



Microplastic Contamination in Indian Mackerel (*Rastrelliger kanagurta*) and Finletted Mackerel (*Megalaspis cordyla*): Exploring Occurrence and Variability in Skin and Gill Tissues

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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

Two marine fish species: Indian mackerel (*Rastrelliger kanagurta*) and finletted mackerel (*Megalaspis cordyla*), were collected from a local fish market near Thiruvalla in Pathanamthitta district, Kerala. Various types of microplastics (such as films, fibres, fragments, and lines) were identified using visual analysis conducted with the aid of light microscopy. This study unveiled a concentration (23%) of microplastics in gill tissues (*Rastrelliger kanagurta*) when compared to the skin (30%) In fish *Rastrelliger kanagurta*, indicating different pathways of uptake. *Megalaspis cordyla* had a greater percentage of microplastics in the skin (25%) than in the gills (22%); also

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indicating different pathways. There was an abundance of microplastics in the form of film at (25%) than in flake, granules, foam, fibre etc. which all stood at 10%, 10%, 10% and 20% abundance, respectively. Notably, Indian mackerels were found to harbour a more significant microplastic load than finletted mackerels, highlighting the existence of inter-species variations in contamination levels. To confirm the presence of polymers, the identified microplastics underwent Fourier-transform infrared (FTIR) spectroscopy analysis, a method that verified the molecular composition of micro plastics by analysing their infrared absorption spectra. Overall, these findings shed light on the prevalence of microplastic pollution in aquatic ecosystems and underscore the necessity for comprehensive strategies to mitigate its far-reaching repercussions.

Keywords: *Microplastics; skin; gill; mackerels; marine ecosystem.*

1. INTRODUCTION

Microplastics, defined as tiny plastic particles measuring less than 5 millimeters, have become a growing concern in environmental science and aquatic ecology. These particles infiltrate marine and freshwater ecosystems through multiple pathways, such as the degradation of larger plastic items and direct discharge, presenting a pervasive global threat [1]. Their small size and persistence have led to their widespread distribution, raising alarms about potential consequences for aquatic life, ecosystems, and human health. Fish species are particularly vulnerable to microplastic ingestion due to their feeding behaviours and the presence of microplastics in their habitats, raising concerns about their impact on aquatic food chains and ecosystems. Microplastics not only enter fish through ingestion but can also accumulate on their surfaces, potentially transferring toxic chemicals and disrupting their normal physiological functions. Research indicates that micro plastics can cause physical harm to fish tissues, affect feeding behaviours, and alter energy allocation for vital processes. Additionally, the bioavailability of microplastics and associated pollutants raises questions about their potential to accumulate up the food chain, eventually affecting human health through seafood consumption [2-4]. The presence of micro plastics in aquatic environments is undeniable, stemming from both primary and secondary sources. Primary sources include microbeads in personal care products, while secondary sources result from the fragmentation of larger plastic items due to environmental factors. These microplastics enter aquatic environments through various pathways, including storm water runoff, wastewater discharge, and atmospheric deposition. In diverse regions such as the Pacific, Atlantic, Indian Oceans, and the Mediterranean Sea, the ingestion of microplastics by fish intended for human

consumption has been documented, even though only a few microplastic particles are typically found per fish. This prevalence raises concerns about broader implications for both aquatic ecosystems and human health. Micro plastics, defined as minute plastic pieces measuring less than five millimetres in length, have garnered increasing attention due to their potential harm to marine ecosystems and aquatic life. These small plastic particles are a complex assemblage of various shapes, such as fragments, fibres, spheroids, granules, pellets, splinters, or beads, with dimensions spanning between 0.1µm and 5000µm [5]. The ingestion of microplastics by fish intended for human consumption has been documented across diverse regions, including the Pacific, Atlantic, Indian Oceans, and the Mediterranean Sea. Despite the detection of only a few microplastic particles per fish, the observed prevalence raises concerns about the broader implications [6].

Microplastics originate from a multitude of sources, including the fragmentation of larger plastic debris that progressively breaks down into smaller fragments. These diminutive particles can easily evade water filtration systems, eventually finding their way into the oceans and Great Lakes, posing potential threats to aquatic organisms [7]. Upon ingestion, microplastics can accumulate within the gastrointestinal system, skin, and gills of fish, subsequently dispersing to other tissues. This accumulation has been linked to various health concerns among fish, including potential transmission of toxic substances and harmful pathogens through microplastics [8]. Humans, in turn, consume fish contaminated with plastic particles, leading to chronic health issues and disease outbreaks [9].

Addressing the issue of microplastic contamination requires a multifaceted approach involving efficient waste management strategies, the extension of plastic product shelf life, and

enhanced awareness campaigns [10]. The adverse effects of microplastics on aquatic life are substantial, encompassing reduced food intake, growth retardation, oxidative damage, and abnormal behaviours. Fish may also experience compromised swimming ability and internal injuries [11]. With microplastics being released directly from commonly used plastic items, as well as through plastic degradation, industrial sources, and wastewater treatment plants, these contaminants infiltrate aquatic environments. Consequently, microplastics enter the food chain, triggering substantial health hazards [12].

In light of the extensive and interconnected implications of microplastic pollution, urgent measures are required to curb its adverse effects on aquatic ecosystems and human health. By comprehending the sources, pathways, and consequences of microplastic contamination, society can strive towards effective mitigation strategies and global cooperation to ensure the preservation of our oceans and the well-being of both aquatic life and humanity.

2. MATERIALS AND METHODS

Fish specimens were collected from a local fish market near Thiruvalla, Pathanamthitta District, Kerala. The study focused on two marine fish species: Indian mackerel (*Rastrelliger kanagurta*) and finletted mackerel (*Megalaspis cordyla*), both commonly consumed by humans. The collected fish samples were immediately stored at a temperature of -20°C. In the laboratory, each frozen fish sample underwent a defrosting process and was subsequently washed using distilled water. Subsequently, the fish were

dissected, and samples of scales and gills were carefully extracted. These samples were then placed in clean petri dishes, covered with aluminium foil, and appropriately labelled for identification. The KOH digestion process, as outlined by [13] was adopted. Each fish sample was individually transferred into a glass conical flask containing a solution of 10% KOH. The flasks were covered with aluminium foil to avoid contamination and labelled accordingly. The digestion process was carried out by placing the flasks in an incubator set at a temperature of 40°C for a duration of 48 to 72 hours. Once removed from the incubator, NaCl solution (1.2 g/mol) was added to each digested sample. These mixtures were stirred for a period of 10 to 20 minutes and left overnight to settle at room temperature. The digested samples were then examined under a light microscope, and detailed observations were made regarding the colour and shapes of the microplastics present. Photographic documentation of the microplastics was also undertaken. To further analyse the samples, Fourier transform infrared spectroscopy (FTIR) was employed. The IR readings obtained were transformed into graphical representations. Using an infrared spectroscopy absorption table, the polymers present in the samples were identified based on their characteristic absorption patterns

3. RESULTS AND DISCUSSION

Two marine fishes (*Rastrelliger kanagurta* and *Megalaspis cordyla*) were analysed to detect microplastics contamination. Both fish samples contained various microplastics. The physical parameters of the sampled fishes are given in the Table 1.

Table 1. Physical parameters of the sampled fishes

Fish species	Length	Weight	Sample weight	
			Skin	Gill
<i>Rastrelliger kanagurta</i>	24 ± 0.28cm	162.47±2.54 g	0.63 ±0.02g	2.16±0.52g
<i>Megalaspis cordyla</i>	24.5±0.51cm	149.62± 3.17 g	0.79 ±0.01g	1.95±0.21g

The microplastics were found in the form of fragments, fibres, granule, flakes and films. The amount of particles was found high in skin than gills.

Table 2. Percentage of various micropalastics found in fish samples

Fish	Samples	Microplastics	Percentage
<i>Rastrelliger kanagurta</i>	Skin	Fibres, film, fragments, Granules	30%
	Gill	Films, fibers	23%
<i>Megalaspis cordyla</i>	Skin	Fibres, fragments, films	25%
	Gill	Flakes, fibres	22%

Displays microplastics in this investigation that were seen under the microscope (Fig.1).

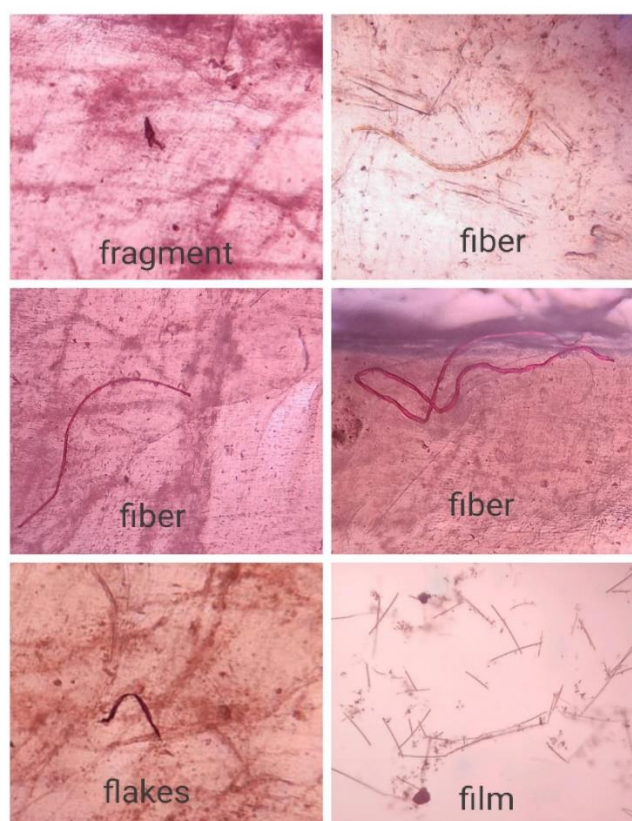


Fig. 1. Showing the presence of MP in skin and gill of *Megalaspis cordyla*

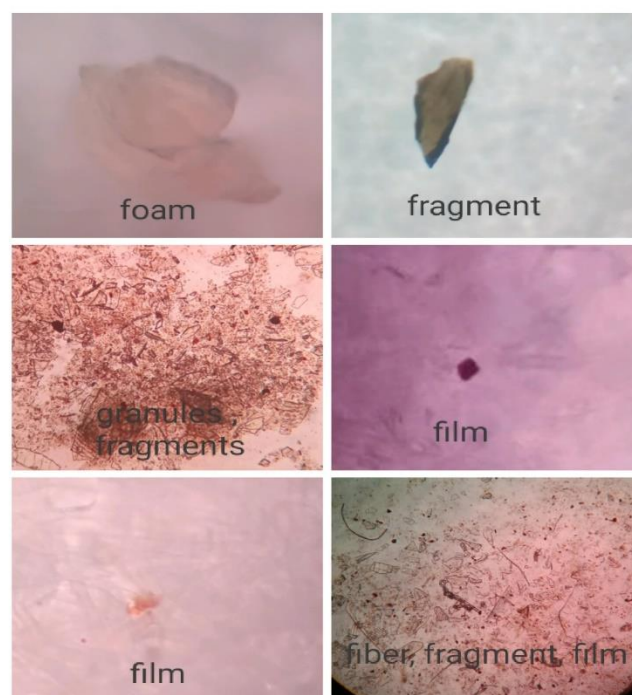


Fig. 2. Showing the presence of MP in skin and gill of *Rastrelliger kanagurta*

The abundance of microplastics in the fish samples did not show a significant correlation with their body weight and length. This lack of correlation is likely a consequence of widespread plastic pollution in water bodies.

Microplastics exhibited a variety of colours and shapes. These minuscule particles appeared in colours such as red, black, grey, orange, and transparent. Among these, black microplastics were the most dominant, constituting 40% of the observed microplastics. Red microplastics followed at 20%, grey at 10%, orange at 20%, and transparent at 10%. In terms of shapes, the microplastics displayed considerable diversity. Fragments, which had irregular shapes and appeared as tiny pieces, were the most abundant type of microplastics. Additionally, fibres and films were prevalent. Fibres were characterized by their elongated, thin, thread-like appearance, while films were thin and possessed irregular shapes. Some particles took on a foam-like structure, resembling a sponge and being larger in size compared to the other microplastics. This array of colours and shapes in microplastics

highlights the complexity of plastic pollution in aquatic environments, as these variations may arise from different sources and degradation processes. The dominance of black and red microplastics might suggest specific sources that contribute to their prevalence in the studied fish samples. Abundance of these particles are described in the Table 3.

Table 3. Abundance of MP in fish species under study

Microplastics	Percentage
Fragments	20%
Fibers	25%
Film	25%
Flakes	10%
Granules	10%
Foam	10%

Identification of microplastics using FTIR spectroscopy: Fourier transform infrared spectroscopy was carried out to identify the monomers of microplastics from the fish samples. The FTIR spectra of fish samples are given below (Fig.3).

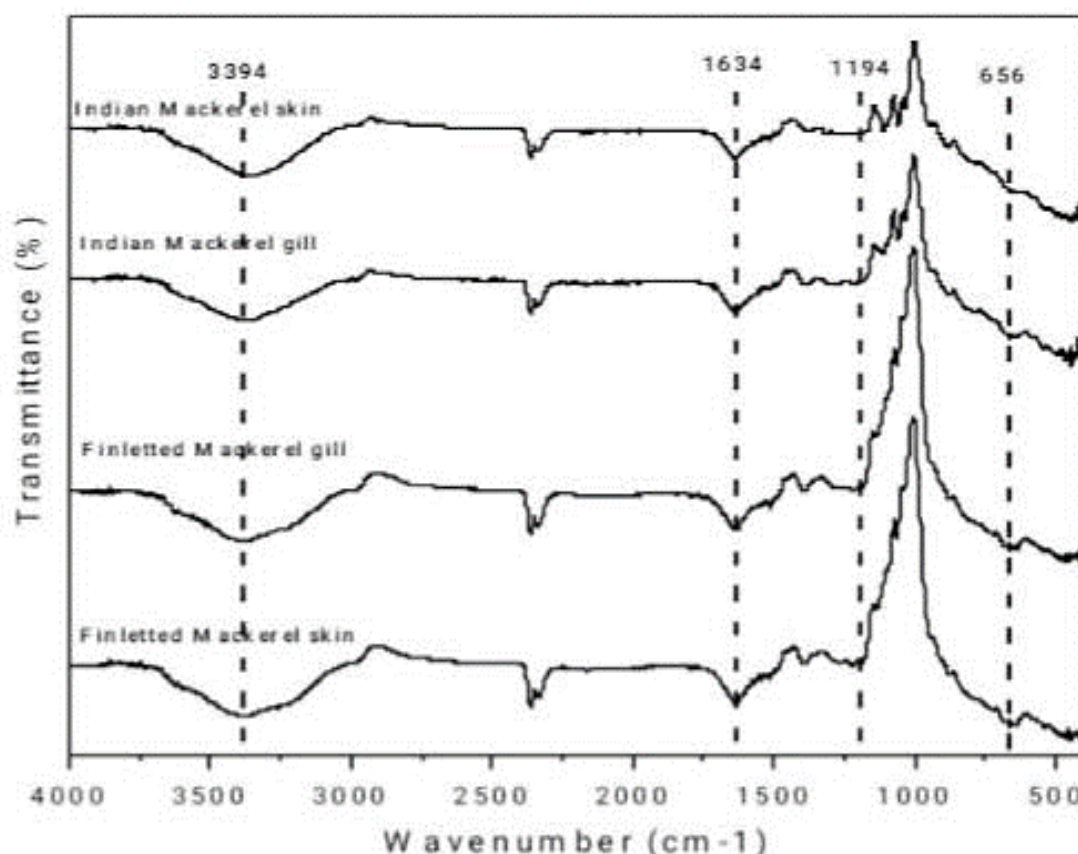


Fig. 3. The FTIR spectra of fish samples

The infrared (IR) spectra of each fish sample revealed distinct bands that corresponded to various functional groups. Notably, a robust N-H stretching band at 3394 cm^{-1} suggested the presence of primary amine groups, possibly indicating the existence of urethane. Urethane serves as the monomer of polyurethane, a subset of plastics known as elastomeric polymers, including rubber. Furthermore, a pronounced C=C stretching band at 1634 cm^{-1} indicated the presence of alkenes like Ethylene, Propylene, and Styrene. These compounds act as the monomers for well-known plastic polymers, namely Polyethylene, Polypropylene, and Polystyrene. Additionally, a medium C-N stretching band at 1194 cm^{-1} suggested the occurrence of amine groups, implying the presence of urethane and amides. These compounds serve as the monomers for polyurethane and polyamides. The presence of a medium bending band of C-H in halo compounds at 656 cm^{-1} pointed to the existence of halogen compounds such as Br, Cl, and I. This indicates the potential presence of toxic substances like carbon tetrachloride and carbon tetra bromide, which can be harmful to both fish and humans.

Numerous studies have demonstrated the threats due to microplastics (MPs) pose to fish. In particular, research on *Danio rerio* has highlighted effects like oxidative stress, reduced mobility, disrupted gene expression, and reproductive organ damage due to MPs intake. *Oryzias melastigma*, the second most studied fish in this context, also faces physical impairments, growth inhibition, weight reduction, and reproductive organ damage. *Sparus aurata*, an important consumable fish, experiences stress, oxidative damage, behavior changes, and damage to key immune system functions due to MPs ingestion.

The presence of MPs in seafood constitutes a significant hazard to human health, considering that seafood is a staple in many diets. MPs' contamination of the gastrointestinal system poses risks of spreading to other body regions. Two common pathways for MPs to enter the human body are endocytosis and adsorption. The toxicological impacts on fish health may have profound implications for human consumers who rely on fish as a significant dietary component. Further investigation into these concerns, accounting for realistic MP and pollutant levels in the ecosystem, is imperative.

Two fish species were subjected to investigation, with each sample revealing contamination by microplastics (MPs). Various types of MPs were detected, encompassing fibres, fragments, films, granules, and flakes, which exhibited diverse shapes and sizes, including black, transparent, red, orange, grey, and transparent. Fragmentary MPs exhibited irregular shapes, while fibers were characterized by elongated, thread-like structures. Foamy particles displayed a larger, sponge-like texture, and film MPs were small, featuring diverse shapes. These findings are congruent with those reported by Solomando et al. [14].

A number of authors have categorized distinct types of MPs into various groupings, such as fibers and fragments (Sun et al. 2019), fibers, fragments, and granules (Hu et al. 2018), and fibers, lamina, fragments, and pellets [15]. Further categorizations include foams, sheets, pellets, films, fibers/lines, fragments (Deepak et al. 2021, Wu et al, 2020). Several studies have examined the size distribution of MPs in marine environments, including the impact of different size classes of MPs on marine food chains [16,17].

Fourier transform infrared spectroscopy was employed to discern functional groups and monomers of microplastics within the fish samples. The outcomes indicated the presence of well-known plastics such as Polyethylene, Polypropylene, Polystyrene, polyurethane, carbon tetrabromide, and carbon tetrachloride, which pose significant hazards to both fish life and human health. These substances can lead to detrimental environmental effects. These findings are parallel to those of another study conducted in the southern coastal region of India by Harikrishnan et al. (2022).

Microplastics exhibit pronounced toxicity to both fish and humans. They result in increased fish population mortality, reduced mobility, inhibited growth, respiratory disruptions, disturbances in various metabolic activities, decreased food intake, weight loss, and damage to reproductive organs. Considering the vital role of seafood in human diets, the presence of toxic microplastics within seafood raises concerns. These substances can infiltrate the human body through seafood consumption, leading to toxicity, tissue damage, immune system alterations, inflammation, and serious illnesses such as cancer, consequently affecting overall human health [18].

Numerous research endeavours have demonstrated the threat posed by MPs to fish, with mortality occurring frequently before fish reach maturity due to MPs ingestion. A majority of these studies have focused on the zebrafish (*Danio rerio*), revealing common effects like oxidative stress, diminished mobility, disrupted gene expression, and damage to reproductive organs. *Oryzias melastigma*, the second most studied fish in this context, experiences physical impairment due to MPs ingestion, leading to growth inhibition, gut dysbiosis, weight reduction, compromised liver antioxidative condition, reproductive organ damage, and growth retardation. Another significant fish species, *Sparus aurata*, commonly consumed by humans, also encounters challenges due to MPs ingestion, resulting in stress, oxidative damage, behavioral alterations, compromised immune system functions, and survival difficulties [19], [20,14]. The toxicological consequences of MPs' presence can extend to fish populations, which holds noteworthy implications for humans who heavily rely on fish consumption as a staple of their diet. Additionally, the impacts could extend to fishing activities themselves, potentially leading to severe consequences [21,22,23]. Given these concerns, further comprehensive investigations are warranted. These investigations should take into account the realistic levels of microplastics and pollutants present in the ecosystem, as the intricate interplay of these factors could contribute to the overall risks associated with microplastics exposure [24-27].

4. CONCLUSION

A comprehensive examination was conducted to uncover the presence of microplastic contamination in two distinct fish species, namely *Rastrelliger kanagurta* and *Megalaspis cordyla*. The results of the investigation unequivocally confirmed the presence of microplastics within both fish species, highlighting the concerning reality of microplastic pollution infiltrating aquatic ecosystems. Among the myriad types of microplastics identified, which encompassed fibers, fragments, films, granules, flakes, and foams, fibers and films stood out as the most frequently encountered. Conversely, foams and flakes granules were found to be the least prevalent. Employing Fourier transform infrared spectroscopy (FTIR) analysis, the presence of notable plastic polymers such as Polyethylene, Polypropylene, Polystyrene, and Polyurethane, in addition to hazardous compounds like carbon

tetrabromide and carbon tetrachloride, was unequivocally established. The implications of such pollutants are severe, posing potential dangers not only to fish populations but also to human health, with the propensity to incite hazardous diseases and an array of detrimental effects. The presence of microplastic pollution in aquatic ecosystems signifies a foreboding future, urging the necessity for proactive intervention. To address this critical concern, imperative steps must be taken to prevent and mitigate the escalating problem. Implementing effective waste management strategies and diligently controlling plastic usage are paramount to curtailing the further dissemination of microplastics into the environment. In so doing, the diverse fish populations, human well-being, and the intricate balance of nature can be preserved and safeguarded. The outcomes of this study underscore the urgency of concerted efforts to combat microplastic contamination. As we confront the challenges posed by this environmental threat, a collective commitment to responsible practices and sustainable actions becomes essential in order to ensure a healthier and more resilient future for both aquatic ecosystems and humanity at large.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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