

Microplastic Analysis in Catfish *Mystus gulio* (Hamilton 1822) and Tilapia *Oreochromis niloticus* (Linnaeus 1758) from Tirunelveli District, Tamil Nadu, India

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ABSTRACT

The microplastics (MPs) are less than 5 mm in length. The freshwater organisms, including fish, can consume microplastics. Here, we investigate the presence of microplastics in *Mystus gulio* and *Oreochromis niloticus* fish species. The fishes were collected from Suthamalli, Thamirabarani River, Tirunelveli. We identified the collected fish at the species level, and fish gastrointestinal tracts were dissected and ground with mortar and pestle. Samples were filtered, and dried in sunlight. The presence of

microplastic particles was observed by the Olympus (CH20i) microscope. We analyzed the morphological variation (fiber, fragment and bead) and different colored (white, red, blue, brown, black and green) microplastics in the gastrointestinal tract of both species. The both fish *Mystus gulio* and *Oreochromis niloticus* Hepatosomatic Index (HSI), Gastro-Somatic Index (GSI), Splenosomatic Index (SSI) and Hypersomatic Index (NSI) were calculated. In this study, MPs present in the gut of *Mystus gulio*, 33.3% of fiber, 61.90% of fragment, and 4.76% of beads were found. In *Oreochromis niloticus*, 36.36% of fiber and 63.63% of fragment were found. The isolated fish gut MPs were characterized by ATR-FTIR. The analysis confirmed the presence of polymers like, polyethylene terephthalate (PET), polypropylene (PP), polyamide (Nylon), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene (PE). Morphometric analysis further revealed that the highest degree of correlation was observed between the total length and the standard length for both fish species. This research provides proof that both fish species are contaminated by the MPs, the river ecosystem is contaminated by plastics.

Keywords Microplastics, *Mystus gulio*, *Oreochromis niloticus*, Thamirabarani river, Gastrointestinal tract.

INTRODUCTION

Plastics are used in a variety of products, these many products produced waste micro and nano-plastics,

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which contaminates various environments. In 2019, 24.1 metric tonnes of plastics have been used in India and 2.0 – 5.6 Metric tonnes were recycled Dhodapkar *et al.* (2023). Nowadays, all sectors are using plastics for packaging various materials (automobiles, cosmetics, food and textiles). The presence of microplastics have been reported in salt pans, fish, groundwater, surface water, lakes and rivers (Ravikumar *et al.* (2023), Kumar *et al.* (2018), Selvam *et al.* (2021), Srinivasalu *et al.* (2021), and Lechthaler *et al.* (2021)). The shape of MPs is like a film, foam, fiber, sheet, fragments, beads and pellets (Sruthy and Ramasamy (2017), and Lusher *et al.* (2020). In Kerala, four different types of polymers were identified: HDPE - High-Density Polyethylene, LDPE - Low-Density Polyethylene, PP – Polypropylene, and PET Polyethylene Terephthalate Mahidev *et al.* (2024). MPs pose a major series threat to marine and freshwater organisms (Kumar *et al.* 2018 and Bhuyan 2022). The *Mystus gulio* is a carnivorous euryhaline fish, occurring mostly in freshwater and less saline brackish water Gupta (2014). Morphometric data provide the larger sample size from the same and different habitats of fish species of tilapia Kosai *et al.* (2014). Therefore, this research aims to analyze the microplastics from gastrointestinal tracts of two different fish species. The ATR-FTIR spectroscopic identification of different types of polymers of both species is reported.

MATERIALS AND METHODS

Study area and identification fish

The fish samples *Mystus gulio* and *Oreochromis niloticus* were collected from Thamirabarani River at Suthamalli (Latitude 8°41'08 N and Longitude 77°38'29 E) (Plate 1), Tirunelveli District, Tamil Nadu, India. The collected fishes were kept under the laboratory condition, after the gut dissection the specimen was stored under 4% formalin. All the collected fish were identified with the help of Inland fishes of India and adjacent countries (Talwar & Jhingran 1991).

Morphometric characters

Twelve morphometric characters were analyzed for the two fish species *Mystus gulio* and *Oreochromis niloticus* there are BDD - Body Depth, CPL - Caudal Peduncle Length, DOH - Dorsal Fin Height, HLL - Head Length, MXL - Maxillary Length, OOL - Orbital Length, PAD - Pelvic-Anal Fin Distance, PCL - Pectoral Fin Length, POL - Preorbital Length, PVL - Pelvic Fin Length, STL - Standard Length and TTL - Total Length (Malley *et al.* 2021).

Visceral organs index calculation

The visceral organs, like the liver, gut, spleen, and



Plate 1. Study area.

kidney, were carefully dissected from the fresh fish. Tissue weight was measured by digital weighing balance, measured weight was calculated by the following.

Hepatosomatic Index (HSI): $HSI (\%) = 100 \times (\text{Liver weight} / \text{Weight of the Fish})$

Gastro-Somatic Index (GSI): $GSI (\%) = 100 \times (\text{Gut weight} / \text{Weight of the Fish})$

Splenosomatic Index (SSI): $SSI (\%) = 100 \times (\text{Spleen weight} / \text{Weight of the Fish})$

Hyperosmotic Index (NSI): $NSI (\%) = 100 \times (\text{Kidney weight} / \text{Weight of the Fish})$

Fish gut removal, morphological observation and ATR- FTIR analysis

The guts of *Mystus gulio* and *Oreochromis niloticus* fish were removed to analyze the microplastics, the guts were dissected from the fish and well ground with the help of a mortar and pestle. After grinding, gut samples were filtered by the filter paper (Whatman No.1), and samples were dried in sunlight. The sample was transferred to petri dish for the identification of microplastics using an Olympus Trinocular Microscope. Visual identification and classification of microplastic particles have been done (Mariano *et al.* 2021). All the samples were characterized by Bruker ALPHA Spectrometer with a universal Zn-Se ATR (Attenuated Total Reflection) accessory in the range $600\text{-}4000\text{ cm}^{-1}$ operated in using OPUS software.



Fig. 1. Gastrointestinal track of catfish *Mystus gulio*.

RESULTS AND DISCUSSION

Taxonomy and morphometric

The two fish species classification and their habits are presented in the Table 1. The fish species body (morphometric) characters: TTL, BDD, CPL, DOH, HLL, MXL, OOL, PAD, PCL, POL, PVL and STL were expressed in cm, weight (gram) was recorded in Table 2. Table 3 shows the regression of total length (TTL) on body depth - (BDD), caudal peduncle length - (CPL), dorsal fin height - (DOH), head length - (HLL), maxillary length - (MXL), orbital length - (OOL), pelvic- anal fin distance - (PAD), pectoral fin length - (PCL), preorbital length - (POL), pelvic in length - (PVL), and standard length - (STL). For *M. gulio* and *O. niloticus*. The correlation coefficients indicate the highest degree of correlation between total length and standard length. For *M. gulio*, the strongest correlation was observed between total length and the pelvic-anal fin distance. Conversely, the correlation coefficients reveal a lower degree of correlation between total length and pectoral fin length, dorsal fin height, head length, and pelvic fin length in *O. niloticus*.

Visceral organs index

Table 4 shows the variation of the hepatosomatic index, gastro-somatic index, splenosomatic index, and hyperosmotic index of two fish species *Mystus gulio* and *Oreochromis niloticus*. Figures 1–2 show the gastrointestinal tract of *Mystus gulio* and *Oreochromis niloticus*.



Fig. 2. Gastrointestinal track of Tilapia *Oreochromis niloticus*.

Table 1. Taxonomy and common name of *Mystus gulio* and *Oreochromis niloticus*.

Sl. No.	Taxonomy and common name	Feeding habit
1	Order: Siluriformes Family: Bagridae Genus: <i>Mystus</i> Species: <i>Mystus gulio</i> (Hamilton 1822) Common name: Long whiskers catfish Tamil name: Kattai-keluthi	Carnivorous - Bottom feeder, most of the food intake by fish - Copepods, insect larvae, crab, gastropods, prawns (Mondal and Mitra (2016) and Gupta (2014))
		
	Catfish (<i>M. gulio</i>)	
2	Order: Perciformes Family: Cichlidae Genus: <i>Oreochromis</i> Species: <i>Oreochromis niloticus</i> (Linnaeus 1758) Common name: Tilapia Tamil name: Jalebi	Omnivorous - Most of the food intake by fish- algae, aquatic plants, small invertebrates. (Tesfahun and Temesgen (2018) and Bonham (2022))
		
	<i>O. niloticus</i>	

Table 2. Morphometric characters of catfish *Mystus gulio* (Hamilton 1822) and tilapia *Oreochromis niloticus* (Linnaeus 1758). * The morphometric characters of *Mystus gulio* (C1– C5) and *Oreochromis niloticus* (T6–T10).

Sl. No.	Morphometric characters (cm)	C1	C2	C3	C4	C5	T6	T7	T8	T9	T10
1	Total length	24	13.4	12.7	20.2	11.6	18.1	14.8	16.7	14.6	17.3
2	Body depth	5	3.5	2.8	4.2	3.5	7.5	5.4	6.2	5	7.8
3	Caudal peduncle length	3	2	1.8	3.3	2	2	1.9	1.9	1.5	2
4	Dorsal fin height	4.5	2.4	1.8	3.6	2.4	2	1.9	1.9	1.7	2
5	Head length	5	3	2.5	4.1	2.8	5	4	4.2	2.7	4.8
6	Maxillary length	3.5	0.6	0.6	1.5	0.6	1.5	1.5	1.5	1	1.5
7	Orbital length	1	0.3	0.3	1	0.3	1	1	1	1	1
8	Pelvic- anal fin distance	4	2.3	2	3.4	2.1	4.7	4	4.2	4	4.4
9	Pectoral fin length	3.5	1.8	1.2	3.1	1.5	6	4	4	4	5.8
10	Preorbital length	2	0.9	1.1	1.8	0.6	2.5	1.8	2	1.5	2
11	Pelvic fin length	2.5	1.5	1.3	2	1.3	4	3	3	3.3	3.7
12	Standard length	19.1	10	10	15.7	9.7	15	12	14.4	12	14.2
13	Sex of the fish	Female	Male	Male	Male	Male	Male	Male	Female	Male	Male
14	Weight of the fish (in grams)	130	21.8	15.6	65	20	112	50.8	56.3	47.7	96

Table 3. The relationship between TTL and the BDD, CPL, DOH, HLL, MXL, OOL, PAD, PCL, POL, PVL and STL of two fish species from Thamirabarni River, Tirunelveli. *Total length – (TTL), Body depth - (BDD), Caudal peduncle length - (CPL), Dorsal fin height - (DOH), Head length - (HLL), Maxillary length - (MXL), Orbital length - (OOL), Pelvic- anal fin distance - (PAD), Pectoral fin length - (PCL), Preorbital length - (POL), Pelvic in length - (PVL), and Standard length - (STL).

Sl. No.	Morphometric Characters	Catfish Correlation r	Tilapia Correlation r
1	TTL vs BDD	0.921	0.936
2	TTL vs CPL	0.902	0.749
3	TTL vs DOH	0.962	0.819
4	TTL vs HLL	0.982	0.875
5	TTL vs MXL	0.934	0.615
6	TTL vs OOL	0.961	0.945
7	TTL vs PAD	0.993	0.949
8	TTL vs PCL	0.974	0.838
9	TTL vs. POL	0.964	0.909
10	TTL vs. PVL	0.990	0.729
11	TTL vs. STL	0.995	0.976

Morphological observation of Microplastics

Three types of microplastics were identified from the gastrointestinal tract of the two fish species under microscope. Totally three different types of microplastics were identified in this study, there are fibers, fragments, and beads, while beads were not present in *O. niloticus* species. Six different colors of MPs

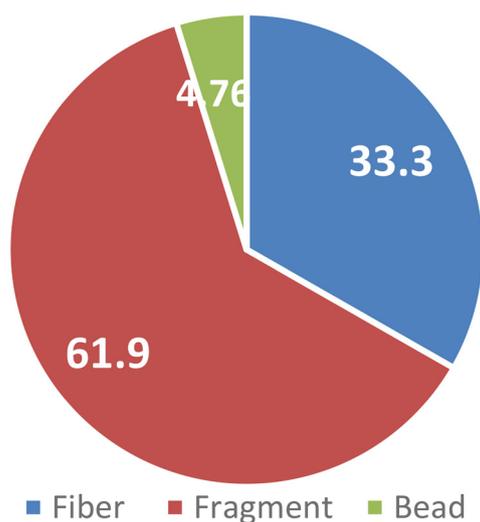


Fig. 3. The different type of microplastic in *M. gulos* gut in percentage (%).

Table 4. Hepatosomatic index, Gastro-somatic index, Splenosomatic index and Hyperosmotic index of two fish species.

Sl. No.	Species name	Hepato-somatic index	Gastro-somatic index	Spleno-somatic index	Hyper-osmotic index
1	<i>Mystus gulos</i>	0.76	0.61	0.15	0.46
2	<i>Oreochromis niloticus</i>	0.25	1.68	0.11	0.38

were founded from *M. gulos* gut (White, Red, Blue, Brown, Black and Green). In *O. niloticus* gut four colors were identified there are White, Blue, Black and Green. The 33.3% of fiber, 61.90% of fragment and 4.76% beads were identified in *M. gulos* and 36.36% of fiber and 63.63% fragment were observed in *O. niloticus* (Figs. 3–4). A total of 21 microplastic particles in *M. gulos* and 11 microplastics particles in *O. niloticus* were identified based on morphological observation (Figs. 5–6).

ATR- FTIR analysis

The ATR-FTIR spectra of MP obtained from the gastrointestinal tract of *M. gulos* and *O. niloticus* are presented in Figs. 7–8, respectively. Polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polystyrene (PS), polyethylene terephthalate

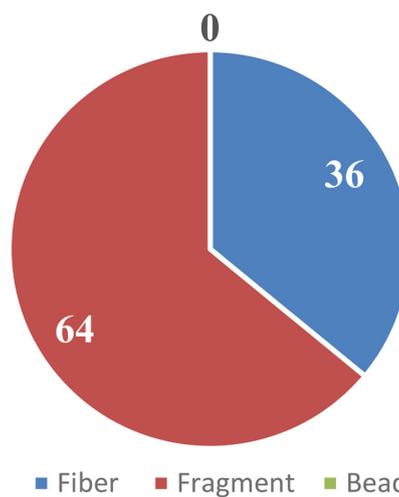


Fig. 4. The different type of microplastic in *O. niloticus* gut in percentage (%).

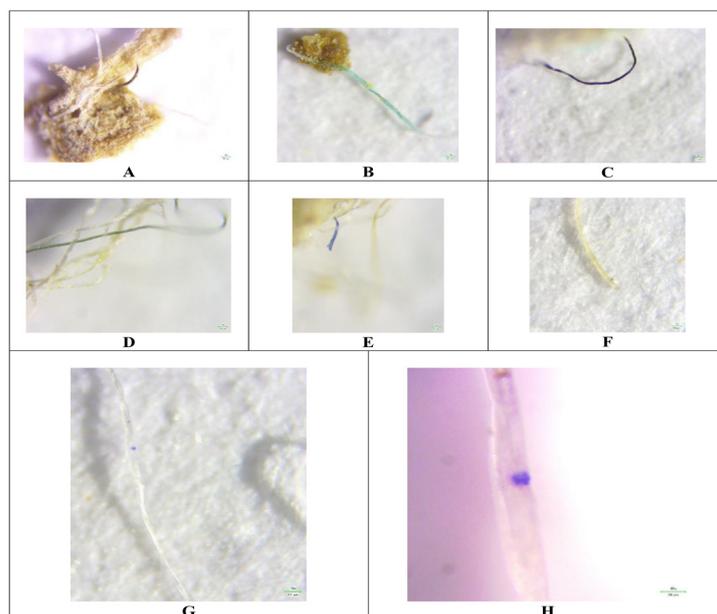


Fig. 5. A-H are capture by *Olympus microscope* and different colors of microplastic identified, and G color less (White) MP and H is inside of G Blue color MP Bead (400X magnification) from *Mystus gulio* gut.

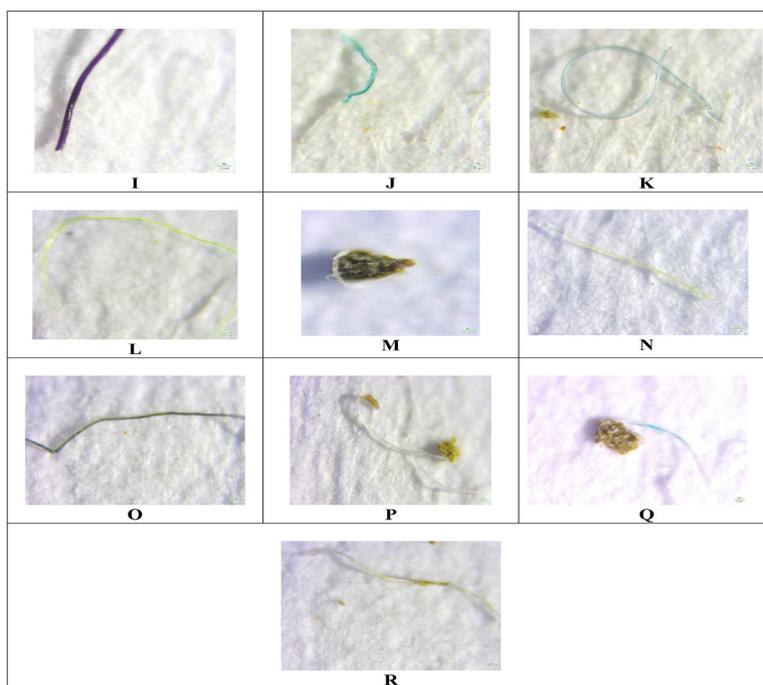


Fig. 6. I-R are capture by *Olympus microscope* and (100X magnification) different colors of microplastic identified from *Orochromis niloticus* gut.

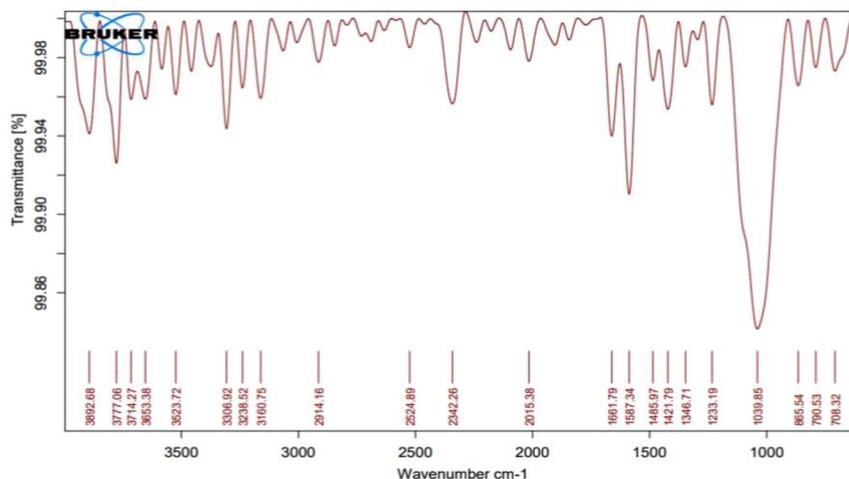


Fig. 7. ATR- FTIR spectra MP's in catfish (*M. gulosus*) fish gastrointestinal tract.

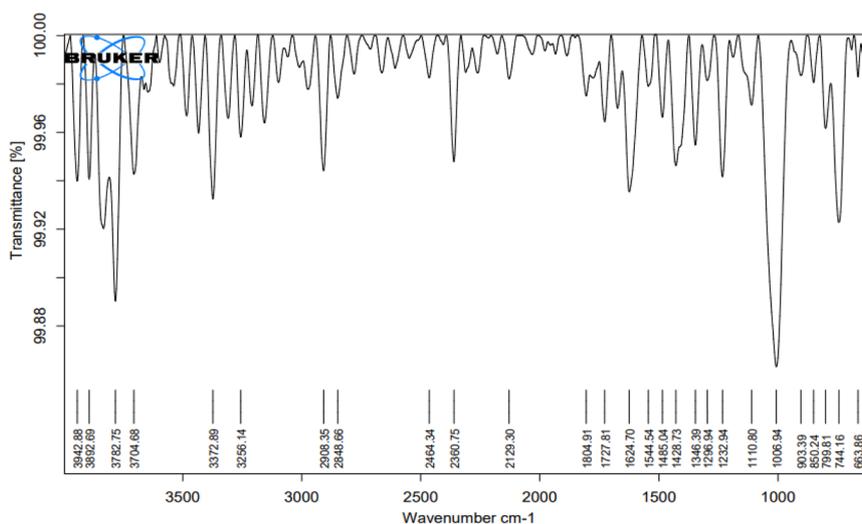
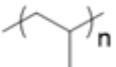
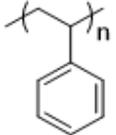
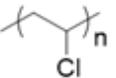
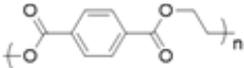
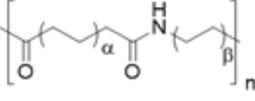


Fig. 8. ATR- FTIR spectra MP's in tilapia (*O. niloticus*) fish gastrointestinal tract.

(PET), and polyamide (PA) were identified. The characteristic bands were assigned to various types of vibrations of different functional group present in the microplastics. The polymers have overlapping bands in the ATR-FTIR spectra hence they could not be resolved for the individual polymers however these bands clearly indicate the presence of MP's in the gastrointestinal tract of the two fishes. The ATR-FTIR bands in some of the MP's have been shifted owing to the polymer degradation due to moist and

acidic environment prevailing in the fish gut. In *M. gulosus*, the bands at 2914.16 cm^{-1} and 2845.66 cm^{-1} are assignable to the C-H asymmetric stretching and C-H (alkane) asymmetric stretching in PE, PP, PS and PA (Jung *et al.* 2018). The bands at 790.53 and 708.32 cm^{-1} are characteristic of CH_2 rocking in PP and PE respectively. The band at 863.54 cm^{-1} is assignable to the C- CH_3 bending in PP. The band at 1661.79 cm^{-1} is assignable to the aromatic ring stretching in PS and the band at 1233.19 is attributed to CH-Cl bending in

Table 5. Characteristic ATR-FTIR bands of microplastic detected in catfish (*M. gulosus*).

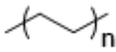
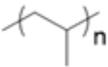
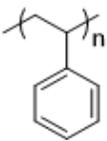
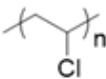
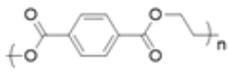
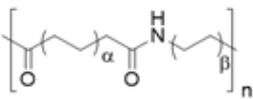
Sl. No.	Type of polymer	Chemical structure	Observed FTIR bands	Functional group and type of vibration
1	Polyethylene (PE)		2914.16	C-H (alkane) asymmetric stretching
			2845.66	C-H (alkane) symmetric stretching
			1485.97	CH ₂ bending
			1421.79	CH ₂ bending
			708.32	CH ₂ rocking
2	Polypropylene (PP)		2914.16	C-H (alkane) asymmetric stretching
			2845.66	C-H (alkane) symmetric stretching
			1346.71	CH ₃ bending
			865.54	C-CH ₃ stretching
			790.53	CH ₂ rocking
3	Polystyrene (PS)		3160.75	Aromatic C-H stretching
			2914.16	C-H (alkane) asymmetric stretching
			2845.66	C-H (alkane) symmetric stretching
			1661.79	Aromatic ring stretching
			1485.97	Aromatic stretching
1039.85	Aromatic C-H out of plane bending			
4	Polyvinyl Chloride (PVC)		1421.79	CH ₂ bending
			1346.71	C-H bending
			1233.19	CH-Cl bending
			1039.85	C-C bending
			708.32	C-Cl bending
5	Polyethylene Terephthalate (PET)		1661.79	(C=O) Carbonyl stretching
			1233.19	C-O stretching
			1039.85	C-O-C stretching
			865.54	Aromatic C-H out-of-plane bending
			708.32	CH ₂ rocking
6	Polyamide (Nylon)		3306.92	N-H stretching
			2914.16	C-H (alkane) asymmetric stretching
			2845.56	C-H (alkane) symmetric stretching
			1661.79	(C=O) Carbonyl stretching
			1587.34	N-H bending
708.32	N-H out of plane bending			

PVC. The band at 3306.92 cm⁻¹ and 1587.34 cm⁻¹ is due to the N-H stretching and bending respectively (Table 5).

In *O. niloticus*, the band at 2908.35 and 2848.66 cm⁻¹ are assignable to the C-H asymmetric stretching

and C-H (alkane) asymmetric stretching in PE, PP, PS and PA (Jung *et al.* 2018). The band at 1485.04 and 1428.73 cm⁻¹ are assignable to the CH₂ bending vibrations in PE. The band at 744.14 cm⁻¹ is assignable to CH₂ rocking vibrations in PE and the band 850.24 cm⁻¹ is attributed to C-CH₃ stretching

Table 6. Characteristic ATR-FTIR bands of microplastic detected in *O. niloticus* fish.

Sl.No.	Type of polymer	Chemical structure	Observed FTIR bands	Functional group and type of vibration
1	Polyethylene (PE)		2908.35	C-H (alkane) asymmetric stretching
			2848.66	C-H (alkane) symmetric stretching
			1485.04	CH ₂ bending
			1428.73	CH ₂ bending
			744.14	CH ₂ rocking
2	Polypropylene (PP)		2908.35	C-H (alkane) asymmetric stretching
			2848.66	C-H (alkane) symmetric stretching
			1346.39	CH ₃ bending
			850.24	C-CH ₃ stretching
			799.81	CH ₂ rocking
3	Polystyrene (PS)		3024.56	Aromatic C-H stretching
			2848.66	C-H (alkane) asymmetric stretching
			1624.70	C-H (alkane) symmetric stretching
			1485.04	Aromatic ring stretching
			1006.94	Aromatic ring stretching
			663.86	Aromatic C-H out of plane bending
4	Polyvinyl Chloride (PVC)		1428.75	CH ₂ bending
			1346.39	C-H bending
			1232.94	CH-Cl bending
			1110.80	C-C bending
			744.16	C-Cl bending
5	Polyethylene Terephthalate (PET)		1727.81 & 1624.70	(C=O) Carbonyl stretching
			1232.94	C-O stretching
			1110.80	C-O-C stretching
			850.24	Aromatic C-H out-of-plane bending
			744.16	CH ₂ rocking
6	Polyamide (Nylon)	 α and β may vary between 1 to 12 depending on monomer	3256.14	N-H stretching
			2908.35	C-H (alkane) asymmetric stretching
			2848.66	C-H (alkane) symmetric stretching
			1624.70	(C=O) Carbonyl stretching
			1544.54	N-H bending
			663.86	N-H out of plane bending

in PP. The bands at 1485.04 and 1006.94 cm^{-1} are attributed to aromatic ring stretching in PS. The band at 1232.94 cm^{-1} is assignable to CH-Cl bending vibrations in PVC. The bands at 1727.81 & 1624.70 cm^{-1} are assignable to the C=O stretching in PET,

the ester functional group in PET would degrade to carboxylic acid and the ester carbonyl usually appear at a higher wavenumber compared to carboxylic acid group, hence C=O stretching vibrations are observed in PET. The bands at 3254.16 and 1544.54 cm^{-1} are

assignable to the N-H stretching and bending vibrations in PA (Table 6).

Chakraborty *et al.* (2023) used Raman spectra to identify the MPs chemical composition in various water sources, like fresh, ground, drinking, ocean, sea, waste and sewage. Bhuyan (2022) reports microplastics cause tissue damage, oxidative stress, neurotoxicity, growth retardation, and behavioral abnormalities in fish. Ripken *et al.* (2021) reported that polyethylene (PE) peaks spanning from 1500 cm^{-1} to 1000 cm^{-1} were found in Okinawa. Sita *et al.* (2024) analyzed the microplastic in Red Snapper and Mackerel commercial fish in the marine environment, their analysis with FTIR confirmed the presence of microplastic. Athukorala *et al.* (2024) reported that 33% of fragments of microplastics were identified from commercial fish intestines in a natural lagoon environment, and primary microplastics are rayon, polyethylene terephthalate, and polypropylene. Willans *et al.* (2023) analyzed those six polymers using ATR-FTIR and reflectance-FTIR. Neelavanan and Sen (2023) reports that the most common microplastics are polyethylene terephthalate (PET), polyethylene (PE), and polypropylene (PP) found in the freshwater ecosystem of India, and mostly water and sediment samples were identified from the spectroscopy technique ATR-FTIR. Sruthy and Ramasamy (2017) report that the PS and PP were found to be dominant, and low-density MPs had PE in their sites. Kosai *et al.* (2014) reports that morphometric data will helpful for taxonomist, biologist and fishiest concerned with this fish species.

CONCLUSION

This study reveals the presence of various microplastics in gastrointestinal tract of two *Mystus gulio* and *Oreochromis niloticus* fish species in Thamirabarni river. Six different types of MP's such as polyethylene terephthalate (PET), polypropylene (PP), polyamide (Nylon), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene (PE) have been identified and their ATR-FTIR spectrum recorded and analyzed. The *Mystus gulio* species gastrointestinal track's have higher number microplastics compared to *Oreochromis niloticus*. These findings emphasize the severe impact of plastic pollution on freshwater ecosystems,

with implications for aquatic organisms and human health through the food chain. Urgent measures are needed to reduce plastic waste dumping in water resources and protect the ecological integrity of the river and its biodiversity.

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