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Evaluation of Microplastic Contamination in Commercially Edible Fresh Water and Marine Fishes Bought from Fish Markets, Tiruchirappalli, Tamil Nadu, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Microplastics have become ubiquitous in aquatic environments worldwide, that there is rising worry about their potential influence on aquatic biota. Data demonstrate widespread plastic contamination in coastal regions all over the world; however, no quantitative studies on the ingestion of microplastics by commercial fish bought from Fish markets which belong to Fresh water and Marine

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environments have been conducted so far. In this work, we looked at the presence and their quantity, features, species - specificity distribution of microplastics in ten commercial fish species among which five species belong to Freshwater habitat and five species belong to marine habitat. Two Hundred fish samples were collected from four different sampling locations (Kallanai, Cauvery paalam fish market, Gandhi Fish Market, Vengur Fish Market). The average abundance of microplastics in commercial fish gastrointestinal tracts was 5mg/species of fish, indicating possible food safety. The majority of microplastics were fragment type with a diameter of less than 5mm. The study findings demonstrate the prevalence of microplastics in fish and pave the path for future research to better elucidate the mechanisms driving the incidence of microplastics in fishes along with possible risk assessment.

Keywords: Micro plastic; commercial fishes; ingestion; fresh water; marine habitats.

1. INTRODUCTION

“Plastics have become part and parcel of modern life, combining unparalleled practical features with low cost. It is projected that over 350 million tonnes of plastics were produced in 2018” (Plastics Europe, 2019). “Between 4.8 and 12.7 million tonnes of plastic flooded the seas in 2010, with this amount anticipated to rise by an order of magnitude by 2025” [1]. “Microplastics (MPs) are small plastic particles less than 5 mm in size that are either produced (e.g., microbeads) or formed by the degradation of larger plastic components” [2-4]. “Because of their great mobility and lengthy residency durations [5], MPs are found internationally”. “MPs were discovered on the sea surface for the first instance in 1972” [6]. However, scientific or public concern about the possible effects on the marine and Freshwater ecosystem has only lately been highlighted. Today, it is apparent that MPs have a harmful impact on marine and freshwater life's health.

Previous research has found “decreased growth, endocrine disruption, decreased feeding and weight loss, liver toxicity and pathology, inflammation, transfer to organs, and decreased reproductive output” [7,8,5,9-12].

“During the manufacturing process, MPs contain various chemical additives (e.g., bisphenol A, phthalates, polybrominated diphenyl ethers, and alkylphenols), and their hydrophobic properties and large adsorption surface area cause them to adsorb organic pollutants and heavy metals from the surrounding environment” [13,14]. “Furthermore, MPs provide a habitat for alien species such as diseases [15], which may subsequently be consumed by marine and freshwater creatures” [16]. “Overall, MPs may have increased toxicological effects when combined with other harmful chemical contaminants and microorganisms. As a result, chemical pollutants and microbial infections are more likely to accumulate gradually in these organism’s biological tissue and induce ecotoxicological consequences” [17,18]. “Furthermore, harmful contaminants may be transported from lower to higher order trophic levels in a food chain via predator and prey interactions” [12].

“Fish and other seafood are key sources of protein, minerals, and vitamins for human health and play a crucial role in the marine and freshwater ecology. Consumption of fishes & fish products is one of the most common ways MPs enter the human body” [19,12]. “As a result, MPs serve as a transporter of hazardous contaminants from marine organisms to the human body, posing a potentially serious threat to human health” [19].

MPs have been found in a wide range of marine and freshwater habitats; the particular aims of this study were to evaluate the prevalence and features of MPs in commercial marine and fresh water fish species in Tiruchirappalli region. This study's findings will be useful for future risk assessments of MPs towards aquatic environment as well as human health risk assessments.

2. MATERIALS AND METHODS

2.1 Fish Collection and Preparation

In December 2021, a total of 200 individual fish, 20 of each species, were collected at four different fish markets in Tiruchirappalli namely, Kallanai, Cauvery Paalam, Gandhi Market and Vengur Market. These species were chosen because of their diverse habitat, year-round availability, and economic significance (www.fishbase.org), According to knowledge of local fishermen, who are living around the shoreline and are quite often consuming the fish species on a regular basis throughout the year. Ten different species of fishes belonging to freshwater and marine habitat were sampled (Fig. 1 & Fig. 2). Table 1 contains information about the fish species. The fishes were wrapped in aluminium foil, stored in an icebox, and brought to the Department of Zoology laboratory, National College, Tiruchirappalli, where they were dissected or frozen (–2^{0}C) and then thawed. Each fish sample was washed in the laboratory with MPs-free distilled water to remove any externally attached plastic, as suggested by Lusher et al. Each fish's body length (cm) and weight (g) were measured. Each fish was then opened on a wooden tray with scissors, a scalpel, and forceps, and the gastrointestinal tract (GIT) was removed before being transported to a Petri plate and weighed (g). Finally, to reduce the danger of contamination, the GIT was put in a 500 mL glass beaker for MPs extraction and covered with aluminium foil.

2.2 Microplastic Extraction

“The GITs were put in 500 mL glass beakers with 200 mL KOH (10%, V/V) and maintained for 2 to 5 days in a thermostatic water bath at 60^{0}C” [20]. “After initial filtering of the supernatant, we added 400 mL saturated sodium chloride solution (gravimetric technique) to fish samples with high clay content and suspended at room temperature overnight” [21]. Following digestion or suspension, the solutions were filtered through 0.45-mm filter papers (Jinteng, Tianjin, China) and air-dried at room temperature before being put on glass plates.

2.3 Microscopy

The microplastics on the filter sheets were measured using an ocular micrometre and examined with a stereomicroscope (NOVEL XP – 213-1001129, INDIA). “Based on the length of the longest segment, the microplastics were classified into six classes: 0.5 mm, 0.5 - 1 mm, 1 - 2 mm, 2 - 3 mm, 3 - 4 mm, and 4 - 5 mm. They were also classified into six colour categories: white, blue, green, red, yellow, and black” [22]. According to prior research [23], “we classified microplastics into four types: fibres, fragments, films, and spheres. Fibres have a unidirectional extension, are supple and strong, and cannot be distorted. Fragments have an uneven form, a particular thickness, and a specific hardness, and they extend in two or three directions. Films are thin microplastics that may be pressed with tweezers and stretch in two directions. Spheres are microplastics that are smooth and spherical. All of these kinds are less than 5 mm long and may be seen using a stereoscopic microscope. We documented the size, colour, and type of all microplastics found in the fish samples based on these parameters”.

Fig. 1. Freshwater fish species collected from different sampling sites – a) *Etroplus suratensis* b) *Cyprinus carpio* c) *Oreochromis mossambicus* d) *Channa striata* e) *Cirrhinus reba*

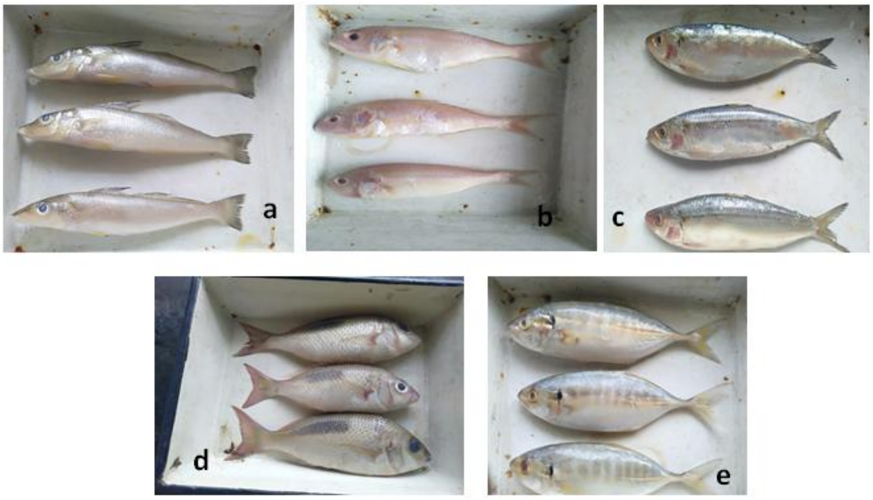


Fig. 2. Marine fish species collected from different sampling sites – a) *Elops machnata* b) *Lutianus madras* c) *Caranx melampygus* d) *Sardinella longiceps* e) *Lethrinus nebulous*

2.4 Microplastic Identification

Microplastic-coated target filter sheets were put on double-sided tape and coated with evaporated gold. The microplastic was identified using a Fourier Transform Infrared (FTIR) spectrometer (BRUKER, VERTEX 70, Germany). However, the samples were too small to be chosen or examined.

2.5 Quality Assurance and Quality Control

Throughout the experiment, medical gloves, glassware, and metal instruments utilised to treat the samples, and the dissecting tools were cleaned three times with distilled water before use. During the experiment, the researchers donned cotton laboratory coats. Distilled water was used to prepare all liquid reagents (10% KOH and saturated sodium chloride solution).

2.6 Statistical Analysis

For statistical analysis and charting, Microsoft Office Excel 2010 and SPSS 16 were used. To investigate interspecies and geographic differences, a one-way ANOVA test was performed at significance level of 0.05.

3. RESULTS

3.1 Characteristics of Microplastics in Fish Samples

A total of two hundred commercial fishes from 10 species, representing both Marine and freshwater habitat were studied and two species belonging to marine habitat (*Elops machnata* & *Lethrinus nebulous*) and one species belonging to Freshwater habitat (*Etroplus suratensis*) were contaminated with micro plastics. The abundance of micro plastics ranged from 1 to 20 (average = 5) items/ species (Table 1). Fig. 3 depicts the common morphologies of the microplastics found in the fish samples. Other pollutants appear to have been incorporated on the surfaces of the different forms of micro plastics. But we didn't know what these contaminants were or what harm they may do to fish.

Microplastics of various hues were found - black and white was the most common (60%) & (30%) with yellow and blue accounting for 3% and 3%, respectively. Green (2%) and red (2%) microplastics were also found in lower amounts (Fig. 4a). The size of the microplastics ranged from 0.05 mm to 5 mm. The bulk (90 percent) was 1 mm in size, and only a few were 7 mm in size (Fig. 4b). The bulk of the microplastics (68 percent) were fragments, followed by films (25 percent), fibres (3 percent), and spheres (3 percent) (Fig. 4c). The FTIR Peaks for Microplastics identified were shown in Fig. 5.

3.2 Microplastics Distribution with Respect to Fish Species

We recorded the average overall length, weight, and GIT weight for each fish species, however there was no apparent association with the amount of microplastics in any species with respect to the morphometric measurements

(Table 1). *Elops machnata,* Marine fish species had an average abundance of microplastics (6 items/individual), with a maximum abundance of 20 items/individual, which was much greater than that of other species (Table 1). Microplastics were discovered in relatively copious levels in the GITs of *Etroplus suratensis*, a freshwater species (4.5 items/individual) and *Lethrinus nebulous*, a marine fish species (5 items/ individual).

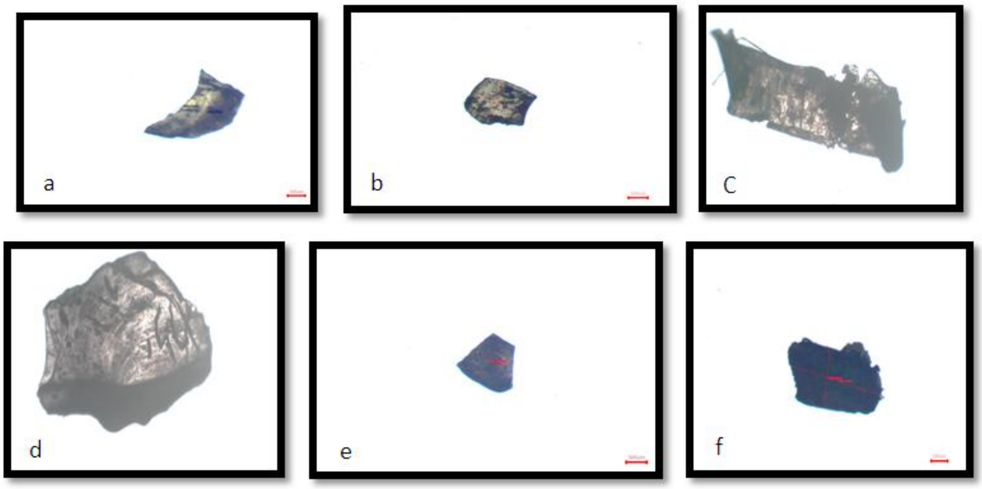


Fig. 3. Shapes of Microplastics in Fish samples from Freshwater and Marine Habitat, Tiruchirappalli: (a, b, d, e) – Fragments; (c, f) – films

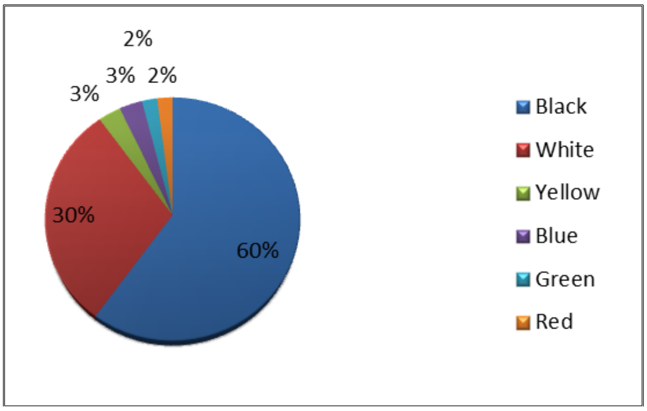


Fig. 4a. Proportion of Colour of Microplastics in GITs of Commercial Freshwater and Marine Fishes from Tiruchirappalli

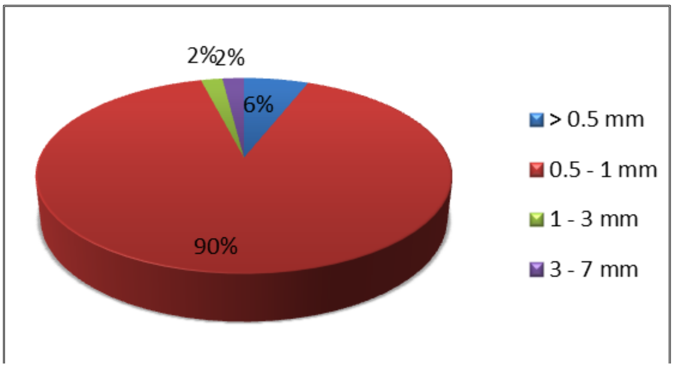


Fig. 4b. Proportion of size of microplastics in GITs of commercial freshwater and marine fishes from tiruchirappalli

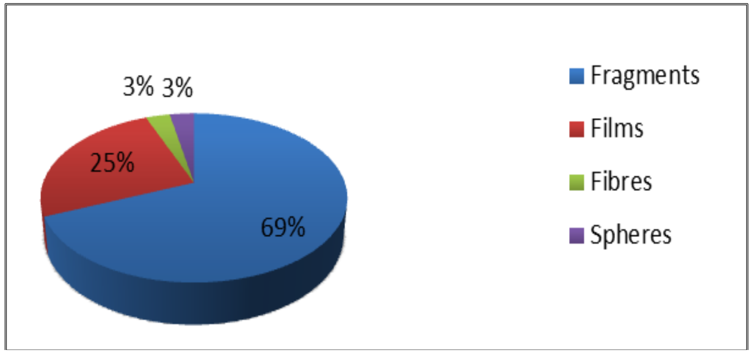
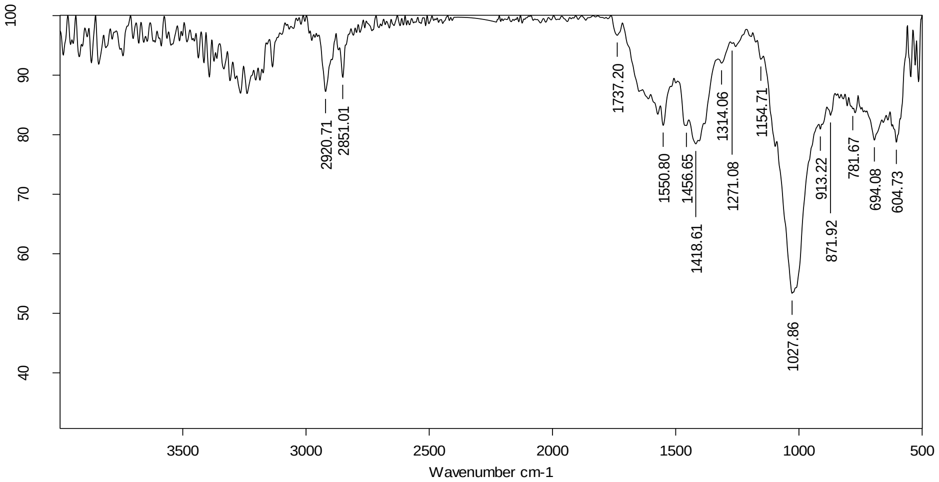


Fig. 4c. Proportion of shapes of microplastics in GITs of commercial freshwater and marine fishes from tiruchirappalli

Table 1. Representing fish morphometrics and abundance of MPs in different fish samples

Species Name	Sample	Habitat	MPs Presence/ Absence (+/-)	Total number of microplastics	Average Weight in (gms)	Average GIT Weight (gms)	Average length in (cm)
Etroplus suratensis	20	Freshwater	+	30	154.8 ± 0.03	54.096 ± 0.02	17.5 ± 0.02
Cyprinus carpio	20	Freshwater	-	NIL	74.15 ± 0.05	46.142 ± 0.05	18 ± 0.04
Oreochromis mossambicus	20	Freshwater	-	NIL	55.16 ± 0.02	32.585 ± 0.03	15.5 ± 0.03
Channa striata	20	Freshwater	-	NIL	195 ± 0.03	62.8 ± 0.05	34 ± 0.05
Cirrhinus reba	20	Freshwater	-	NIL	23.6 ± 0.04	13.651 ± 0.04	16.5 ± 0.01
Elops Machnata	20	Marine	+	50	54 ± 0.02	23.234 ± 0.02	17 ± 0.06
Lutjanus madras	20	Marine	-	NIL	50.15 ± 0.05	14.539 ± 0.04	17.1 ± 0.04
Caranx melampygus	20	Marine	-	NIL	83.3 ± 0.03	29.443 ± 0.03	22.6 ± 0.03
Sardinella longiceps	20	Marine	-	NIL	30 ± 0.02	31 ± 0.05	15.5 ± 0.02
Lethrinus nebulous	20	Marine	+	40	160 ± 0.01	30 ± 0.02	22.4± 0.01



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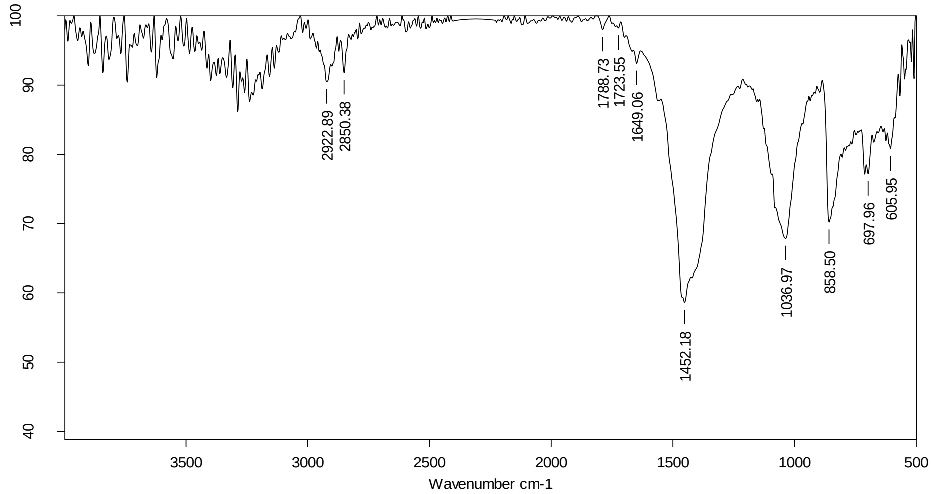

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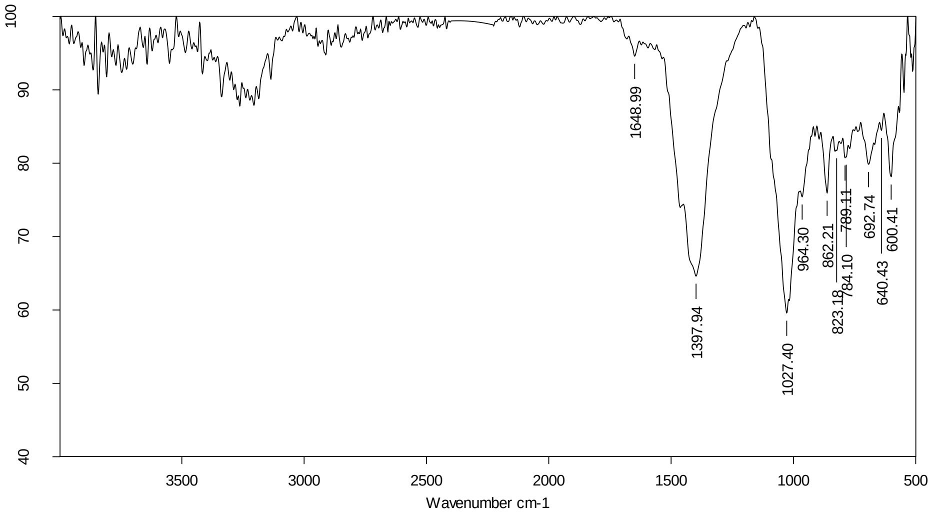

c

Fig. 5. AT – FTIR Peak predicting the MPs to be Polyethylene a) *Elops machnata* b) *Lethrinus nebulous* c) *Etroplus suratensis*

4. DISCUSSION

4.1 Microplastic Characteristics in Fish Samples

"The based on the proportion of microplastics in polluted fish GITs in this study was 6 items/individual, which was higher than levels documented in deep-sea fish from the South China Sea (1.96 ± 1.12 items/individual in the stomachs and 1.77 ± 0.73 items/individual in the intestines) [24], but lesser than those found in fish samples around Nanxun Reef in the South China Sea (3.1 items/individual)" [20,25]. These findings show that freshwater fish may be more polluted than fish in coastal and marine areas. In Central Texas, USA, a similar condition was found [26].

"The substantial accumulation of microplastics in wild fishes may result in a loss in fisheries resources, affecting the structure and stability of the food chain" [27-29]. "Furthermore, these commercial fish are widely consumed by humans, which may jeopardise human food security, safety, and health" [30,29]. When it comes to the hue of microplastics, transparent and white are frequently confused. Because we couldn't tell the difference between transparency and white in our investigation, we lumped them into the "white" group. Similarly, grey and black were labelled as "black," whereas orange and yellow were denoted as "yellow." This may explain why these three hues have the highest percentages in our data. Blue was the most common microplastic colour found in fish GITs in artificial reefs in the Ma'an Archipelago [31]. "Transparent and white microplastics accounted up 70.5 percent of the GITs of wild freshwater fish from the Pearl River watershed, whereas grey and black comprised up 8.5 percent [23], which was comparable to our findings". The primary source determines the colour of microplastics, although it is also impacted by UV radiation, weathering, and microbial deterioration throughout the transfer process. White, translucent, and blue colours are comparable to plankton, which is a key food source for fish.

"Another frequent criterion for measuring microplastics is size, however there is presently no unified standard. It was not surprise that the proportion of microplastics 1 mm was considerable, as previously reported" [24]. "Microplastics smaller than 0.01 mm, on the other hand, are impossible to see or study. According to Samuel Roch et al. >95 percent of microplastic particles are believed to be smaller than 4 mm, which are not detected by researchers but are crucial to fish health and consumer exposure" [32]. We anticipate the development of automated detection systems capable of detecting microplastic particles at the micron and even nanometre levels in the future.

Sun et al. discovered "three types of microplastics: fibres, pellets, and fragments [27], whereas Hu et al., detected fibres, fragments, and granules" [33]. Arias and colleagues divided "particles into four types: fibres, lamina, pieces, and pellets" [34]. Fibers, films, pellets, and pieces were identified by Wenke Yuan et al [35]. De - la Torre and colleagues detected pieces, foams, sheets, pellets, films, and fibers/lines [36,37]. Recycled microplastics are also classified into two types based on their shape: fragments and fibres [38,22]. Fibres, in general, are the most prevalent kind discovered in all research. The distinction between films and fragments is ambiguous. In this study, we separated them mostly based on thickness. Fragments are microplastics that have an uneven form, a certain thickness, and a hardness that extends in two or three directions. Films are thin microplastics that may be pressed with tweezers and stretch in two directions. Pellets, spheres, and granules are the names given to smooth and spherical microplastics. We used the word "spherical" in this investigation, and these were seen at a very low proportion (3 percent). We advocate precise descriptions of microplastic morphologies to enable categorization and comparisons because there is a lot of variation in morphology types employed and no standardisation.

Human health is negatively impacted by micro plastics. Inhaling micro plastics may trigger cancer, hormone disturbances, and respiratory and endocrine disorders. During pregnancy, they may have a deleterious effect on brain development. Micro plastic exposure may result in oxidative stress, DNA damage, and inflammation.

Increased MP build-up in oceans and other aquatic habitats, such as aquaculture ponds, is certain to have negative effects on fish stocks that are already in poor health. For example, MPs pose a threat to aquatic creatures, which increases the energy needed to choose nutritious prey or organic material and it leads to less amount of energy available for reproduction and development. This results in lower mean sized specimens and reduced population sizes of commercial fishing stocks, which raise the probability of fisheries failure and have negative economic and social effects on fishing communities and fishing industry.

4.2 Fish as a Marker of Microplastic Contamination

When selecting fish to serve as indicators of microplastic contamination, various factors must be considered, including the fish's occurrence, mobility, commercial value, distribution, and habitat. In this study, we collected fish samples from several marketplaces that had a particular economic worth and a lot in common, thus we chose ten different fish species as prospective indicators of microplastic contamination. In conclusion, we propose that *Elops machnata* & *Lethrinus nebulous* from marine environement and the *Etroplus suratensis* from freshwater environment be regarded as three species ideal for monitoring microplastics ingestion in seabed and water column populations.

5. CONCLUSION

In this study, we discovered the degree of microplastic contamination in commercial Freshwater and Marine fish from Tiruchirappalli, Tamil Nadu. A total of 200 Freshwater and Marine fishes from Ten distinct species (5 Freshwater Species & 5 Marine Species) were examined. Microplastics were found in 2 Marine Species (*Elops machnata* & *Lethrinus nebulous*) and 1 Freshwater Species (*Etroplus suratensis*), with an average abundance of 6 pieces per species. The microplastics found in the GITs of wild sea fish were predominantly fibres and whitish in appearance. The majority of the microplastics found were 1 mm in size. Microplastics were found to be species-specific and spatially distributed in terms of quantity, colour, size, and form. We spoke about microplastics standards. To accurately identify and quantify microplastics in various environmental substrates, standard analytical procedures and automated detection technologies should be established as soon as feasible. Fish might give data on biome monitoring and provide data on ecological niches that are not currently observed. We propose that sample protocols be established that are compatible with spatial and seasonal sampling. We recommend that *Elops machnata* and *Lethrinus nebulus*, from the marine environment, as well as *Etroplus suratensis* from the freshwater environment, be considered as three species excellent for monitoring microplastics intake in seabed and water column populations. Effective measures must be implemented globally, especially in the Global South, to reduce the amount of plastic waste that is dumped into the ocean. In reality, enforcing regulations to restrict the emissions of debris to soil and water bodies, including the ocean, will be necessary if research finds increased evidence of the potential for ecological and human health damage with rising concentrations of MPs. Furthermore, increased public awareness is required to reduce single-use plastics, plastic consumption, and plastic input into the water system. It is necessary to stimulate a new management approach towards accumulation and recycling of plastic materials, as well as towards efficient utilisation water resources.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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