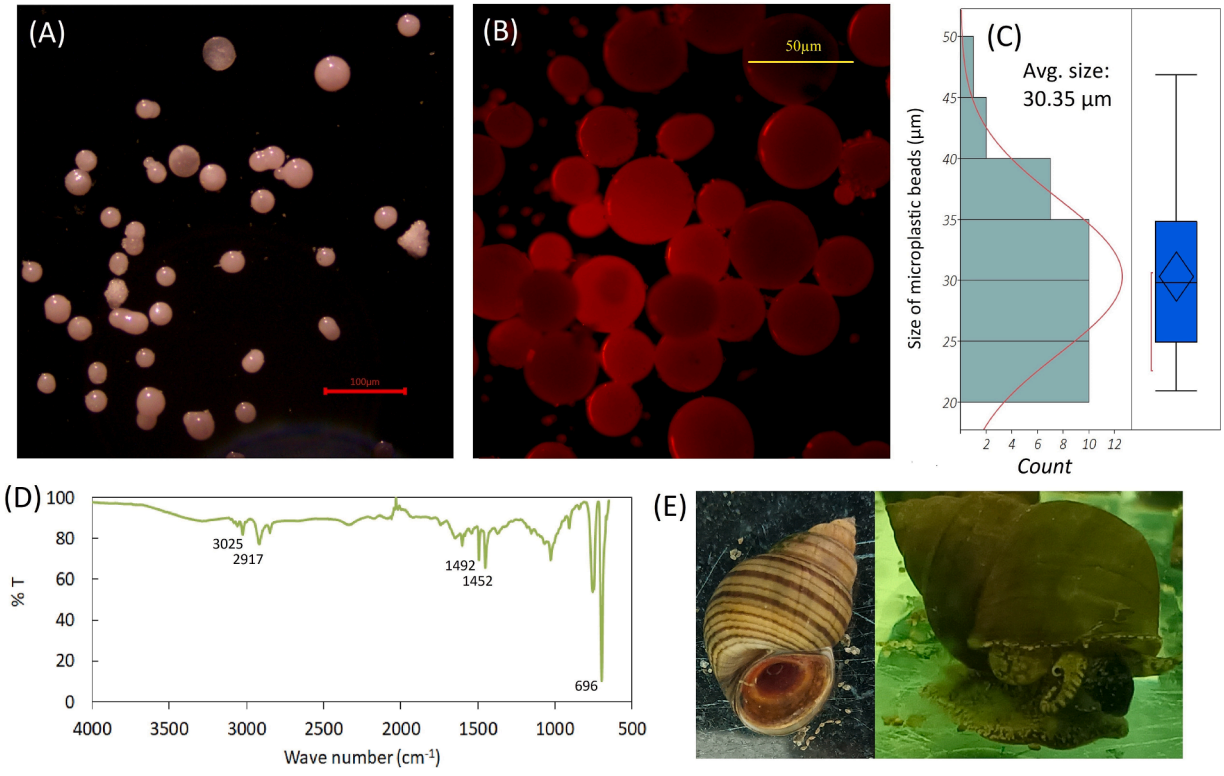


Fig. 1. Optical (A) and fluorescence (B) photomicrographs of synthesised polystyrene microsphere; the average particle size (C) and FT-IR spectra (D) of the microsphere; the test organism, *Filopaludina bengalensis* (E).

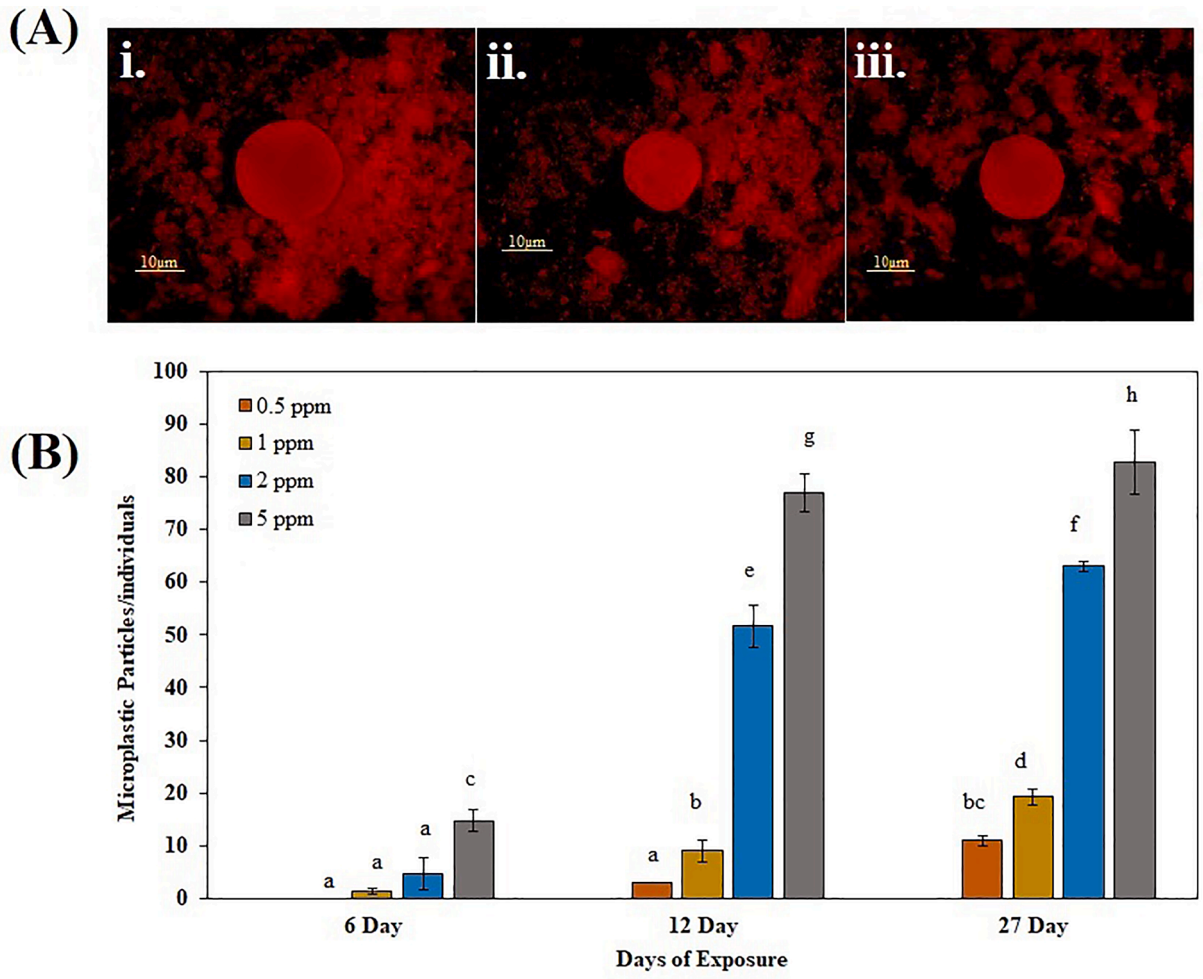


Fig. 2. Fluorescence photomicrographs of microsphere extracted from *F. bengalensis* (A). Increase in the count of microplastic beads in different concentration of microplastic treatments along with incubation time (days) (B).

0.5 ppm to 82 ± 6.02 particles /individuals at 5 ppm concentration.

3.2. Enzymatic antioxidant assay

3.2.1. SOD activity

The results of the enzymatic antioxidant, SOD, did not show any significant trend throughout the concentrations on the 6th, 12th and 27th day (Fig. 3A). On the 6th day, compared to the control, the effects of the antioxidant assay increased in all the concentrations except at 5 ppm (1.05 ± 0.0.015 mmol/min/mg protein), where the activity lowered. The maximum activity on the 6th day was observed at 2 ppm concentration (1.03 ± 0.025 mmol/min/mg protein). The variation of SOD activity in the treatment group was more or less similar and lower than the control group on the 12th day. On the 27th day of the exposure, the SOD activity decreased from 1 ppm (1.12 ± 0.015 mmol/min/mg protein) to 5 ppm (0.88 ± 0.020 mmol/min/mg protein) compared to the control group.

3.2.2. Catalase activity

The activities of the catalase enzyme ceased on the 6th day of sampling at the 5 ppm concentration (0.00 U/mg protein) (Fig. 3B). Compared to the control (0.11 ± 0.04 mmol/min/mg protein), the maximum activity was observed at 2 ppm concentration (0.11 ± 0.015 mmol/min/mg protein) on the 6th day. The activities gradually decreased compared to the control (0.12 ± 0.025 mmol/min/mg protein) on the 12th day but showed an abrupt elevation at 5 ppm concentration (0.14 ± 0.016 mmol/min/mg protein). On the 27th day, there was a marginal increase in the activity at 5 ppm compared to the control. The changes within the concentrations on the 27th day did not show many deviations in the results.

3.3. Non-enzymatic antioxidant assay

3.3.1. DPPH

The non-enzymatic DPPH assay showed a distinct result throughout the experiment. On the 6th day, the DPPH activity increased throughout the treatment as compared to the control (Fig. 4A). On the 12th day post-exposure as compared to control, there was an increase in some of the concentrations exposed, but a reduction in the value was also observed in 1 ppm (19.76 ± 0.67 U/mg protein). On the 27th day, the activity of DPPH ceased at 5 ppm concentration.

3.3.2. ABTS

The antioxidant activity using the ABTS method was increased in all concentrations on the 6th day compared to the control (69.14 ± 2.96 U/mg protein) (Fig. 4B). The results showed that the activity increased from 0.5 ppm to 5 ppm. The activity peaked at 0.5 ppm, 2 ppm and 5 ppm on the 12th day, whereas at 1 ppm concentration, the activity decreased (52.53 ± 0.905 U/mg protein) compared to the rest of the concentrations. On the 27th day, the activity showed a sudden decrease throughout the concentrations, showing the lowest at 1 ppm concentration (42.11 ± 1.05 U/mg protein). There was a reduction of

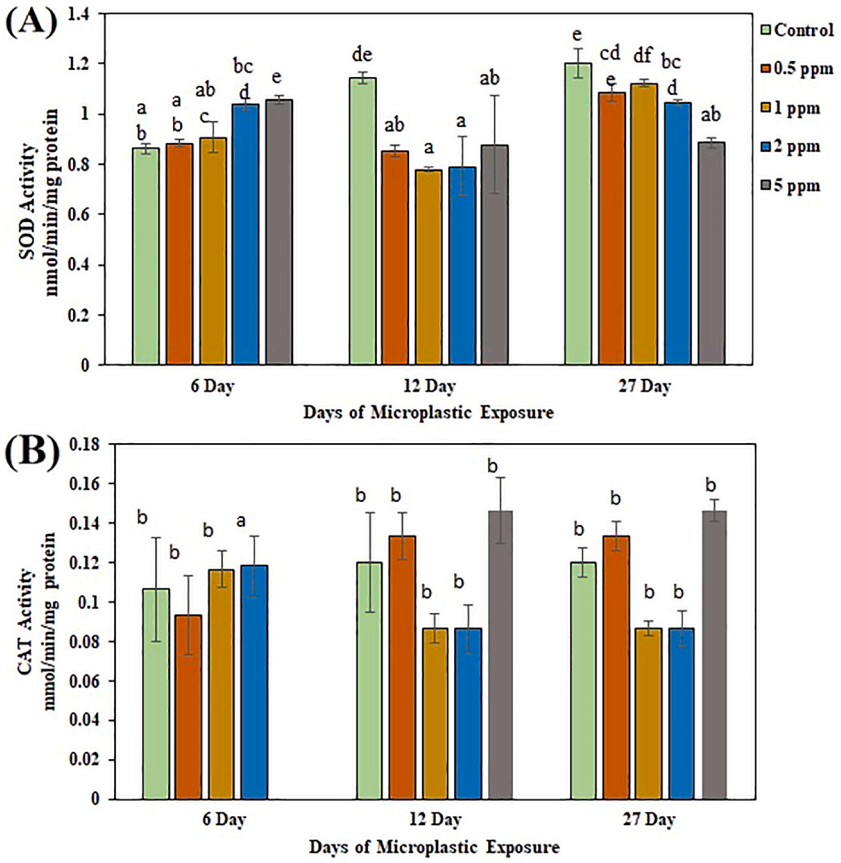


Fig. 3. Changes in SOD (A) and catalase (B) enzymes in PS microsphere treated organism on different days, data represents Mean ± SE along the concentrations (N = 9).

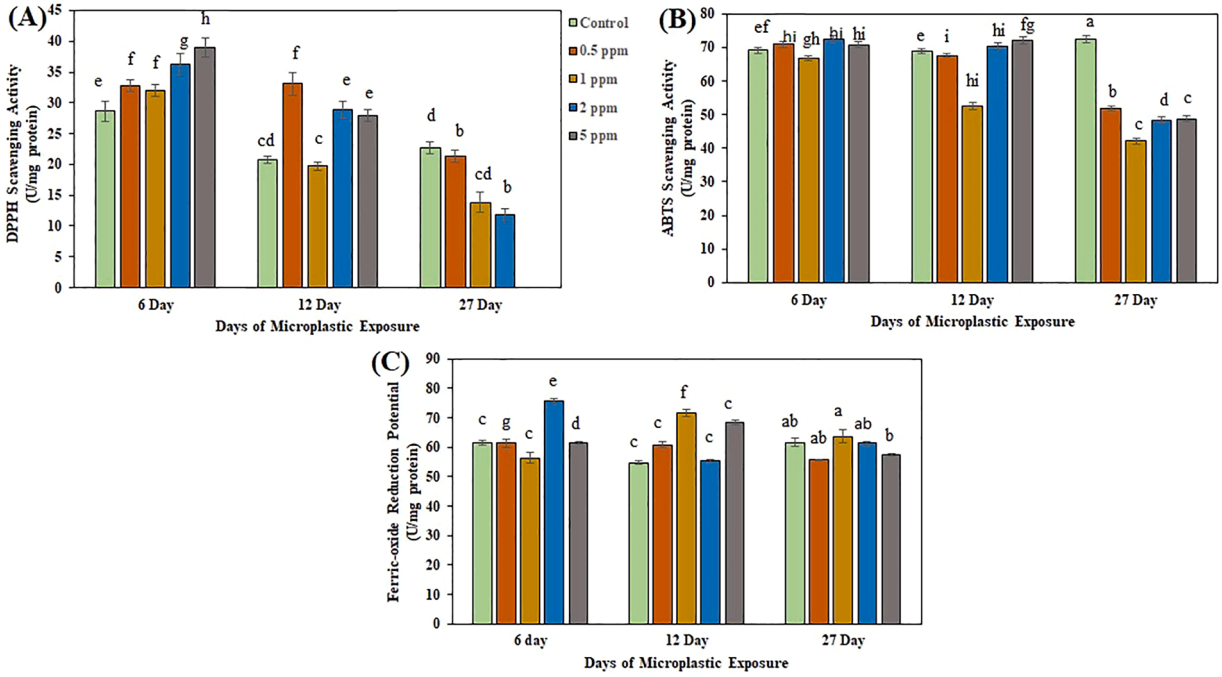


Fig. 4. Changes in DPPH (A), ABTS (B) and FRAP (C) non-enzymatic antioxidant profile with variations in dose and exposure of PS microsphere, data representing Mean ± SE along the concentrations (N = 9).

antioxidant level within the concentration at the 27th day compared to the 6th day.

3.3.3. FRAP

On the 6th day, there was a decrease in the ferric oxide reduction potential in all the concentrations except 2 ppm (75.72 ± 0.88 U/mg protein) as compared to the control (Fig. 4C). It was noticed that there was no distinct variation observed throughout the concentration on the 12th day of exposure, except for a sudden elevation at 1 ppm (71.61 ± 1.05 U/mg protein). Similarly, on the 27th day, marginal changes were observed compared to the control. There was a reduction in antioxidant profile from the 6th day to the 27th day within the concentration.

3.4. Acetylcholinesterase activity

AChE was increased within the concentrations compared to control on the 6th day, showing a maximum range at 0.5 ppm concentration (Fig. 5). The value of AChE decreased through the days showing a distinct reduction over the exposure levels. Initially, the choline esterase activity was increased with an increase in the PS microsphere concentration but subsequently decreased on the 12th day and 27th day over the same concentrations tested. The choline esterase activity decreased from 0.5 ppm to 5 ppm from the 6th to 27th day within the concentrations compared to the control.

3.5. Statistical analysis

Statistical analysis showed that there is a negative correlation between PS microsphere concentration and DPPH and AChE activity (r= –401, r = –523; p<0.01) (**Table S1**). SOD showed a significantly negative correlation with ABTS (r = –356). DPPH was positively correlated with the other parameter like ABTS, FRAP and AChE (r = 664, r = 491 and r = 544) (**Table S1**). Through the computation of ANOVA, a significant difference ($p < 0.05$) was observed within the days, especially on the 12th day and 27th day of ABTS and DPPH assay (**Tables S4 and S5**), while FRAP showed a significant difference ($p < 0.05$) on the 6th and 12th days (**Table S6**). In the case of AChE, a significant difference ($p < 0.05$) was observed on 12th and 27th day (**Table S7**).

4. Discussion

The impact of pollution in the freshwater ecosystem creates a major threat to the aquatic biota. Thus, for the first time, the present study establishes the assemblage of microsphere beads in the soft tissues of freshwater gastropods (*F. bengalensis*) and the alterations in the antioxidant profile and neurotransmitter enzyme (AChE).

4.1. Accumulation of PS microsphere in F. bengalensis

The present study found that PS microbeads of average 30 μm size can readily accumulate in the benthic test organism, amounting to more than 80 particles per individual within 27 days. The test organism's major entry route was postulated to be ingested through food channels and gill. Research on the entry of MPs in the aquatic organisms at different strata of the trophic has been depicted in different literature (Bordós et al., 2019; Slootmaekers et al., 2019). The route of MPs intake by aquatic mussels is primarily by respiration through gills and ingestion through feeding (Weis, 2019). Various other research showed MPs accumulation in the liver and stomach of marine mussels (Wegner et al., 2012; Watts et al., 2016). The accumulation of MPs in the vital organs of freshwater Zebra mussels (*Dreissena polymorpha*) reduced feed intake and decreased energy. Further, these workers reported changes in histological architecture in the biological tissues of Zebra mussel (*Dreissena polymorpha*) which could be used as biomarkers for MP detection (Magni et al., 2018).

Recently, many studies have been undertaken to assess the toxicity of microplastics towards aquatic organisms. For example, the acute effect of polyamide MPs in combination with polybrominated diphenyl ethers (PDBE) against pond snail *Lymnaea stagnalis* revealed that polyamide MPs did not cause mortality and significantly influenced PDBE uptake by the snail. Similar observations were noticed in the present study, where no mortality of the test organisms was recorded. A previous study revealed that polystyrene MPs of size 5–90 μm were ingested and egested by L. *stagnalis*, showing no chronic toxicity in the organism's physiology (Weber et al., 2021). However, in contradiction to the effect of MPs on adult L. *stagnalis*, a significant growth reduction was observed on veliger larvae of Slipper Limpets (*Crepidula onyx*) when exposed to 2–5 μm polystyrene spheres at a concentration of 60,000 and 140,000 particles ml/l (Lo and Chan, 2018). It might be due to the rapid excretion of MPs, supported by a different study where smaller PS microsphere was excreted faster than larger ones in a marine pelecypoda, *Mytilus galloprovincialis*, subjected to different sized PS sphere (1,10 and 90 mm) (Kinjo et al., 2019). The study showed that 90% of the PS microbeads spheres were recovered from the digestive tract, affecting the regulation of antioxidant enzymes. Our study was conducted for one month and revealed the MPs accumulation in a lesser quantity, as reported by the other workers, indicating a slow accumulation process in the tissue levels.

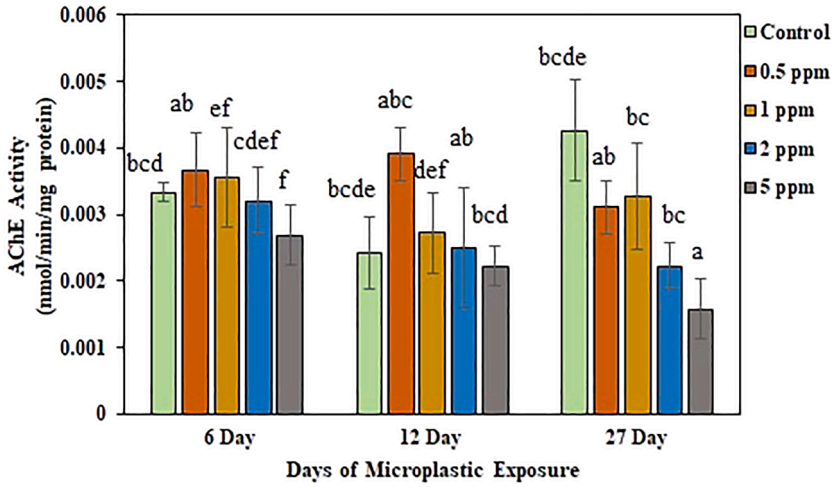


Fig. 5. Changes in AChE activity with dose variations and exposure of PS microsphere, data represents Mean ± SE along the concentrations (N = 9).

4.2. SOD and CAT activities

Antioxidants protect cellular tissue mechanisms from the harmful effects of oxidative stress and reactive oxygen species (ROS) and are common enzymes used for the detection of stress levels in organisms. The increase in ROS production may cause damage in the lipid cell membranes called lipid peroxidation, which involves their oxidative degradation (Hampel et al., 2016). Though in our present study, cellular damage on the lipid was not studied, but other muscles antioxidant defences might have caused lipid peroxidation and potentially other adaptive mechanisms were able to counter act the ROS formation. Lompré et al. (2021) reported that activation of SOD and CAT in *Ruditapes decussatus* due to Terbium causing sufficient cellular damage. It was reported that MPs enhanced reactive oxygen species (ROS) production and oxidative stress in marine mussels and lugworms (Avio et al., 2015). To reduce oxidative stress, the molluscs (*Pomacea canaliculata*) depend on enzymatic and non-enzymatic antioxidants (Giraud-Billoud et al., 2013). SOD and CAT can be potential biomarkers for oxidative stress underlying effects on the molluscan physiology, *Amphioctopus fangsiao*, (Zheng et al., 2022) and which could be corroborated in our present study.

Additionally, PSMPs dramatically boosted SOD and CAT activities in freshwater gastropods, indicating the production of oxidative stress. The MPs pollution affects the higher vertebrates like fish, and other invertebrates, including macrobenthic organisms. Zebra mussels were used as primary indicators for microplastic (polystyrene beads) pollution in the marine environment (Magni et al., 2018). The beads accumulated in the tissue and hemolymph and caused cellular damage, oxidative stress and neuro-genotoxicity, whereas the antioxidant enzymes like SOD did not show significant variations, and the CAT activity increased with the treatment. In the present study, the activities in SOD increased over the concentration on 6th day and decreased on the 12th and 27th days as compared to the control. Similar results were reported in *Mytilus galloprovincialis* when exposed to MPs beads for 7 days (Avio et al., 2015). The CAT activity of control increased over the period depicting the highest at 2 ppm concentration on the 27th day; similar results were reported in *Mytilus coruscus*, where the CAT activity increased upon exposure to higher concentration of PS MPs (Wang et al., 2020).

4.3. DPPH, ABTS and FRAP antioxidant activities

The activities of the non-enzymatic antioxidants during the experimental period decreased compared to the control groups. Non-enzymatic antioxidant molecules play an essential role in the nutrition of any organism. The free radical scavenging capacity from the hemolymph of Garden Snail (*Helix aspersa maxima*) showed 59.54% DPPH free radical scavenging and 62.31% inhibition of ABTS (Raynova et al., 2015). The fluctuations of antioxidant capacity in an organism have been considered an indicator for monitoring the environmental pollution caused by anthropogenic activities in the freshwater ecosystem. The scavenging potential of antioxidants like DPPH, ABTS and FRAP to maintain the cellular ROS activity in freshwater Pachychilidae, *Brotia costula*, were reported (Rout et al., 2022). According to them, DPPH showed a scavenging potential of 54.14%; ABTS showed 52.86% and FRAP recovery was estimated at an organism8259.39 μMol $FeSO_4$ /mg in 100% ethanol extract. The absorbance of DPPH radical diminishes through the scavenging of hydroxyl ions, developing an interaction between antioxidant molecules and radicals. A study reported that the DPPH activity in the freshwater Apple Snail, *Pila globosa*, lowered during monsoon seasons than in summer (Panda et al., 2022). In the present study, a definite trend was not observed in terms of duration as well as concentration, rather, variations were observed from individual to individual. The exposure of *F. bengalensis* to 4 different concentrations of PS microsphere showed lowered antioxidant activities, referring to toxicity enhancing oxidative stress. From the 6th to the 27th day, DPPH activity decreased and gradually reduced to 0 activity on the 27th day at 5 ppm concentration. The antioxidant activities were reduced as measured through ABTS and FRAP assay throughout the experiment. Earlier reports available on pollution exacerbating the antioxidant activities in molluscs acting as essential biomarkers of riverine and stream waters (Gorinstein et al., 2006). The present findings are corroborated by the work on Rapana *venosa* (gastropods) and *Mytilus galloprovincialis* (mussel) collected from polluted and non-polluted zones of the Bulgarian coast of the Black Sea where the activities were higher in *R. venosa* collected from the polluted zone than *M. galloprovincialis* from the non-polluted zone (Moncheva et al., 2011). The present study pertaining to non-enzymatic antioxidants due to MPs accumulation showed a negative effect which was reported for the first time in freshwater gastropods. Thus, non-enzymatic antioxidants could be a biomarker for MP pollution in the aquatic ecosystem. Through the investigations based on former researches, the mechanism of such negative effects could not be established in the present study. However, a complex phenomenon in relation to cellular damage and ROS might be involved in such process which warrants through investigation.

4.4. Acetylcholine-esterase activities

AChE is an essential neuropeptidase for neuromuscular juncture, which helps transmit cholinergic motor neurons through the synaptic cleft (Beltran and Pocsidio, 2010). Acetylcholine esterase helps in the catalysis of acetylcholine (neurological transmitter) which causes enzymatic and behavioural changes in the organism (Kukkola et al., 2021). Stress caused due to exposure to a contaminant inhibits AChE activity and thus disrupts the neural transmission (English and Webster, 2012). AChE inhibitions are reportedly triggered by pesticide exposures and metals (Brown et al., 2004; Peric et al., 2017). Nevertheless, in the present study, AChE was affected by MPs upto 5 ppm concentration. Previously, exposure to PS MPs was reported to inhibit acetylcholine activities (Guo et al., 2021). AChE activity decreased rapidly and significantly in bivalve (*Mytilus galloprovincialis*) gill tissues in response to polystyrene MPs (Avio et al., 2015). Earlier researchers showed decrease in AChE biomarkers in the gills, digestive system and gonad of Asian Clam (*Corbicula fluminea*) due to the cumulative toxic effects of MPs and Cadmium (Parra et al., 2021). A study that evaluated the AChE activity in the gills of *Scrobicularia plana* revealed neurotoxicity endpoints in the visceral mass (without gills), showed a possible decrease due to MPs accumulation (Ribeiro et al., 2017). The combined effect of Ciprofloxacin and PS MPs caused a reduction in AChE secretion, leading to neurotoxicity in Asian Clam, *Corbicula fluminea*, (Guo et al., 2021). In relevance to the former study, the current experiment showed that the AChE activity decreased during the exposure and was less than the control which might be due to ingestion of MPs and causes an inflammatory response in the lysosomal membrane and destabilises the cellular responses of the host (Von Moos et al., 2012).

5. Conclusion

The present study showed that polystyrene microplastics of 30 μm readily accumulate in the *Filopaludina bengalensis*, a common freshwater edible snail of the river Ganga. The investigations also revealed that increased exposure duration and concentrations enhanced the microplastic accumulation with significant changes in the enzymatic and non-enzymatic antioxidant profiles. Exposure to the PS microsphere lowers non-enzymatic antioxidant activities because MPs lead to oxidative stress in the target organism, which can be served as a biomarker for MP pollution for gastropod species. The AChE activity also gets reduced due to MPs exposure for a short duration; however, a dose-dependent trend was not found. The present study highlights the role of microplastic particles in bringing out biochemical, enzymatic and oxidative changes in the freshwater gastropod, indicating the toxic role of microplastic pollution in a freshwater aquatic ecosystem.

CRediT authorship contribution statement

Shreya Roy: Methodology, Investigation, Formal analysis, Writing – original draft. **Dhruba Jyoti Sarkar:** Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – review & editing. **Nabanita Chakraborty:** Formal analysis. **Kausik Mondal:** Data curation, Validation, Supervision. **Basanta Kumar Das:** Conceptualization, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Basanta Kumar Das reports financial support, equipment, drugs, or supplies, and travel were provided by National Mission For Clean Ganga under Ministry of JalSakti, Govt. of India.

Data availability

Data will be made available on request.

Acknowledgement

The authors are grateful to National Mission for Clean Ganga for providing us with the platform to perform the experiments and carry out the research activities and to ICAR- Central Inland Fisheries Research Institute for providing us with all the research support and facilities.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2023.106697.

References

APHA, Baird, R.B., Eaton, A.D., Rice, E.W., Clesceri, L.S., 2017. Standard Methods for the Examination of Water and Wastewater, 23. American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), pp. 1–1546.

Arnao, M.B., Cano, A., Acosta, M., 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 73, 239–244.

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargelloni, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198, 211–222.

Beltran, K.S., Pocsidio, G.N., 2010. Acetylcholinesterase activity in *Corbicula fluminea* Mull., as a biomarker of organophosphate pesticide pollution in Pinacanauan River, Philippines. Environ. Monit. Assess. 165, 331–340.

Bergmann, M., Gutow, L., Klages, M., 2015. Marine Anthropogenic Litter. Springer Nature, p. 447.

Besseling, E., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans, A.A., 2017. The effect of microplastic on the uptake of chemicals by the lugworm *Arenicola marina* (L.) under environmentally relevant exposure conditions. Environ. Sci. Technol. 51, 8795–8804.

Bordós, G., Urbányi, B., Micsinai, A., Kriszt, B., Palotai, Z., Szabó, I., Hantosi, Z., Szoboszlay, S., 2019. Identification of microplastics in fish ponds and natural freshwater environments of the Carpathian basin, Europe. Chemosphere 216, 110–116.

Bouwmeester, H., Hollman, P.C., Peters, R.J., 2015. Potential health impact of environmentally released micro-and nanoplastics in the human food production chain: experiences from nanotoxicology. Environ. Sci. Technol. 49, 8932–8947.

Brown, K.M., Varza, D., Richardson, T.D., 1989. Life histories and population dynamics of two subtropical snails (Prosobranchia: viviparidae). J. N. Am. Benthol. Soc. 8, 222–228.

Brown, R.J., Galloway, T.S., Lowe, D., Browne, M.A., Dissanayake, A., Jones, M.B., Depledge, M.H., 2004. Differential sensitivity of three marine invertebrates to copper assessed using multiple biomarkers. Aquat. Toxicol. 66 (3), 267–278.

Carbery, M., O'Connor, W., Palanisami, T., 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. Environ. Int. 115, 400–409.

Das, B.K., Mukherjee, S.C., 2003. Toxicity of cypermethrin in Labeo rohita fingerlings: biochemical, enzymatic and haematological consequences. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 134 (1), 109–121.

Dhara, K., Saha, S., Chukwuka, A.V., Pal, P., Saha, N.C., Faggio, C., 2022. Fluoride sensitivity in freshwater snail, *Bellamya bengalensis* (Lamarck, 1882): an integrative biomarker response assessment of behavioral indices, oxygen consumption, haemocyte and tissue protein levels under environmentally relevant exposure concentrations. Environ. Toxicol. Pharmacol. 89, 103789.

Dietz, A.A., 1977. Colorimetric Determination of Serum Cholinesterase and Its Genetic Variants by the Propionyl Thiocholine Dithiobis (Nitrobenzoic Acid) Procedure, 8. American Association for Clinical Chemistry, pp. 41–46. Cooper G.R. and King J.S., eds.

Dillon, R.T., Wethington, A.R., Rhett, J.M., Smith, T.P., 2006. Freshwater Gastropoda. The Mollusks: A Guide to Their study, Collection, and Preservation. The American Malacological Society, Pittsburgh, PA, pp. 251–259.

English, B.A., Webster, A.A., 2012. Acetylcholinesterase and its inhibitors. Primer on the Autonomic Nervous System. Academic Press, pp. 631–633.

Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ. Pollut. 177, 1–3.

Gbogbo, F., Takyi, J.B., Billah, M.K., Ewool, J., 2020. Analysis of microplastics in wetland samples from coastal Ghana using the Rose Bengal stain. Environ. Monit. Assess. 192, 1–10.

Giraud-Billoud, M., Vega, I.A., Tosi, M.E.R., Abud, M.A., Calderón, M.L., Castro-Vazquez, A., 2013. Antioxidant and molecular chaperone defences during estivation and arousal in the South American apple snail *Pomacea canaliculata*. J. Exp. Biol. 216, 614–622.

González-Palma, I., Escalona-Buendía, H.B., Ponce-Alquicira, E., Téllez-Téllez, M., Gupta, V.K., Díaz-Godínez, G., Soriano-Santos, J., 2016. Evaluation of the antioxidant activity of aqueous and methanol extracts of *Pleurotus ostreatus* in different growth stages. Front. Microbiol. 7, 1099.

Gorinstein, S., Arancibia-Avila, P., Moncheva, S., Toledo, F., Trakhtenberg, S., Gorinstein, A., Goshev, I., Namiesnik, J., 2006. Changes in mussel *Mytilus galloprovincialis* protein profile as a reaction of water pollution. Environ. Int. 32, 95–100.

Gorsd, M.N., Blanco, M.N., Pizzio, L.R., 2012. Synthesis of polystyrene microspheres to be used as template in the preparation of hollow spherical materials: study of the operative variables. Procedia Mater. Sci. 1, 432–438.

Goswami, P., Vinithkumar, N.V., Dharani, G., 2021. Microplastics particles in seafloor sediments along the Arabian Sea and the Andaman Sea continental shelves: first insight on the occurrence, identification, and characterization. Mar. Pollut. Bull. 167, 112311.

Gowd, S.S., Reddy, M.R., Govil, P.K., 2010. Assessment of heavy metal contamination in soils at Jajmau (Kanpur) and Unnao industrial areas of the Ganga Plain, Uttar Pradesh, India. J. Hazard. Mater. 174, 113–121.

Gu, Q.H., Husemann, M., Wu, H.H., Dong, J., Zhou, C.J., Wang, X.F., Gao, Y.N., Zhang, M., Zhu, G.R., Nie, G.X., 2019. Phylogeography of Bellamya (Mollusca: gastropoda: viviparidae) snails on different continents: contrasting patterns of diversification in China and East Africa. BMC Evol. Biol. 19, 1–13.

Guo, X., Cai, Y., Ma, C., Han, L., Yang, Z., 2021. Combined toxicity of micro/nano scale polystyrene plastics and ciprofloxacin to *Corbicula fluminea* in freshwater sediments. Sci. Total Environ. 789, 147887.

Hampel, M., Blasco, J., Díaz, M.M., 2016. Biomarkers and effects. Marine Ecotoxicology. Academic Press, pp. 21–165.

Horton, A.A., Newbold, L.K., Palacio-Cortés, A.M., Spurgeon, D.J., Pereira, M.G., Carter, H., Gweon, H.S., Vijver, M.G., van Bodegom, P.M., da Silva, M.A.N., Lahive, E., 2020. Accumulation of polybrominated diphenyl ethers and microbiome response in the great pond snail *Lymnaea stagnalis* with exposure to nylon (polyamide) microplastics. Ecotoxicol. Environ. Saf. 188, 109882.

Imhof, H.K., Laforsch, C., 2016. Hazardous or not–are adult and juvenile individuals of *Potamopyrgus antipodarum* affected by non-buoyant microplastic particles? Environ. Pollut. 218, 383–391.

Jeyavani, J., Sibiya, A., Gopi, N., Mahboob, S., Riaz, M.N., Vaseeharan, B., 2022. Dietary consumption of polypropylene microplastics alter the biochemical parameters and histological response in freshwater benthic mollusc *Pomacea paludosa*. Environ. Res. 212, 113370.

Jung, M.R., Horgen, F.D., Orski, S.V., Rodriguez, V., Beers, K.L., Balazs, G.H., Jones, T.T., Work, T.M., Brignac, K.C., Royer, S.J., Hyrenbach, K.D., 2018. Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. Mar. Pollut. Bull. 127, 704–716.

Kinjo, A., Mizukawa, K., Takada, H., Inoue, K., 2019. Size-dependent elimination of ingested microplastics in the Mediterranean mussel *Mytilus galloprovincialis*. Mar. Pollut. Bull. 149, 110512.

Kukkola, A., Krause, S., Lynch, I., Smith, G.H.S., Nel, H., 2021. Nano and microplastic interactions with freshwater biota–current knowledge, challenges and future solutions. Environ. Int. 152, 106504.

Lai, V.W.M., Kanaki, K., Pergantis, S.A., Cullen, W.R., Reimer, K.J., 2012. Arsenic speciation in freshwater snails and its life cycle variation. J. Environ. Monit. 14, 743–751.

Lebreton, L., Van Der Zwet, J., Damsteeg, J.W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world's oceans. Nat. Commun. 8, 1–10.

Li, J., Green, C., Reynolds, A., Shi, H., Rotchell, J.M., 2018. Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. Environ. Pollut. 241, 35–44.

Liu, Y., Hu, J., 2020. Investigation of polystyrene-based microspheres from different copolymers and their structural color coatings on wood surface. Coatings 11, 14.

Lo, H.K.A., Chan, K.Y.K., 2018. Negative effects of microplastic exposure on growth and development of *Crepidula onyx*. Environ. Pollut. 233, 588–595.

Lompré, J.S., Moleiro, P., De Marchi, L., Soares, A.M., Pretti, C., Chielini, F., Pereira, E., Freitas, R., 2021. Bioaccumulation and ecotoxicological responses of clams exposed to terbium and carbon nanotubes: comparison between native (Ruditapes

decussatus) and invasive (Ruditapes philippinarum) species. Sci. Total Environ. 784, 146914.
Magni, S., Gagné, F., André, C., Della Torre, C., Auclair, J., Hanana, H., Parenti, C.C., Bonasoro, F., Binelli, A., 2018. Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in freshwater Zebra mussel *Dreissena polymorpha* (Mollusca: bivalvia). Sci. Total Environ. 631, 778–788.
Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. JBC 247, 3170–3175.
Moncheva, S., Namiesnik, J., Apak, R., Arancibia-Avila, P., Toledo, F., Kang, S.G., Jung, S.T., Gorinstein, S., 2011. *Rapana venosa* as a bioindicator of environmental pollution. J. Chem. Ecol. 27, 31–41.
Naidu, S.A., Ranga Rao, V., Ramu, K.J.E.G., 2018. Microplastics in the benthic invertebrates from the coastal waters of Kochi, Southeastern Arabian Sea. Environ. Geochem. Health 40, 1377–1383.
Naji, A., Nuri, M., Vethaak, A.D., 2018. Microplastics contamination in molluscs from the northern part of the Persian Gulf. Environ. Pollut. 235, 113–120.
Nakashima, E., Isobe, A., Kako, S.I., Itai, T., Takahashi, S., Guo, X., 2016. The potential of oceanic transport and onshore leaching of additive-derived lead by marine macro-plastic debris. Mar. Pollut. Bull. 107, 333–339.
Oehlmann, J., Schulte-Oehlmann, U., 2003. Molluscs as bioindicators. In: Trace Metals and other Contaminants in the Environment, 6, pp. 577–635.
Omidi, M.H., Alibeygi, M., Piri, F., Masoudifarid, M., 2017. Polystyrene/magnetite nanocomposite synthesis and characterization: investigation of magnetic and electrical properties for using as microelectromechanical systems (MEMS). Mater. Sci. Pol. 35, 105–110.
Panda, F., Pati, S.G., Anwar, T.N., Samanta, L., Paital, B., 2022. Seasonal variation of water quality modulated redox regulatory system in the apple snail *Pila globosa* and its use as a bioindicator species in freshwater ecosystems across India. Water J. 14, 3275.
Parra, S., Varandas, S., Santos, D., Félix, L., Fernandes, L., Cabecinha, E., Gago, J., Monteiro, S.M., 2021. Multi-biomarker responses of Asian clam *Corbicula fluminea* (Bivalvia, Corbiculidea) to cadmium and microplastics pollutants. Water J. 13, 394.
Perić, L., Nerlović, V., Žurga, P., Žilić, L., Ramšak, A., 2017. Variations of biomarkers response in mussels Mytilus galloprovincialis to low, moderate and high concentrations of organic chemicals and metals. Chemosphere 174, 554–562.
Qu, H., Ma, R., Barrett, H., Wang, B., Han, J., Wang, F., Chen, P., Wang, W., Peng, G., Yu, G., 2020. How microplastics affect chiral illicit drug methamphetamine in aquatic food chain? From green alga (*Chlorella pyrenoidosa*) to freshwater snail (*Cipangopaludian cathayensis*). Environ. Int. 136, 105480.
Raynova, Y., Marchev, A., Doumanova, L., Pavlov, A., Idakieva, K., 2015. Antioxidant Activity of *Helix aspersa maxima* (Gastropod) Hemocyanin. Acta Microbiol. Bulg. 31, 127–131.
Ribeiro, F., Garcia, A.R., Pereira, B.P., Fonseca, M., Mestre, N.C., Fonseca, T.G., Ilharco, L.M., Bebianno, M.J., 2017. Microplastics effects in *Scrobicularia plana*. Mar. Pollut. Bull. 122, 379–391.
Risso, A., Pellegrino, F.J., Corrada, Y., Schinella, G., 2021. Evaluation of total antioxidant activity and oxidative stress in seminal plasma from dogs supplemented with fish oil and vitamin E. Int. J. Fertil. Steril. 15, 15.
Rout, B., Mehta, N.K., Tripathi, H.H., Sharma, S., Majumdar, R.K., Priyadarshini, M.B., 2022. Preliminary investigations on antimicrobial, antioxidant and nutritional properties of freshwater snail *Brotia costula* (Rafinesque, 1833). Indian J. Fish. 69, 100–110.
Santos, D., Félix, L., Luzio, A., Parra, S., Cabecinha, E., Bellas, J., Monteiro, S.M., 2020. Toxicological effects induced on early life stages of Zebrafish (*Danio rerio*) after an acute exposure to microplastics alone or co-exposed with copper. Chemosphere 261, 127748.
Sarkar, D.J., Sarkar, S.D., Das, B.K., Manna, R.K., Behera, B.K., Samanta, S., 2019. Spatial distribution of meso and microplastics in the sediments of river Ganga at eastern India. Sci. Total Environ. 694, 133712.
Sarkar, D.J., Das Sarkar, S., Das, B.K., 2022. Occurrence, Fate and Removal of Microplastics in Wastewater Treatment Plants (WWTPs) and Drinking Water Treatment Plants (DWTPs). Microplastics Pollution in Aquatic Media: Occurrence, Detection, and Removal. Singapore, Springer Singapore, pp. 223–245.
Sarkar, D.J., Das Sarkar, S., Kumar, S., Kundu, S., Das, B.K., 2022b. Microplastic pollution In Ganga: present status and future need. Sci. Cult. 88, 400–405.
Sarkar, D.J., Sarkar, S.D., Das, B.K., Praharaj, J.K., Mahajan, D.K., Purokait, B., Mohanty, T.R., Mohanty, D., Gogoi, P., Kumar, S., Behera, B.K., 2021b. Microplastics removal efficiency of drinking water treatment plant with pulse clarifier. J. Hazard. Mater. 413, 125347.
Sarkar, D.J., Sarkar, S.D., Das, B.K., Sahoo, B.K., Das, A., Nag, S.K., Manna, R.K., Behera, B.K., Samanta, S., 2021a. Occurrence, fate and removal of microplastics as heavy metal vector in natural wastewater treatment wetland system. Water Res. 192, 116853.
Sarkar, T., 2022. Diversity and conservation status of malacofauna from the river Teesta, West Bengal, India. Asian J. Conserv. Biol. 11, 240–246.
Sarkar, T., Debnath, S., Das, B.K.D.M., 2021. Edible fresh water molluscs diversity in the different water bodies of Gangarampur Block, Dakshin Dinajpur, West Bengal. Ecol. Environ. Conserv. 27, S293–S296.
Scherer, C., Weber, A., Stock, F., Vurusic, S., Egerci, H., Kochleus, C., Arendt, N., Foeldi, C., Dierkes, G., Wagner, M., Brennoholt, N., Reifferscheid, G., 2020. Comparative assessment of microplastics in water and sediment of a large European river. Sci. Total Environ. 738, 139866.
Sengupta, M.E., Kristensen, T.K., Madsen, H., Jørgensen, A., 2009. Molecular phylogenetic investigations of the Viviparidae (Gastropoda: caenogastropoda) in the lakes of the Rift Valley area of Africa. Mol. Phylogenet. Evol. 52, 797–805.
Shim, W.J., Song, Y.K., Hong, S.H., Jang, M., 2016. Identification and quantification of microplastics using Nile Red staining. Mar. Pollut. Bull. 113, 469–476.
Sil, M., Aravind, N.A., Karanth, K.P., 2019. Role of geography and climatic oscillations in governing into-India dispersal of freshwater snails of the family: viviparidae. Mol. Phylogenet. Evol. 138, 174–181.
Slootmaekers, B., Carteny, C.C., Belpaire, C., Saverwyns, S., Fremout, W., Blust, R., Bervoets, L., 2019. Microplastic contamination in gudgeons (*Gobio gobio*) from Flemish rivers (Belgium). Environ. Pollut. 244, 675–684.
Takahara, S., Hamilton, H.B., Neel, J.V., Kobara, T.Y., Ogura, Y., Nishimura, E.T., 1960. Hypocatalasemia: a new genetic carrier state. J. Clin. Investig. 39, 610–619.
Tanaka, K., Takada, H., Yamashita, R., Mizukawa, K., Fukuwaka, M.A., Watanuki, Y., 2015. Facilitated leaching of additive-derived PBDEs from plastic by seabirds' stomach oil and accumulation in tissues. Environ. Sci. Technol. 49, 11799–11807.
Vass, K.K., Das, M.K., Srivastava, P.K., Dey, S., 2009. Assessing the impact of climate change on inland fisheries in River Ganga and its plains in India. Aquat. Ecosyst. Health Manag. 12, 138–151.
Von Moos, N., Burkhardt-Holm, P., Köhler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ. Sci. Technol. 46 (20), 11327–11335.
Wagner, M., Lambert, S., 2018. Freshwater Microplastics: Emerging Environmental Contaminants? Springer International Publishing, Cham, Heidelberg, New York, Dordrecht, London.
Wang, X., Huang, W., Wei, S., Shang, Y., Gu, H., Wu, F., Lan, Z., Hu, M., Shi, H., Wang, Y., 2020. Microplastics impair digestive performance but show little effects on antioxidant activity in mussels under low pH conditions. Environ. Pollut. 258, 113691.
Watts, A.J., Urbina, M.A., Goodhead, R., Moger, J., Lewis, C., Galloway, T.S., 2016. Effect of microplastic on the gills of the shore crab *Carcinus maenas*. Environ. Sci. Technol. 50, 5364–5369.
Weber, A., von Randow, M., Voigt, A.L., von der Au, M., Fischer, E., Meermann, B., Wagner, M., 2021. Ingestion and toxicity of microplastics in the freshwater gastropod *Lymnaea stagnalis*: no microplastic-induced effects alone or in combination with copper. Chemosphere 263, 128040.
Wegner, A., Besseling, E., Foekema, E.M., Kamermans, P., Koelmans, A.A., 2012. Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). Environ. Toxicol. 31, 2490–2497.
Weis, J.S., 2019. Improving microplastic research. AIMS Environ. Sci. 6, 326–340.
Wright, S.L., Kelly, F.J., 2017. Plastic and human health: a micro issue? Environ. Sci. Technol. 51, 6634–6647.
Zheng, J., Li, C., Zheng, X., 2022. Toxic effects of polystyrene microplastics on the intestine of *Amphioctopus fangsiao* (Mollusca: cephalopoda): from physiological responses to underlying molecular mechanisms. Chemosphere 308, 136362.