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Microplastics Contamination in the Edible Fish Mozambique Tilapia (*Oreochromis mossambicus*) from the Selvampathy Wetland of Coimbatore, Tamil Nadu, India

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Abstract

The present study investigated the microplastics (MPs) contamination in the gastrointestinal (GI) tract, gills and muscle of the Mozambique tilapia, *Oreochromis mossambicus* sampled from the Selvampathy Lake of Coimbatore, Tamil Nadu. MPs abundance was found in 10 to 28, 8 to 27, and 4 to 12 particles and their size ranged between 4.4 and 210, 4.6 to 180, and 4.5 to 194 µm in the GI tract, gills and muscle, respectively. MPs were dominantly shaped as fibres (95%) and fragments (5%) with the following colour pattern of blue > black > pink > transparent > and others. Extracted MPs polymer nature were polyethylene (54%), polyamide (38%) and polypropylene (8%). The present study reveals that the edible fish *O. mossambicus* had MPs that can be transferred to consumers. Moreover, urban discharges, including domestic wastes, agricultural and rainwater runoff, might be possible MPs sources to the studied wetland.

Keywords Plastics · Fish · Wetland · Muscle · India · Selvampathy Lake

Introduction

Plastics are omnipresent in our environment due to their durability, lightweight, strength and inexpensiveness (Li et al. 2021). It has been estimated that about 79% of plastic wastes are incorrectly discarded or treated and dumped into landfills or other natural environments (Geyer et al. 2017). Nearly 1.15 to 2.14 million tons of plastic waste enter oceans via rivers, and the accumulation of plastics in the aquatic environment is directly proportional to the linear use of plastics and accretion on land (Jambeck et al. 2015). This ongoing process occurs when the plastic particle is weathered into small particles (Cole et al. 2011). The dominant factors for the fragmentation of plastics include physical mechanisms like weathering by ultraviolet radiation from sunlight, mechanical stresses such as abrasion, wave action and turbulence, and thermal and chemical actions like plastic surface chemistry changes induced by photo-oxidative reactions (Arp et al. 2021). The degradation of these plastics poses a significant risk as it becomes hard to identify and remove the MPs.

Microplastics (MPs), as a size < 5 mm, have a great concern among environmentalists and pollution biologists due to their wide distribution on the earth and occurrence in different parts of an organism. The smaller size of the MPs overlaps with the feed size of many aquatic organisms, making them straightforwardly ingested by aquatic organisms (Walkinshaw et al. 2020). Aquatic organisms such as zooplankton and benthic feeders like bivalves, corals, and vertebrates, including fishes, water birds, and mammals, can uptake the MPs, and it injures the digestive tracts of the fish and can even lead to internal blockages (Prinz et al., 2020; Li et al. 2023). The ingestion of MPs is shown to have detrimental effects on feeding disruption, metabolic changes, reproductive ailments, etc., on various fishes, crustaceans, and molluscs (Anbumani and Kakkar 2018; Aljaibachi and Callaghan 2018; Pandey et al. 2022). However, the inland

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parts of the countries with freshwater fish consumption are less investigated than the coastal areas.

The present study area, Selvampathy Lake (11° 0' 10.8"N and 76° 56' 16.8" E), is an ecologically significant Lake of Coimbatore, Tamil Nadu, India, and it is considered the city's heart, covering an area of 40 acres (Dineshkumar and Natarajan 2020). This wetland has been serving as a livelihood for local communities due to the presence of edible fishes *Catla catla, Oreochromis mossambicus*, and *Crypnius carpio*. Also, this wetland serves as a habitat for water birds, including migratory birds (Hemambika et al. 2014). This lake is a natural habitat for 15 floral species, 29 Avifaunal species belonging to 17 families, and four edible fish species from Cyprinidae, Channidae, and Cichlidae families. The primary water source for Selvampathy Lake is Krishnampathy Lake, which was recorded with poor water quality caused by agricultural and mixed urban wastes (Janaranjani et al. 2017). Despite these, this wetland's health is affected by nearby urban activities, building constructions, municipal sewage disposal, and urban runoff (Ragunath and Sundar 2017). Observing and conserving the wetland from these pollutants is inevitable because it carries the chance of potential MPs transfer to its consumers. Mozambique tilapia, *O. mossambicus*, is a major edible fish used by inland fisheries, including lakes and ponds, around the globe for its high nutritional and medicinal properties. The fisheries sector in India aimed to enhance fish production through tilapia fish cultivation to achieve an annual growth rate of fish production of up to 9.0% (MoST, 2022). Due to their availability, extensive production in inland aquaculture and low cost, nearby lake residents show more interest in its consumption. Moreover, the reports on the occurrence of MPs in the edible freshwater fishes from the urban wetlands in India are limited. Hence, the present study aimed to evaluate the occurrence of MPs and their morphological properties (size, shape, and colour) with their polymer nature in the gills, gastrointestinal tracts (GI tracts), and muscle tissues of *O. mossambicus* individuals to address the MPs pollution status of Selvampathy Lake.

Materials and Methods

A total of 22 individual fish were caught at Selvampathy Lake (Fig. 1) with the help of local fishermen, kept frozen in a high-pressure-steam sterilized sample container and transported to the laboratory for further analysis. The fish were washed using distilled water to remove external plastic contamination, and the morphometry was measured. The length and weight of fish ranged from 17.4 to 22.7 cm and 114 to 195 g, respectively. Further, fish species were initially confirmed with the data from the Fish Base website and the taxonomy of the collected fish, the standard manuals (Strauss and Bond 1990; Froese and Pauly 2022). Besides, the genomic DNA was isolated to confirm the species using the salting out method protocol (Russell and Sambrook, 2001). 16s rRNA fragments (~ 570 bp) from three *O. mossambicus* individuals were amplified using a universal primer (Palumbi 1996). The PCR was done with initial denaturation (94 °C for 5 min), denaturation (94 °C for the 30s), annealing (52 °C for 30s), extension (72 °C for 45s) for 35 cycles and final extension at (72 °C for 10 min). PCR results were visualised on agarose gel electrophoresis (1.5%) containing ethidium bromide, using Gel Doc™ XR+ (Bio-Rad, India), and the Applied Biosystems 3730xl DNA analyzer used to sequence the amplified PCR products. Obtained sequences were edited with clustalW multiple alignments (Thompson et al. 2003), aiding BioEdit version 5.0.9 (Hall 1999), deposited in GenBank, with the following accession numbers: OQ439744 - OQ439746.

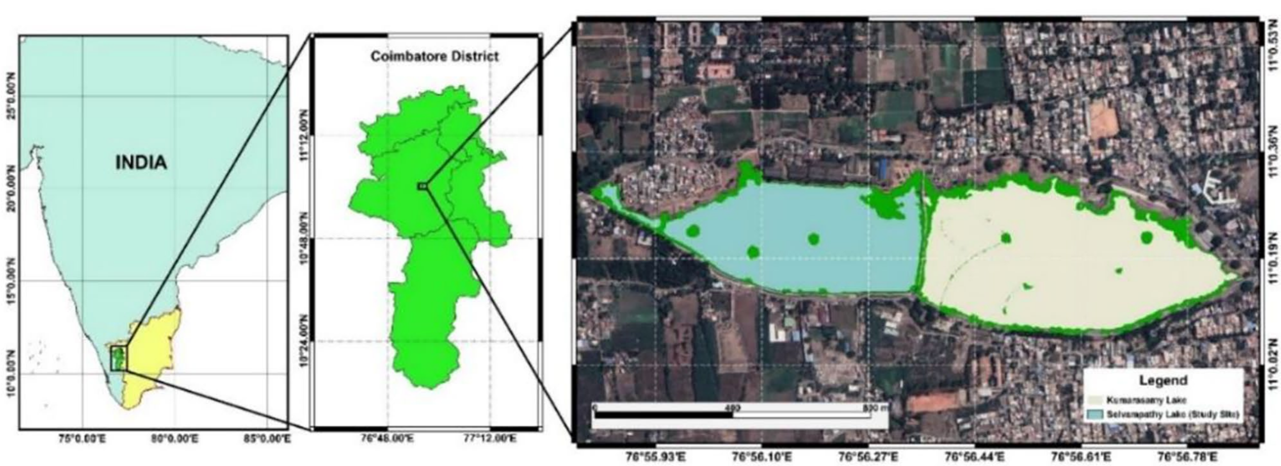

Fig. 1 Fish sampling sites of Selvampathy Lake

Gill, GI tract, and muscle parts were dissected, weighed, and transferred to a clean glass container for further inspection. The fish's different organs, GI tracts, gills, and muscles were digested using 10% of KOH (≥ 95%) (w/v) incubation at 40 °C for 72 h to achieve digestion without affecting the MPs, and the remains were separated using 0.7 µm nylon membrane from the mixture. Sodium iodide (NaI) with ≥ 95% was prepared at 4.4 M, and 15 ml was added with the beaker containing filter membrane for 5 min to separate the MPs on their density. Sonication was done at 50 Hz for 5 min in an ultra-probe sonicator. Later, the sample was treated by agitation by an orbital shaker at 200 rpm for 5 min and centrifuged at 500×g for 2 min. The residue was treated with the sequential order of sonication, agitation, centrifugation, and vacuum filtration was conducted to obtain the supernatant using a 0.7 µm pore filter membrane (Karami et al. 2017). Filter membranes were observed under a stereomicroscope with 20-40X magnification for MPs inspection. Suspected particles were treated with a hot needle to test and observe the melting point of the MPs (De Witte et al. 2014). Nile red stain was prepared at 10 µg ml^{-1} in acetone and used to treat the particles observed under a fluorescent microscope to confirm the MPs separated from the muscle tissues of fish (Maes et al., 2017). Identified and filtered MPs images were captured using a Magcam DC5 camera, and their size was measured using Magvision 4.11 software. The morphometric properties of MPs were examined using the following distinguishing criteria: shape, size, and colour, as stated by (Hidalgo-Ruz et al. 2012).

Isolated MPs from the fish organs (> 100 µm) from the filter membrane were considered to identify their polymer nature due to the size range of extracted MPs from the fish. FTIR analysis used the Shimadzu IR Affinity-1 Spectrometer (Shimadzu, India). Thirty scans were taken per particle with a spectral resolution of 4 cm^{-1} and a spectral range 400-4000 cm^{-1} in Attenuated Transmittance Reflectance (ATR) mode. Obtained spectra were compared with the Lab IR Solutions polymer library to identify the polymer type of MPs from various natural and synthetic polymer databases. Polymer matches with > 80% were considered for the confirmation of MPs.

All standard operating procedures were adopted for quality control management during the entire MPs extraction and characterisation process. Detailed information is stated in the supplementary materials section. The data were stated as mean ± SD. Significant variations in the MPs' number, length, shape and colour were determined by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. The level of significance was fixed at $p < 0.05$. The entire statistical analysis was performed using SPSS (26.0) software.

Results and Discussion

In the present study, 865 MPs particles were isolated from the GI tract, gills, and muscles of 22 individuals of *O. mossambicus*. Among them, the GI tract accounted for a higher number of MPs (379), followed by the gills (314) and muscles (172). Organ-wise distribution of MPs ranged between 10 and 28 MPs in the GI tract, 8 to 27 MPs in the gills, and 4 to 12 MPs in the muscles. MPs per individual fish, the GI tract accounted for more MPs (17.23 ± 4.3) than the other two organs, like gills (14.73 ± 5.3) and muscle (7.82 ± 2.06). A significant difference ($P < 0.05$) was observed in the number of MPs in the GI tract of the other two organs of *O. mossambicus* (Fig. 2a). These results suggest that the omnivore-feeding nature of *O. mossambicus* made the possibility of the higher ingestion of MPs through false feeding and fed on MPs ingested prey as direct and indirect ingestion respectively, which led to accumulation in GI tract and muscle. Besides, MPs get adhered within the gills from the water through non-selective water exchange by fish (Yin et al. 2022). Similarly, fishes like *Arius maculates*, *Etroplus suratensis*, and *Etroplus maculatus* from Vembanad Lake, Kerala, India, shown the GI tracts were accounted for with MPs (Nikki et al. 2021), which recorded lower than the present study. Moreover, MPs abundance in the GI tracts of freshwater fish *Sarotherodon melanotheron* and *Chrysichthys nigrodigitatus* from Densu River, Ghana (Blankson et al. 2022) has been reported, which showed lower than the present study.

Meanwhile, the organ-wise accumulation of MPs in the fish per gram ranged from 0.80 to 3.54 g^{-1}, 0.73 to 2.71 g^{-1}, and 0.8 to 2.4 g^{-1} in gills, GI tract and muscle, respectively. Likewise, the accumulation of MPs was significantly higher ($P < 0.05$) in gills (2.14 ± 0.83), after that, GI tracts (1.73 ± 0.61) and muscles (1.56 ± 0.41), which indicates that the direct exposure of gills to environmental matrices that led to more adherence of MPs from water (Fig. 2b, Fig. S1). Moreover, the occurrence of MPs in GI tracts and muscle tissues suggested that studied fish were vulnerable to MPs in the sampled urban wetland. The feeding behaviour, the structure of the GI tract, MPs size, transfer through gills to the circulatory system, and the physical retention of MPs in the GI tract of fishes might accumulate MPs in the tissues of fishes (Lu et al. 2016; Miller et al. 2020). Therefore, the MPs in the edible and inedible parts of fishes denote the risk of transfer to the diet of fish consumers, including humans. Previously, edible parts of shellfish, *Uroteuthis duvaucelii* and *Portunus pelagicus* were reported with a lesser accumulation of MPs than this study (Daniel et al. 2021). Similar to the abundance, fishes *Clupanodon thrissa*, and *Terapon jarbua* from Guangdong, South

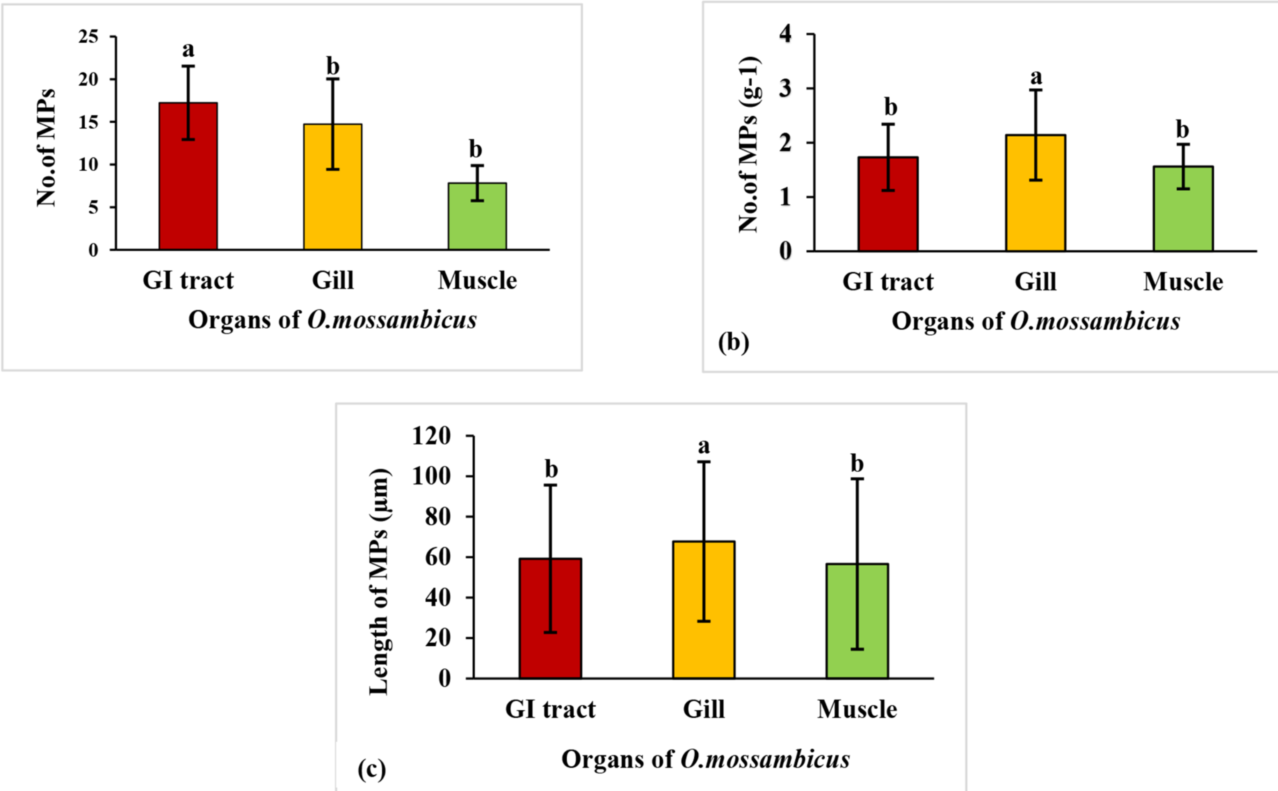

Fig. 2 **(a)** Abundance, **(b)** Accumulation per (g^{-1}), and **(c)** Length of MPs in the different organs of fish *O. mossambicus* individuals. Mean values of different bars with mean number of microplastic abundance sharing different alphabetical letters are statistically significant at P < 0.05

China, were also recorded with more MPs in GI tracts than other organs (Pan et al. 2021). Furthermore, Park et al. (2020) reported that the false ingestion of MPs by freshwater fishes like *C. carpio, Lepomis macrochirus, Carassius cuvieri, Micropterus salmoides*, and *Silurus asotus* from South Korea accounted for a higher number of MPs in their GI tracts than other organs of the examined fishes.

The size of MPs is important to determine the bioaccumulation and transfer to other tropics. In the present investigation, the length-wise distribution of total MPs ranged between 4.4 and 210 µm in *O. mossambicus*. MPs distribution among the organs showed a higher length in gills, followed by the GI tracts and muscles (Fig. 2c). This suggests that gills that are highly exposed to water lead to the entrapment and adherence of larger MPs from water. Moreover, different-sized MPs in the GI tract and muscle of *O. mossambicus* might be due to the non-intentional feeding of MPs from various food sources due to its omnivore feeding habit. Similar to the present study, 0.3 to 0.6 mm MPs have been observed in *C. carpio*, *C. cuvieri*, *L. macrochirus, M. salmoides, S. asotus*, and *Channa argus* from Han River, South Korea (Park et al. 2020). Besides, fish *S. trutta, W. attu*, and *C. naziri* from the Swat River of Pakistan were found to be 500 to 1000 µm in length MPs, which is higher than the present study, it denotes the ingestion of MPs can be varied in their habitats and their feeding habits (Li et al. 2021; Bilal et al. 2022).

Primary and secondary MPs have been reported with various shapes like fibres, fragments, pellets, foam, etc. The shape of the MPs in every ecosystem is mainly affected by other severe environmental conditions like photo-oxidative reactions and wave actions. In our study, the shape dimension of every isolated particle was observed and revealed as dominant in fibre shape and remaining as fragments. All examined organs shared the same fate in the shape of MPs. Fibres were occupied at 94, 96, and 96% in the GI tracts, gills, and muscles, respectively. Fragments accounted for 6% of the GI tracts, 4% of the gills, and 4% of the muscles of *O. mossambicus*. Moreover, 23 number of fragments were recorded in the GI tract than in other organs (Fig S2, Fig S4). These results revealed that these fibres and fragments could be discharged from primary sources like textile fibres, tire dust, personal care products, and engineered plastic pellets. Secondary MPs sources like macro plastic debris originated from disposed consumer plastic wastes. Previously, the riverine fishes *Chanos chanos, Chelon macrolepis, Chanda*

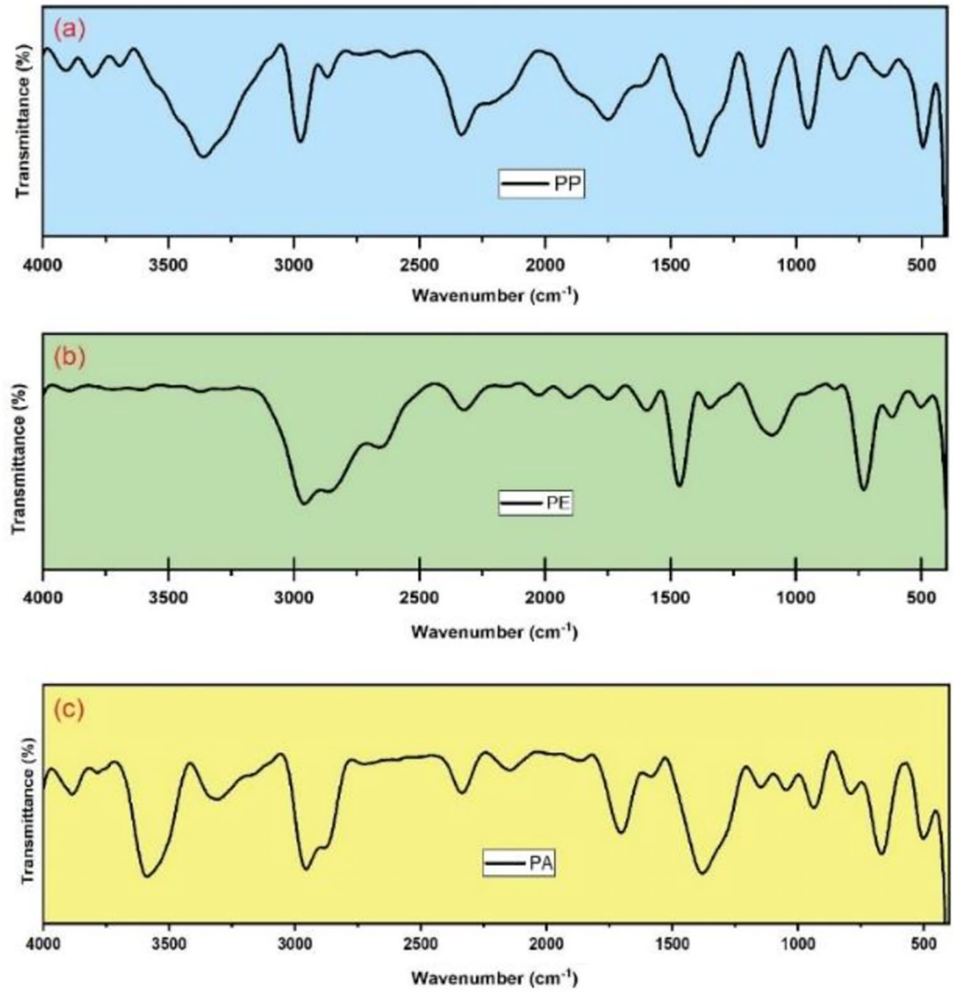

Fig. 3 FT-IR spectra of the identified MPs polymers from the organs of *O. mossambicus* **(a)** PP - Polypropylene, **(b)** PE – Polyethylene, and **(c)** PA – Polyamide (Nylon)

nama, Gerrus filamentosus and *Carangoides malabaricus* were also accounted with dominant fibre and fragments-shaped MPs (Anandhan et al. 2022). Besides, fishes such as *S. cephalus*, *C. carpio*, and *Alburnus mossulensis* accounted for nearly 88% of fibres and 6% of fragments (Atamanalp et al. 2022). Meanwhile, the GI tract of fish *Piaractus brachypomus* from the Vembanad Lake consisted of fibres, fragments, and foam (Devi et al. 2020).

Challenges in identifying and differentiating MPs from organic matter due to their smaller size were tackled using the coloured patterns of isolated MPs. In the present study, isolated MPs from the different organs of *O. mossambicus* showed 11 different coloured patterns: blue, black, pink, green, red, white, yellow, brown, grey, purple and transparent. Blue (36%) and black (24%) were predominant, over the other colours in all examined organs of fish (Fig S3, Fig S4). The dominant level of blue and other coloured MPs in all studied organs of the GI tract of *O. mossambicus* suggests these coloured fibres and fragments could be derived from the wetland's textile wastes and fishing gears. Previous studies also reported that a similar pattern of MPs was observed in the gills and GI tracts of *Gambusia affinis* from the Brantas River, Indonesia (Buwono et al. 2021). All colour patterns of MPs in this study match MPs observed in the fishes *Gobio gobio* and *Rutilus rutilus* of the Widawa River in Poland (Kuśmierek and Popiołek 2020). Gills and GI tracts contents of *Tilapia sparrmanii* individuals from the delta region of Botswana were accounted for predominantly translucent (40.0%) blue (22.1%), black (20.0%), and red (17.9%), which share the same colour pattern of the present study (Ditlhakanyane et al. 2022). From the Nile River, *Oreochromis niloticus* and *Bagrus bajad* ingested black and red coloured MPs more than other coloured MPs (Khan et al. 2020). It indicates that the colours of their prey or food, light conditions in the wetland, food availability, and feeding habits of fish influenced the false ingestion of these predominant dark-coloured MPs (Wu et al., 2020; Okamoto et al. 2022).

Apart from the morphometric analysis of MPs, the polymer composition of MPs is required to find the occurrence of MPs in complex environmental and biological samples. Polymer analysis will define the total contamination of MPs origin in the studied ecosystems. In the present study, the extracted MPs were constituted with polyethylene (PE), polyamide (PA), and polypropylene (PP) polymer types (Fig. 3). They were majorly dominated by the PE (54%), PA (36%) and PP (10%) (Fig. S5) in all examined organs of *O. mossambicus*. These identified polymers were commercial ones mostly applied in many industrial applications like packaging, machinery parts, and textile fibres production and the same were found in the GI tracts of fishes, crustaceans, and water birds from global water bodies (Obbard et al. 2014; Rummel et al. 2016). Similarly, fishes from the southern coastal regions of India were detected with PE, PP, and PA (Harikrishnan et al., 2023). In freshwater bodies, PE and PP dominated the other polymers listed for commercial use (Bordós et al., 2019). As secondary MPs, The identified polymer types from the different organs might be derived from large plastic particles' degradation and breakdown process. Also, the GI tracts of *P. brachypomus* from the Vembanad Lake, Kerala, had the same polymer composition of MPs like PE, PA, and PP (Devi et al. 2020).

Conclusion

The present study revealed the occurrence of MPs in the GI tracts, gills, and muscles of *O. mossambicus* sampled from Selvampathy Lake, Coimbatore, Tamil Nadu, which serves as an ecological habitat for flora and faunal communities. The results indicate the MPs pollution of the selected wetland and the vulnerability of the edible fish *O. mossambicus* to MPs pollution. A higher occurrence of MPs was recorded in the GI tracts than in other organs, which reveals that the omnivore-feeding nature of *O. mossambicus* had facilitated more MPs through diets. Besides, the occurrence of MPs in the edible muscle part of studied fish suggests the possible transfer of MPs to consumers. MPs pollution in the selected wetland is highly encouraged by the city's municipal wastes, which are associated with synthetic textiles, fishing gear fibres, and other plastic fragments. Hence, our study reveals primary evidence about the MPs pollution in Selvampathy Lake and the possible risk of MPs transfer to other tropics from the MPs contaminated fish. Besides, regular monitoring and regulatory measures are recommended to manage the plastic pollution in the studied wetland.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00128-023-03839-w.

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Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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