Full Length Research Article

© 2024, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

National Conference on "Emerging Technologies Focusing on Food Safety and Security"
Volume 15; (March 2024); pp 74–80

Identification and Characterization of Microplastics in Tilapia Fish from Chennai Freshwater Bodies

Renu Agarwal[1] and Sri Varshni J*[2]

[1-2] Department of Home Science - Food Science Nutrition and Dietetics, Shrimathi Devkunvar Nanalal Bhatt Vaishnav College for Women, Affiliated with the University of Madras, Chromepet, Chennai, Tamil Nadu, India

Abstract

Emerging contaminants known as "Microplastics," which are 5 mm or smaller, are harmful to human health. This study aims to analyze the prevalence of Microplastic (MP) contamination in Tilapia, collected from two freshwater bodies in Chennai. With secondary data stating the polluted level of these waters, it is thus necessary to determine, if the organisms surviving in this environment are also affected by these MP pollutants. Tilapia (*Oreochromis mossambicus)*, called Jalebi in Tamil is a highly consumed freshwater fish by the Chennai people as evidenced by the survey conducted among 20 fishermen. A total of 12 samples were collected from the Puzhal and Retteri fish markets and measured for their morphological features. The analysis methods included dissection, digestion, filtration, and FTIR (Fourier Transform Infrared Spectroscopy) characterization of the MP particles obtained. Both the edible tissues and gastrointestinal tracts of the fish were analyzed. Results showed the presence of MPs in all 12 Tilapia samples in the form of filaments, fibers, and fragments of color yellow, white, and black. The study's result is an indicator of contaminant risk for fish consumers. Consumption of such foods may pose serious threats to the human body.

Key words**:** Microplastics, Tilapia, Edible tissue, Gastrointestinal, FTIR

In the modern era, plastic has become an essential component of daily life, with many different uses [16]. Humans' use of plastic has resulted in the presence of it in water, air, and land. They are extremely resistant to decomposition and persist in the environment for a long time, making it simple for Microplastics to enter the human food chain through beverages, food additives, and plastic food packaging. Microplastics (MPs) are synthetic materials with a diameter of less than 5mm and come from primary sources such as polyethylene, polypropylene, and polystyrene particles of consumer products, toothpaste, cosmetics, scrubs, and abrasives [6] [2] and from secondary sources due to the biological, chemical, and physical processes causing plastic debris to fragment [15] [3]. They are classified into 5 shapes such as film, fiber, pellet, filament, and fragment [13]. These particles enter the aquatic ecosystem through various pathways such as water body littering, estuaries, and fishing activities [9]. Food commodities such as beer, sugar, honey, table salt, and seafood are found to be contaminated with MP particles.

The freshwater bodies being close to urban and industrial centers can expose humans to MPs through the consumption of fish captured from such environments. Approximately, 20% of the protein intake by humans corresponds to seafood. It is also rich in unsaturated fatty acids, vitamins, and trace elements. It is easily digestive and is also considered to be a reliable good by the rich as well as the poor [12]. However, in recent studies, there has been a very strong belief stating Microplastic contamination in fish species. Thus, an analysis of MP contamination of the highly commercial fish species of Chennai freshwater bodies has been carried out. The GI tracts of the fish are commonly contaminated with MPs. However, a potential for leaching and accumulation of the associated chemical particles can occur in the edible tissues. Taking this into account, the relative MP levels are estimated in both the GI tracts and edible tissues of fish samples using Fourier Transform Infrared (FT-IR) Spectroscopy coupled with the microscopic examination. FTIR technique was developed to identify and quantify MPs [10]. Spectroscopy is the field involving the study of the interaction between matter and electromagnetic radiation. The IR spectrometer studies the absorbance of matter. It has been extensively used in the research of MPs since 2004 as it compares the identified polymer types of different MPs with the spectral library.

The sample selected for the analysis was *Oreochromis mossambicus* as shown in (Fig 1). It is an African-mouth brooder called Mozambique Tilapia. This species was introduced to India from Sri Lanka in the year 1952 and is now a part of the fish fauna in most of the Indian rivers such as Godavari, Cauvery, Krishna, and Ganga [11].

Studies done previously have stated that MPs have been found in the blood, gills, gonads, intestines, liver, muscles, and stomach of this fish. Another study by [4] proved 139 fiber MP particles of colors black, blue, red, and white from 15 Tilapia fishes.

This fish being a major consumed variety can thus impact the food security of the individual [1].

***Correspondence to:** Sri Varshni J, E-mail: srivarshnij2907@gmail.com; Tel: +91 9659722299

The main objectives of the study are:

- To identify the highly consumed freshwater fish among Chennai people
- To collect and examine the presence of Microplastics in fish samples, from Chennai

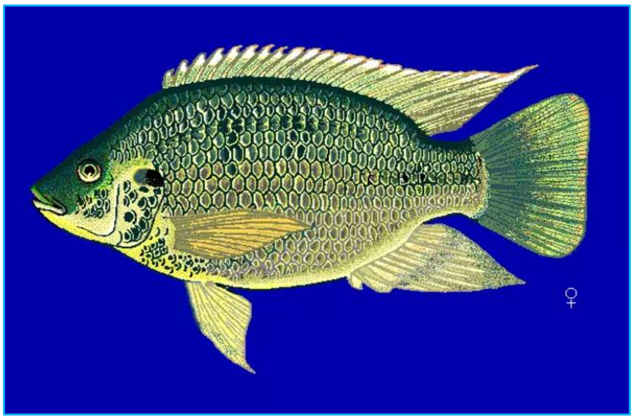

Fig 1 *Oreochromis mossambicus - Fish*

MATERIALS AND METHODS

Study area

Samples were obtained from the fish markets where fish were caught from the two major freshwater bodies of Chennai, Tamil Nadu. The major freshwater body for fishing as well as the primary source of drinking water, Puzhal located in the Tiruvallur district of Tamil Nadu has been dumped with plastic wastes along the reservoir bed all these years together and has clogged nearly 10 to 12 tonnes of garbage. Another freshwater body located on the Grand Northern Trunk, Retteri Lake is surrounded by built-up urban areas which can pose a threat to the lake through pollution activities. These water bodies have a diverse marine ecosystem [14]. The fish samples for analysis were obtained from these locations.

Secondary data obtained from [5] states the pollution level of these water bodies. According to the data collected, an average amount of six pieces of plastic were found in every 1 litre of water, and an average quantity of 27 particles was identified for every 1kg of sediment. High-density polymers such as polyethylene (PE) and low-density polymers such as polypropylene (PP), and polystyrene (PS) were found. This points to the garbage dumped into the water body. Such particles in these locations can pose a threat to the aquatic organisms present, especially fish. Hence, they must be analyzed for any threat due to Microplastics.

Sampling

The most popular freshwater fish consumed by Chennai people was assessed by the survey conducted with 20 fishermen. The demographic data of the surveyed fishermen included gender, age, and experience in years. Based on the statements obtained from them, four freshwater fish varieties such as Yerivavaal (Pomfret), Catla (Catfish), Rohu (Carp fish), and Jalebi (Tilapia) were found to be popular among Chennai people. The most preferred fish variety was taken for Microplastic contamination analysis. About 6 samples were collected from River 1 (Puzhal) and River 2 (Retteri). They were named as P1, P2, P3, P4, P5, and P6 for river 1 samples. River 2 samples were named R1, R2, R3, R4, R5, and R6. The summarized illustration is given in (Fig 2).

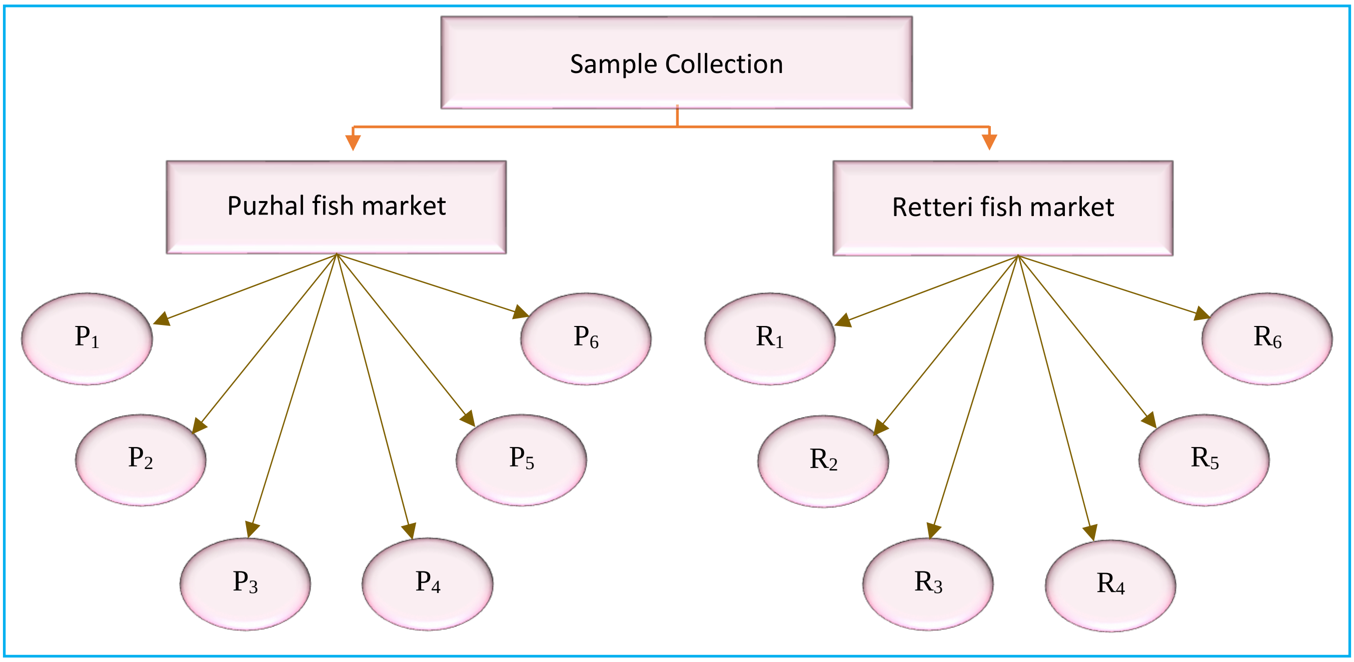

Fig 2 Sample naming and classification

All the fish were purchased whole to measure the morphological characteristics and transported on ice to the laboratory to be stored in the freezer at a temperature of -20ºC, until further processing and also to avoid decomposition.

The flow of the study

The (Fig 3) depicts the illustrations of the processes carried out in this study to identify the Microplastics present in the Tilapia samples after collection from fish markets by measurement, dissection, digestion, and visualization of the microscopic view and spectral structure.

Dissection and digestion

The fish samples were thawed before dissection and the entire organ system was removed, weighed, rinsed in ultrapure water, and placed separately respective to each sample with a label. The physical examination for the presence of Microplastics was done. All the organic materials were homogenized and digested. For the process of digestion, 30mL of 10% KOH (potassium hydroxide) solution was used and the contents were continuously stirred for 1 hour @350rpm [8].

This step was followed by the addition of 5mL of 30% H_2O_2 (hydrogen peroxide) with stirring of the contents @350rpm for 30 minutes. The digested solution was allowed to sit overnight. The residue obtained was treated with 20mL Fenton reagent (0.05M $FeSO_4.7H_2O$-30% H_2O_2 [1:1]) for 30mins @350rpm. This final solution was filtered through a sieve and examined stereotypically. The color and type of the Microplastics were recorded.

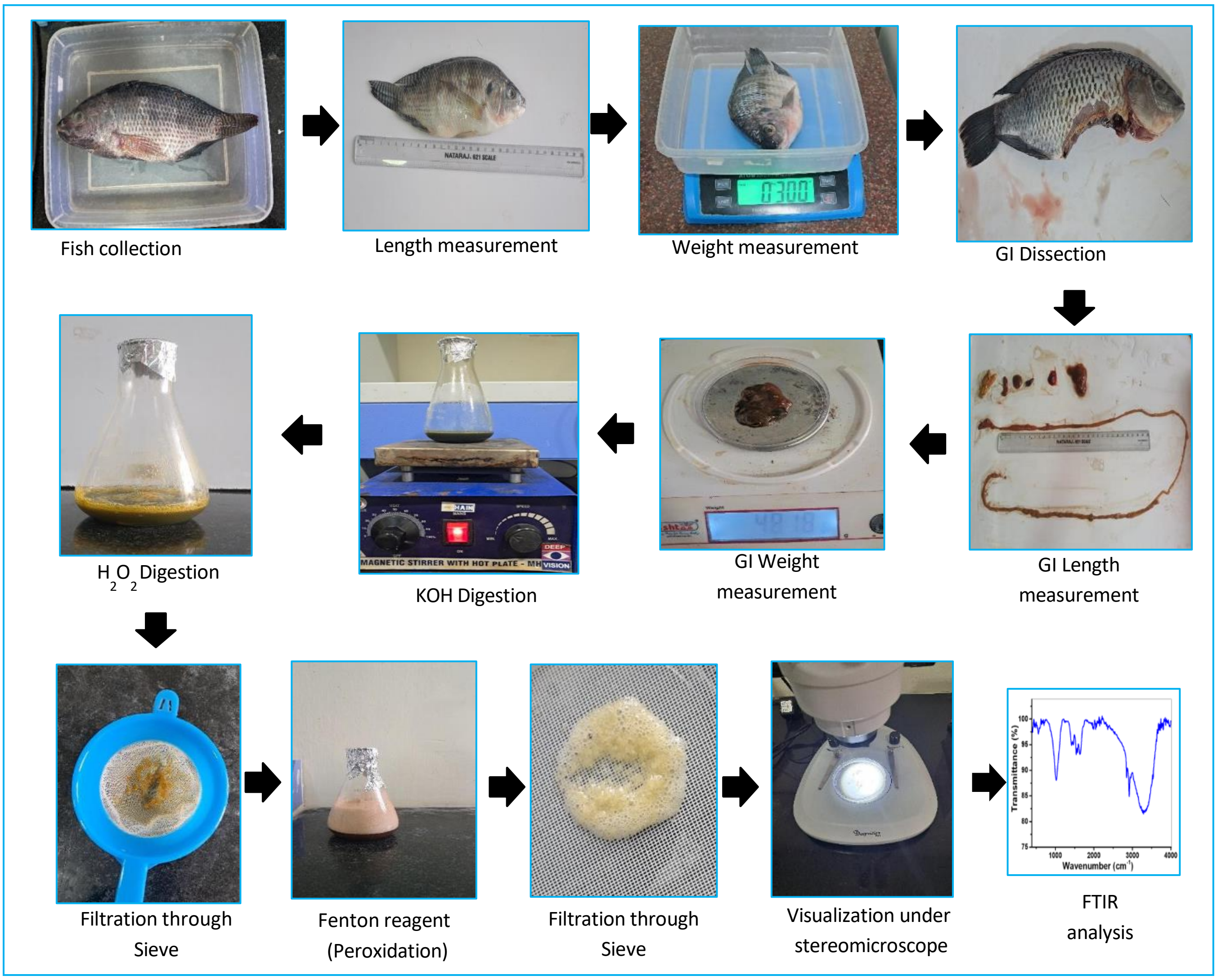

Fig 3 The flow of fish processing for MP visualization and analysis

FTIR analysis

One sample collected from each fish market was subjected to FTIR (Fourier Transform Infrared Spectroscopy) analysis for polymer-functional group identification. The spectrum of the particles was recorded with the FT/IR-6300 from JASCO due to its advanced capabilities for high performance. The polymer isolated was characterized based on the peaks formed in the IR spectra. Peak signifies the functional group of polymers. The graph's peak values were recorded for each of the two samples, allowing for composition confirmation through comparison with the reference library [7]. This suggests that the FTIR approach is capable of identifying the specific functional group of polymers. This technique was further used to confirm the presence of MP particles in the collected fish samples. IR Pal software was used to interpret the IR Spectra of the functional groups from the graph obtained in FTIR.

RESULTS AND DISCUSSION

The most popular freshwater fish consumed by Chennai people was assessed by the survey conducted with 20 local fishermen. Through the survey, the demographic profile of the fishermen was compiled and represented as shown in (Table 1). (Table 1 Demographic profile of fishermen).

Table 1 Demographic profile of fishermen

Parameters	N = 20	Percent (%)
Gender		
Male	19	95.0
Female	1	5.0
Age		
30-40	5	25.0
41-50	11	55.0
51-60	4	20.0
Experience (years)		
<10	2	10.0
10-20	5	25.0
20-30	6	30.0
>30	7	35.0

These fishermen were surveyed for their statements to identify the commercial freshwater fish varieties preferred by the people of Chennai. Based on the data collected through the survey, Table 2 depicts the percentage of fishermen identifying the highest consumed freshwater fish.

Table 2 Percentage of fishermen identifying the highly consumed freshwater fish in Chennai

Fish variety	Percentage of fishermen identifying the highly consumed freshwater fish	Mean ± SD
Jalebi	90	0.90±0.31
Catla	85	0.85±0.37
Rohu	10	0.10±0.31
Yerivavaal	65	0.65±0.49

It was estimated that with a percentage of 90%, Jalebi fish (Oreochromis mossambicus) is the freshwater fish variety highly preferred by the people of Chennai followed by Catla (85%), Yerivavaal (65%), and Rohu (10%). Figure 4 gives an illustration of the above-tabulated data.

Table 3 Microplastic dry weight

Station	Total fish	% Ingestion	Mean±SD (MP dry weight in grams)
Locale 1	6	100%	0.09±0.05
Locale 2	6	100%	0.19±0.22
Total	13	100%	0.14±0.16

A total of 12 fish samples belonging to the order Perciformes, genera Oreochromis, and species mossambicus were collected from the fish markets of Puzhal and Retteri in Chennai, Tamil Nadu, India. The common name of *Oreochromis mossambicus* is Tilapia and the vernacular name (Tamil) is called the 'Jalebi meen'. The samples obtained were analyzed for Microplastics by following the procedure as illustrated in Figure 3. Following the method of [8], the analysis of Microplastics from the collected fish samples was carried out. The Microplastic particles isolated from the samples originating from Puzhal and Retteri were visualized with the naked eye. All six samples collected were found to show the presence of Microplastic particles. The total fish analyzed and the ingestion percent of Microplastics from each locale obtained are summarized in (Table 3).

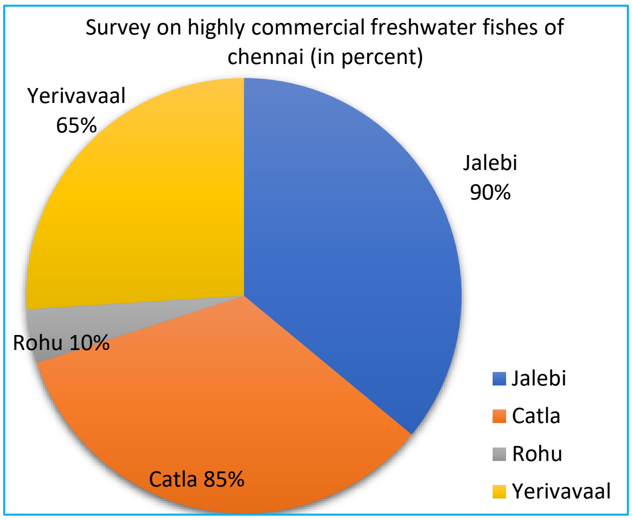

Fig 4 Highly commercial freshwater fishes of Chennai

Based on the tabulation, it was stated that 100% of Microplastic ingestion has occurred in the collected samples. The stereomicroscopic imaging of the obtained plastic particles from locale 1 can be seen as illustrated in (Fig 5-6) illustrates the stereomicroscopic imaging of locale 2 samples.

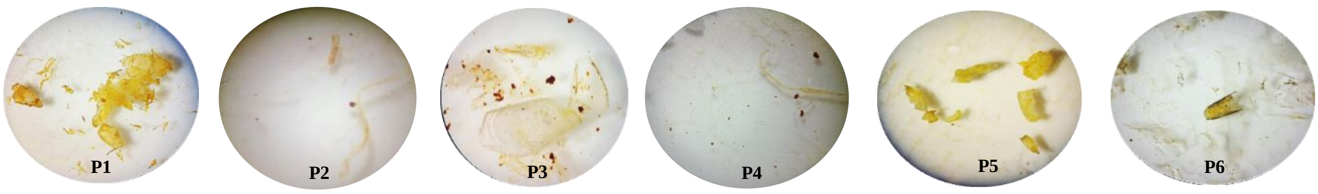

Fig 5 Stereomicroscopic imaging of MPs from locale 1 samples

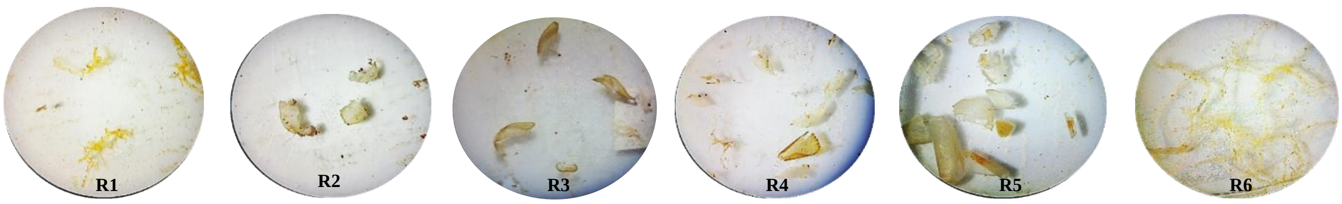

Fig 6 Stereomicroscopic imaging of MPs from locale 2 samples

The stereomicroscopic imaging showed the number, color, and type of Microplastics present in the samples as tabulated below in (Table 4). More than 60 plastic particulates were isolated from the 6 fishes of locale 1. Out of the isolated plastics, 32 medium to large-sized fragments in yellow, 17 intact yellow films, 5 broken transparent films, and 9-minute black fibres were identified as summarized in (Table 4).

Table 4 Presence of microplastics, their color, type, and number of particles from Locale 1 samples

Species	Sampling location	Sample	Plastics	Color	Type of microplastic	No of plastics identified
Oreochromis mossambicus	Puzhal	P_1	+	Yellow	Fragments	24
				Yellow	Film	4
		P_2	+	Yellow	Film	2
				Black	Minute Film	1
		P_3	+	Yellow	Film	10
		P_4	+	Black	Minute fibre	9

				Yellow	Film	1
		P_5	+	Yellow	Large fragment	7
		P_6	+	Yellow	Large fragment	1
				White	Broken film	5
	Total		6+			64

Note: '+' indicates the presence of microplastics

Table 5Presence of microplastics, their color, type, and number of particles from Locale 2 samples

Species	Sampling location	Sample	Plastics	Color	Type of microplastic	No of plastics identified
Oreochromis mossambicus	Retteri	R_1	+	Yellow	Broken film	3
		R_2	+	White	Fragment	3
				Yellow	Fragment	4
		R_3	+	Black	Fibre	2
				White	Fibre	1
				Yellow	Fragment	1
		R_4	+	Yellow	Broken film	2
				Transparent	Film	5
		R_5	+	Yellow	Fragment	3
				Transparent	Fragment	9
		R_6	+	Yellow	Film	≈16
	Total		6+			≈49

Note: '+' indicates the presence of microplastics

More than 50 plastic particulates were isolated from the 6 fishes of locale 2. Out of the isolated plastics, 3 white fragments, 8 yellow fragments, 5 broken yellow films, 1 white fibre, 2 black fibre, 5 transparent films, 5 broken yellow films, and numerous strands of films were identified. This is summarized in (Table 5). Followed by stereomicroscopic imaging and quantitative analysis, FTIR analysis was employed with the FT/IR-6300 from JASCO to reveal spectra of the polymers as shown in (Fig 7). One sample from each locale was taken for spectrum identification as illustrated below in (Fig 7).

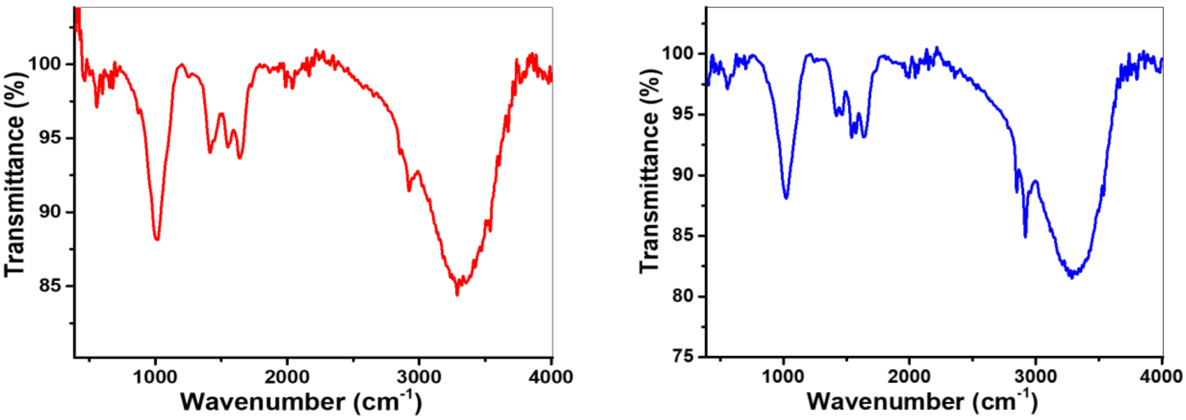

Fig 7 FTIR imaging of Microplastics from samples of (i) locale 1 (ii) locale 2

Table 6 Polymer peaks identified with FTIR and IR Pal software for sample from Locale 1

S. No.	Derived wavelength	Structure	Wave length range
1.	1000 cm^{-1}	RCO-OH (C-O stretch)	1320-1000 cm^{-1}
2.	1400 cm^{-1}	RCO-O- (C-O stretch)	1405-1395 cm^{-1}
3.	1540 cm^{-1}	RCONHR`	1550-1530 cm^{-1}
4.	1625 cm^{-1}	Ar-CH=CHR (Ar-CH=CHR)	1630-1620 cm^{-1}
5.	2850 cm^{-1}	-CH2-(-CH2)	2855-2845 cm^{-1}
6.	2960 cm^{-1}	-CH3 (-CH3)	2965-2955 cm^{-1}
7.	3200 cm^{-1}	ArO-H bonded (ArO-H H-bonded)	3500-3200 cm^{-1}

Table 7 Polymer peaks identified with FTIR and IR Pal software for sample from Locale 2

S. No.	Derived wavelength	Structure	Wave length range
1.	1320 cm^{-1}	RCO-OH (C-O stretch)	1320-1000 cm^{-1}
2.	1400 cm^{-1}	RCO-O- (C-O stretch)	1405-1395 cm^{-1}
3.	1540 cm^{-1}	RCONHR`	1550-1530 cm^{-1}
4.	2925 cm^{-1}	-CH2-(-CH2)	2930-2920 cm^{-1}
5.	3270 cm^{-1}	RC#C-H (#C-H stretch)	3330-3270 cm^{-1}
6.	3400 cm^{-1}	R3COH (O-H stretch)	3600-3400 cm^{-1}

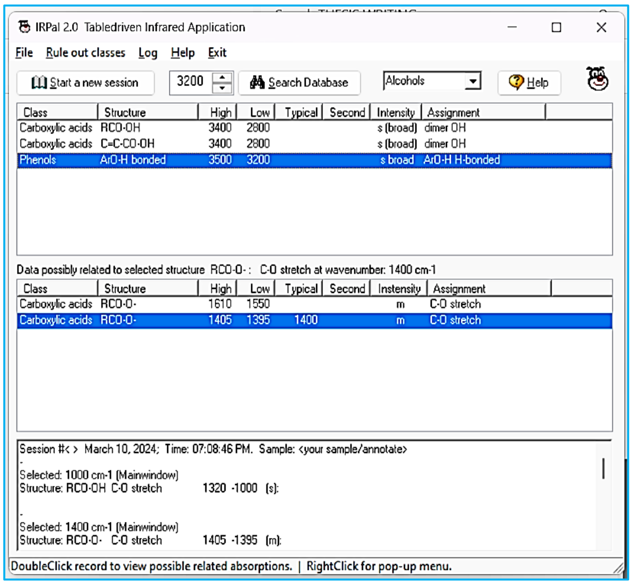

Fig 8 IR Pal software to identify the polymer peak category

The IR Pal software was used to interpret the IR Spectra of the functional groups from the graph obtained by FTIR. Each peak obtained signifies a particular functional group. Hence, based on the peaks the respective functional groups at each range of wavelength can be obtained. The IR Pal software used to analyze the data is shown below in Figure 8. The wavelength obtained in the graph was searched for its database in the software. The relevant class matching the wavelength entered was noted to interpret the structure.

(i) Based on the graph from the FTIR reading, the wavenumbers are entered in IR Pal to determine the presence of functional groups. The polymer peak of Microplastics of the sample from Puzhal was identified at 1000 cm-1, between 1000 cm^{-1} and 2000 cm^{-1}, and between 3000 cm^{-1} and 3500 cm^{-1}. The readings obtained for the Puzhal sample are given in Table 6.

(ii) Based on the above graph from FTIR reading, the wavenumbers are entered in IR Pal to determine the presence of functional groups. The polymer peak of Microplastics of the sample from Puzhal was identified at 1000 cm-1, between 1000 cm^{-1} and 2000 cm^{-1}, and between 3000 cm^{-1} and 3500 cm^{-1}. The following readings mentioned in Table 7 were obtained for the Retteri sample.

Inference

These stretching and bonding obtained from IR readings confirm the presence of functional groups of the polymers providing sufficient data for the presence of Microplastic polymers. The 'Ar' symbol with the hydroxyl group from the readings of the graph represents the 'aromatic' functional groups of polymers. An example of an aromatic polymer includes PET which is Polyethylene terephthalate, PEN called Polyethylene naphthalate. These polymers are plastic polymers with a source stating to plastic bottles and plastic food containers. The functional group CH_2 present can be a confirming factor for PE called Polyethylene. Thus, it can also be concluded that Microplastics analyzed from edible tissues, GI, and other organs of the selected fish species showed a 100% presence of MPs.

CONCLUSION

The rapid expansion of MP pollution in surface and underground water supplies has made it an imminent threat to our ecology in India and other emerging nations. Identifying and characterizing the MPs in freshwater fish samples was the study's primary objective. About 12 Tilapia fish were collected from the fish markets of Chennai. These fishes were particularly caught from the freshwater bodies, Puzhal and Retteri. According to the study's findings, MP was present in every sample that was gathered. Three different forms of plastic particles, including fragments, fibres, and films of colors white, yellow, and black were detected by stereomicroscopic imaging. FTIR analysis results showed the presence of functional group peaks of aromatic polymers such as polyethylene terephthalate and also the presence of polyethylene. This proved the presence of polymer particles. The quantity of plastic pollution in freshwater bodies is shown by the presence of such particles in the tilapia fish that were captured. There may be a risk of microplastic contamination for those who consume this fish. Therefore, future studies aimed at reducing plastic pollution in aquatic ecosystems can use this study as a baseline.

LITERATURE CITED

1. Aryani, D., Khalifa, M. A., Herjayanto, M., Solahudin, E. A., Rizki, E. M., Halwatiyah, W., Istiqomah, H., Maharani, S. H., Wahyudin, H., & Pratama, G. (2021). Penetration of Microplastics (Polyethylene) to Several Organs of Nile Tilapia (Oreochromis niloticus). IOP Conference Series: Earth and Environmental Science, 715(1). https://doi.org/10.1088/1755-1315/715/1/012061
2. Bergmann, M., Gutow, L., & Klages, M. (2015). Marine Anthropogenic Litter. Springer Cham XVIII, 447 https://doi.org/10.1007/978-3-319-16510-3
3. Clark, J. R., Cole, M., Lindeque, P. K., Fileman, E., Blackford, J., Lewis, C., Lenton, T. M., & Galloway, T. S. (2016). Marine microplastic debris: a targeted plan for understanding and quantifying interactions with marine life. In Frontiers in Ecology and the Environment (Vol. 14, Issue 6, pp. 317–324). Wiley Blackwell. https://doi.org/10.1002/fee.1297
4. E. Martinez-Tavera, A. M. D.-M. S. B. S. P. F. R.-E. G. R.-O. N. E. (2021). Microplastics and metal burdens in freshwater Tilapia (Oreochromis niloticus) of a metropolitan reservoir in Central Mexico: Potential threats for human health. Chemospere, 266, 128968.
5. Gopinath, K., Seshachalam, S., Neelavannan, K., Anburaj, V., Rachel, M., Ravi, S., Bharath, M., & Achyuthan, H. (2020). Quantification of microplastic in Red Hills Lake of Chennai city, Tamil Nadu, India. Environmental Science and Pollution Research, 27(26), 33297–33306. https://doi.org/10.1007/s11356-020-09622-2
6. Horton, A. A., & Dixon, S. J. (2018). Microplastics: An introduction to environmental transport processes. Wiley Interdisciplinary Reviews: Water, 5(2). https://doi.org/10.1002/WAT2.1268
7. Jung, M. R., Horgen, F. D., Orski, S. V., Rodriguez C., V., Beers, K. L., Balazs, G. H., Jones, T. T., Work, T. M., Brignac, K. C., Royer, S.-J., Hyrenbach, K. D., Jensen, B. A., & Lynch, J. M. (2018). Validation of ATR FT-IR to identify polymers of

plastic marine debris, including those ingested by marine organisms. Marine Pollution Bulletin, 127, 704–716. https://doi.org/10.1016/j.marpolbul.2017.12.061

8. Karthik, R., Robin, R. S., Purvaja, R., Ganguly, D., Anandavelu, I., Raghuraman, R., Hariharan, G., Ramakrishna, A., & Ramesh, R. (2018). Microplastics along the beaches of southeast coast of India. Science of the Total Environment, 645, 1388–1399. https://doi.org/10.1016/j.scitotenv.2018.07.242
9. Lebreton, L. C. M., Van Der Zwet, J., Damsteeg, J. W., Slat, B., Andrady, A., & Reisser, J. (2017). River plastic emissions to the world's oceans. Nature Communications, 8. https://doi.org/10.1038/ncomms15611
10. Löder, M. G. J., Kuczera, M., Mintenig, S., Lorenz, C., & Gerdts, G. (2015). Focal plane array detector-based micro-Fourier-transform infrared imaging for the analysis of microplastics in environmental samples. Environmental Chemistry, 12(5), 563–581. https://doi.org/10.1071/EN14205
11. Mushtaq Ahmad Ganie, Mehraj Din Bh Invasion of the Mozambique tilapia, Oreochromis mossambicus (Pisces: Cichlidae; Peters, 1852) in the Yamuna river, Uttar Pradesh, India at, Mohd Iqbal Khan, Muni Parveen, M. H Balkhi and Muneer Ahmad Malla. Journal of Ecology and the Natural Environment, 5(10), 310-317. https://doi.org/10.5897/JENE2013.0397x
12. Ravikanth, L., & Kavi Kumar, K. S. (2015). CAUGHT IN THE "NET": FISH CONSUMPTION PATTERNS OF COASTAL REGIONS IN INDIA. www.mse.ac.in
13. Singh, R., Kumar, R., & Sharma, P. (2021). Microplastic in the subsurface system: Extraction and characterization from sediments of River Ganga near Patna, Bihar. In Advances in Remediation Techniques for Polluted Soils and Groundwater (pp. 191–217). Elsevier. https://doi.org/10.1016/B978-0-12-823830-1.00013-4
14. Srihari, S., Subramani, T., Prapanchan, V. N., & Li, P. (2023). Human health risk perspective study on characterization, quantification, and spatial distribution of microplastics in surface water, groundwater, and coastal sediments of thickly populated Chennai coast of South India. *Human and Ecological Risk Assessment*, *29*(1), 222–244. https://doi.org/10.1080/10807039.2022.2154635
15. Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W. G., Mcgonigle, D., & Russell, A. E. (n.d.). Lost at Sea: Where Is All the Plastic? www.sciencemag.org/cgi/content/full/304/5672/838/
16. Zheng, Y., Li, J., Cao, W., Liu, X., Jiang, F., Ding, J., Yin, X., & Sun, C. (2019). Distribution characteristics of microplastics in the seawater and sediment: A case study in Jiaozhou Bay, China. Science of The Total Environment, 674, 27–35. https://doi.org/10.1016/j.scitotenv.2019.04.008