Microplastic in Selected Tissues of Freshwater Fish in River Chaliyar, Kerala, India

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Abstract

Microplastics are found in every environment, from marine, freshwater, terrestrial, and even atmospheric condition. The microplastic debris can be ranked as primary or secondary. Primary microplastics largely consist of microbeads. Secondary microplastics vary in form and origin. Mostly fibrous and fragmented microplastics are secondary plastics. Species traits and tropic level may be important factors related to fish microplastic concentrations and could influence whether microplastics accumulate in the gills or gastrointestinal tissues. The current study observed the presence of microplastics in selected tissues of two different species of commercial fishes: *Epinephelus chlorostigma* and *Scatophagus argus* from river Chaliyar in Kerala by using Scanning electron microscopy. In both species, MP was deposited in the form of fibers, fragments, and beads, but their size and amount vary with respect to tissue and species difference. No depositions were observed in the gill of *Epinephelus chlorostigm.* Very high depositions were observed in the gill of the *Scatophagus argus* in the form of large filaments.

Keywords: Microplastic, Tissue accumulation, SEM, Freshwater fish,

Introduction

The contamination of aquatic environments with microplastics and their ingestion by freshwater organisms is of increasing concern. Microplastics were found in every environment, from marine, freshwater, terrestrial, and even atmospheric conditions. Microplastic is less than 5 mm in size and is derived through the degradation of larger plastics into smaller fragments or fibers. One of the main sources of microplastic for freshwater ecosystems is wastewater treatment plants and urbanization. In remote locations, the breakdown of plastic waste and subsequent distribution of particles by wind can lead to high levels of contamination [1]. Microplastic concentrations in freshwater ecosystems have high magnitude than in marine environments, leading to high consequences for aquatic food webs. It is having high physiological effects [2]. Microplastics are composed of synthetic organic polymers that can contain hazardous chemical additives and can serve as vectors for persistent organic pollutants [3]. As plastic degrades, particle size decreases, allowing ingestion by a larger range of organisms. Harmful chemicals in plastics include cadmium, lead, and polyolefins. Polyolefins are a broad polymer group that includes polyethylene, ethylene-vinyl acetate, synthetic rubber, polypropylene, and polystyrene, it can be absorbed directly into the consumer leading to physiological impacts on reproductive success, behavior, and growth [4].

Plastic debris can be ranked as primary or secondary. Primary microplastics largely consist of microbeads. Secondary microplastics vary in form and origin and often result from the breakdown of macroplastics through ultraviolet, microbial, and physical degradation [5, 6]. Further categorization includes grouping based on shape as beads, fibers, and fragments. Microplastic fibers can be primary or secondary in nature and mainly originate from items such as clothing, fishing nets, and plastic bags [7, 8].

Fragmented microplastics are secondary plastics often originating from plastic objects that do not fray into fibers [7, 9].

Species traits and tropic level may be important factors related to fish microplastic concentrations and could influence whether microplastics accumulate in the gills or gastrointestinal tissues [1, 10]. Microplastic ingestion has been documented for an increasing number of marine species. However, to date, only a few studies have investigated their biological effects. So far, only a few studies provide evidence for the presence of microplastic in rivers and lakes [11]. Hundreds of marine and freshwater species encounter microplastics in their environment [12]. This may include Copepods, Fishes, Mollusca, Birds, and higher Mammals and among these Fishes and Mollusca contribute to the major element in both commercial and nutritional value [13].

Microplastics being highly toxic and ultra-hazardous may have direct and indirect impacts on aquatic organisms. The biochemically unstable monomers of these deadly polymers have the potential to reach out from the polymer matrix and can reach different natural resources and have the power to accumulate in different levels of the food web and tropic levels. Microplastics get lodged within the animal tissues by ingestion and respiration. The presence of microplastics can be detected in gastrointestinal tracts, muscle tissues, and even the gills of aquatic organisms. The direct ingestion of microplastics might be the major route for primary herbivores and secondary consumers while apex predators are additionally prone to indirect ingestion of microplastics via prey through the food web. Microplastics are known to induce significant detrimental effects (both physical and chemical) on the growth, fecundity, and survival of exposed organisms [14, 15].

A wide variety of species have been reported to be negatively impacted by plastic debris: marine birds, sea turtles, cetaceans, fur seals, sharks, and filter feeders are just some of those documented. Plastic ingested by these animals persists in the digestive system and can lead to decreased feeding stimuli, gastrointestinal blockage, and decreased secretion of gastric enzymes, and decreased levels of steroid hormones, leading to reproduction problems [16]. The detrimental effect of microplastics arises due to the migration of chemicals and sorbed contaminants from plastic polymer to the body of an organism. The amount of plastic waste in Kerala will threaten aquatic life because all type of contaminants finally enters into the aquatic environment. Data on the presence of microplastics in the body of fish is still very limited, despite the level of plastic pollution in Kerala is high. Therefore, it is very important to conduct research on the content and characteristics of microplastics in fish, by considering fish as an important food item, having good sources of PUFA, and being widely consumed by man.

Materials and methods

The present study was conducted in River Chaliyar of Feroke, the fourth longest river in Kerala having 169 km in length, and finally empties into the Arabian Sea at Beypore port. Two different species of edible fishes were collected from the study area that includes: *Epinephelus chlorostigma*(brown spotted grouper) and *Scatophagus argus* (Spotted scat).

	
Epinephelus chlorostigma	*Scatophagus argus*

The tissues like intestine, liver, muscle, and gills were dissected out from the two species and preserved using glutaraldehyde fixative. The fixed tissues were then undergone Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) analysis. Specifications for ZEISS Gemini SEM 300 used were: Resolution: 0.6 nm @ 30 kV (STEM),0.7 nm @ 15 kv, 1.2 nm @ 1 kv, 1.1 nm @ 1kV TD. In lens BSE Resolution: 1.2 nm @ 1 kV. Resolution in Variable Pressure mode (30 Pa): 1.4 nm @ 3 kV and 1.0 nm @ 15 kv. Acceleration Voltage: 0.02 - 30 kV. Probe Current: 3 pA - 20 nA (100 nA configuration also available). Magnification: 12 – 2,000,000. Electron Emitter: Thermal field emission type, stability better than 0.2 %/h. Detectors were available in the basic configuration: High-efficiency VPSE detector (included in variable pressure option) Store Resolution: Up to 32k × 24k pixels. Specimen Stage: 5-axes motorized eucentric specimen stage, X = 130 mm; Y = 130 mm, Z = 50 mm, T = -4º to 70º, R = 360º.

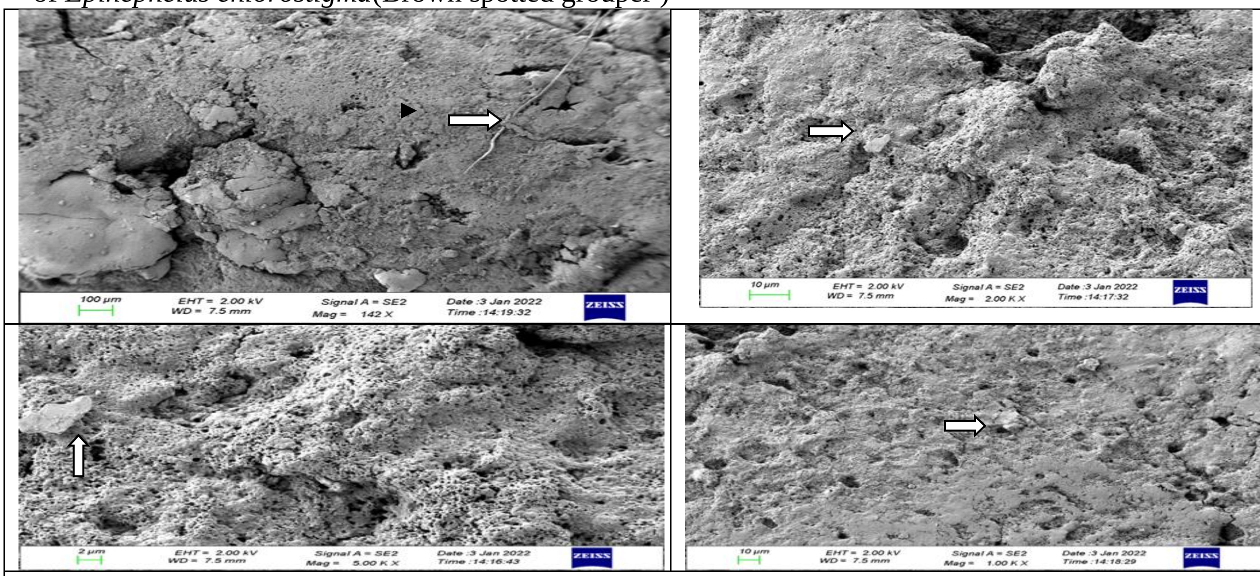
Results

Presence of microplastics was determined in selected tissues like the liver, muscles, intestine, and gills of *Epinephelus chlorostigma* (brown spotted grouper) and *Scatophagus argus* (Spotted scat) collected from River Chaliyar in Feroke region, Kerala with the help of SEM photographs. The detected microplastics were in the form of fibers, small beads, and fragments. In the hepatic tissue of *Epinephelus chlorostigma* microplastic was deposited in the form of long fibers and fragments in all tissue samples analyzed. Fibers are in the form of elongated threads and tend to pearsed into the tissue. Deposition of microplastics in the form of fragments was frequently observed in all tissue samples. They are freely observed on the surface of the tissue (fig.1). In the *Scatophagus argus* liver, microplastics were deposited in small quantities in the form of small beads over the surface of the hepatic tissue of all samples analyzed. The frequency of occurrence of microplastic was low when compared to *Epinephelus chlorostigma* (fig. 5). In the muscle tissue of *Epinephelus chlorostigma* microplastic deposition is in the form of small and large fragments. The size and shape of the fragment varies between samples. The fragments were observed on the surface of myofibrils and muscle bands (fig. 2). But in *Scatophagus argus* muscle, the deposition of microplastic frequency is low when compare to *Epinephelus chlorostigma*. In *Scatophagus argus* microplastics were deposited in the form of small fragments (fig. 6).

In the intestinal tissue of *Epinephelus chlorostigma* microplastic deposition is in the form of small beads and fragments. They are found projecting over the tissue (fig.3). The intestinal tissue of *Scatophagus argus* microplastic was deposited in the form of fragments. But the frequency of occurrence of fragments is high when compared to *Epinephelus chlorostigma*(fig.7). In the gill tissue of *Epinephelus chlorostigma* no considerable depositions of microplastics were observed (fig.4). In *Scatophagus argus* gill the deposition of microplastic was very high. They are deposited in the form of large filaments over the gill rackers (fig. 8).

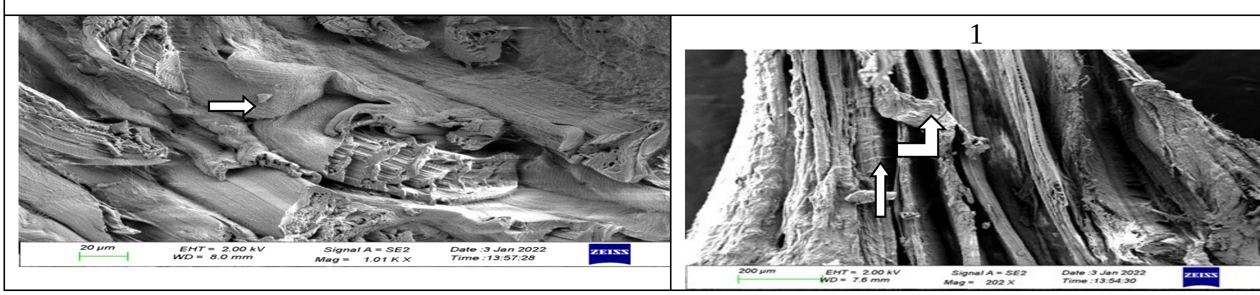
There are differences in the type of microplastic found in the tissues between species. In the gill, they are in the form of filaments, but in other tissues, they are in the form of small beads/ large or small fragments. Microplastics were present in both species studied, but the predicted frequency of occurrence of plastic ingestion varied depending on the species. *Epinephelus chlorostigma* has a high frequency of occurrence of microplastic in various tissues when compared to *Scatophagus argus.* In *Epinephelus chlorostigma* high frequency of accumulation of microplastic was observed in the liver and muscles. But in *Scatophagus argus* high frequency of occurrence was in the gills rather than in the liver and muscles.

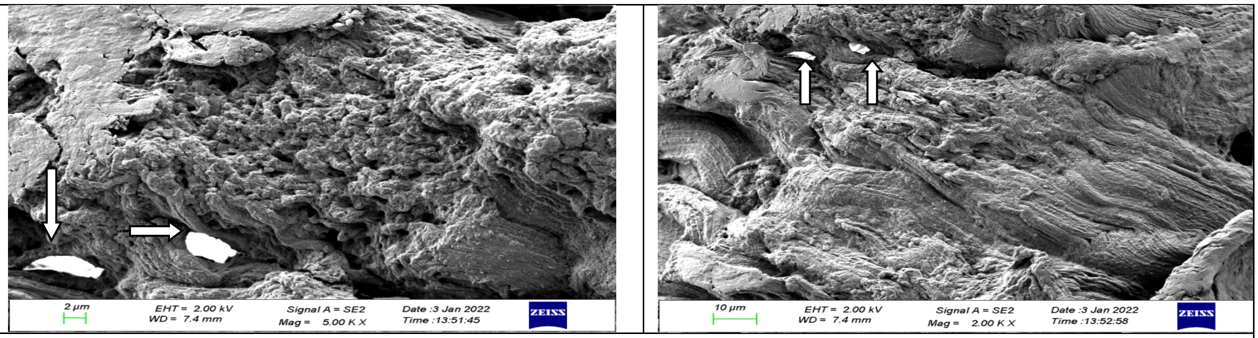
SEM picture showing the presence of microplastics in selected tissues (liver, muscles, intestine, and gills) of *Epinephelus chlorostigma*(Brown spotted grouper)



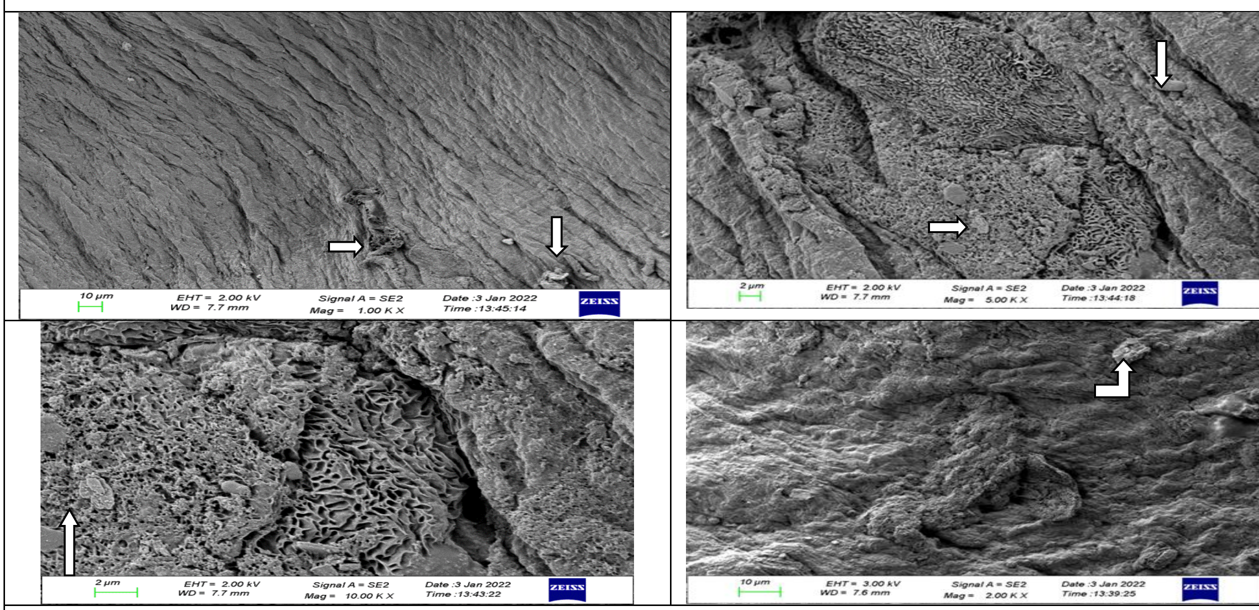
SEM picture showing the presence of microplastics in Grouper Liver: Fig.1

1

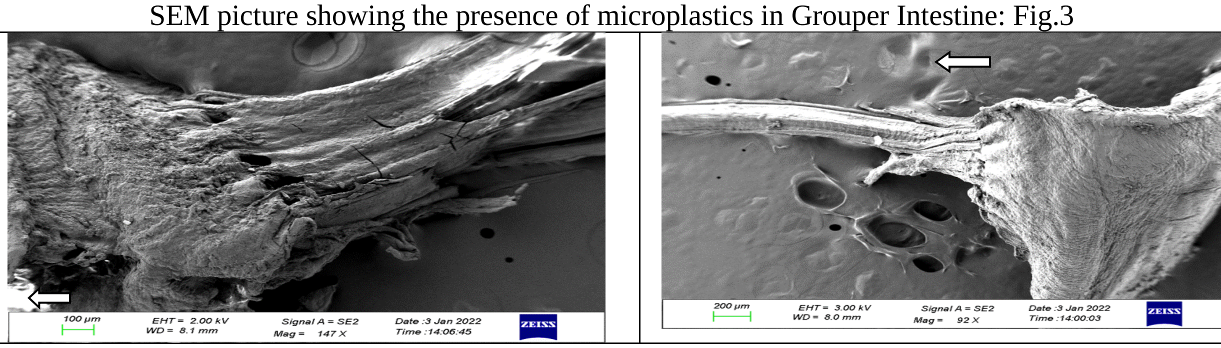


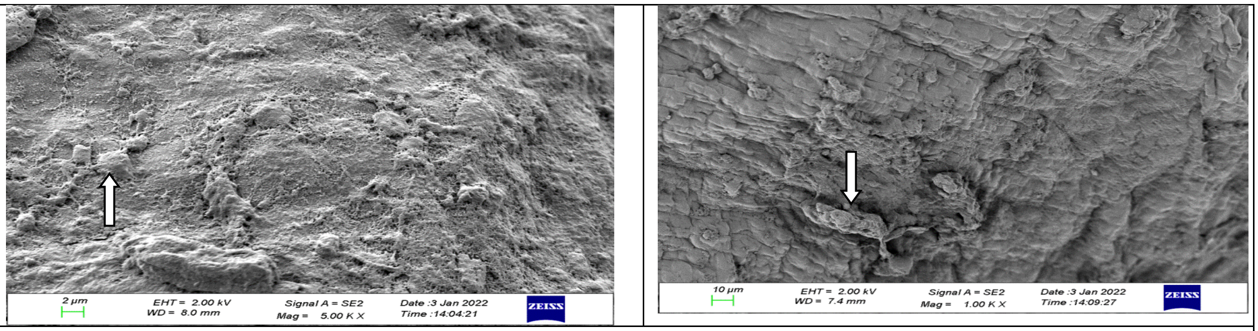


SEM picture showing the presence of microplastics in Grouper Muscle: Fig.2



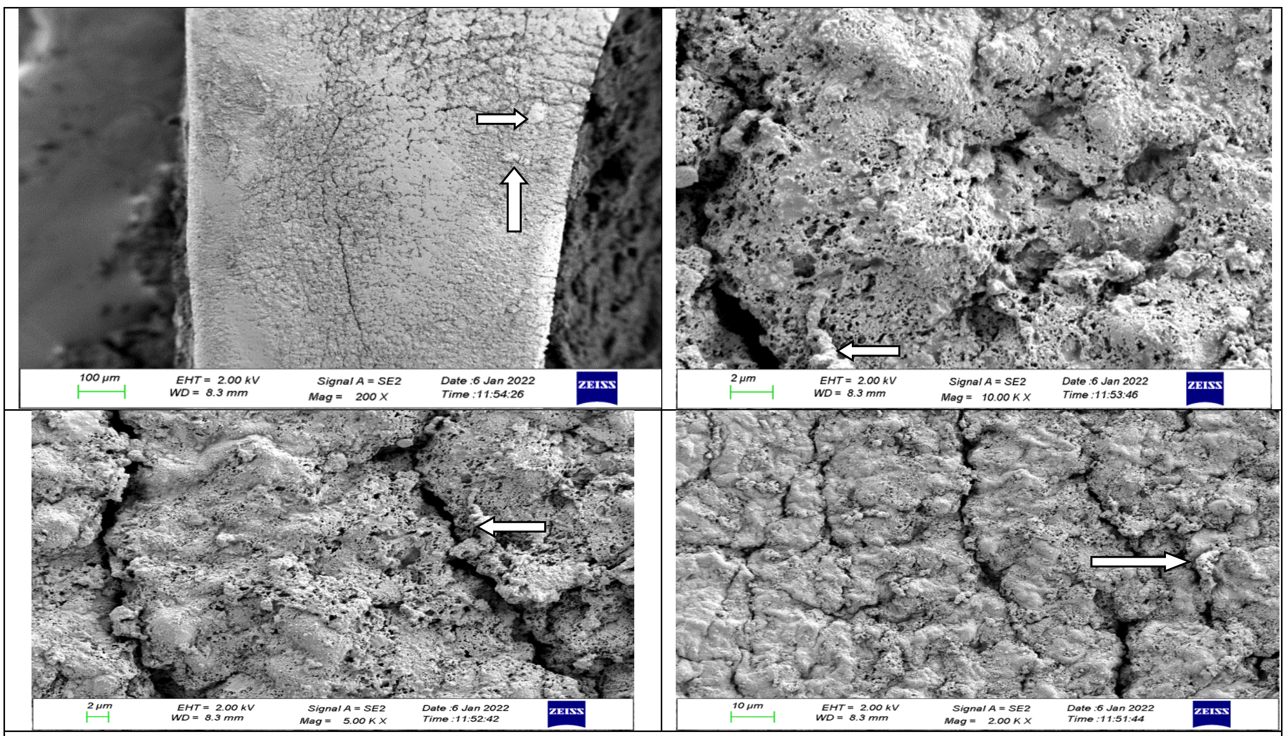
SEM picture showing the presence of microplastics in Grouper Intestine: Fig.3





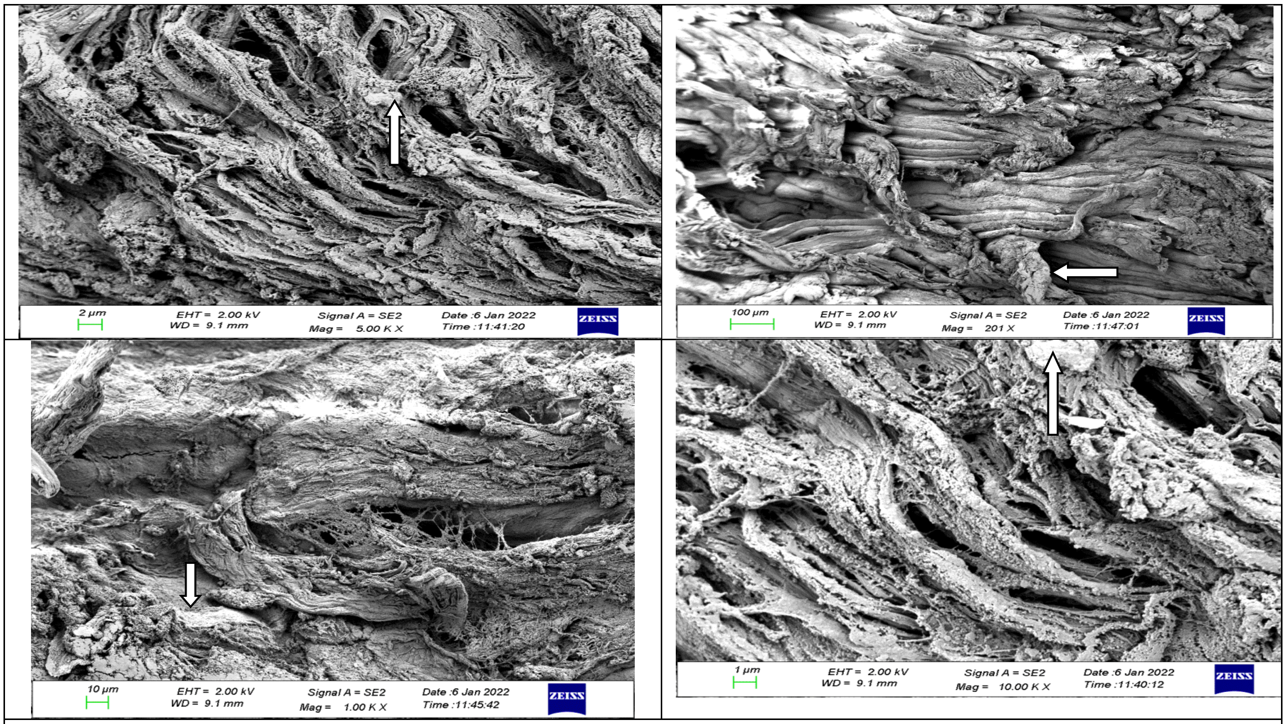
SEM picture showing the presence of microplastics in Grouper Gill: Fig 4.

SEM picture showing the presence of microplastics in selected tissues (liver, muscles, intestine, and gills) of *Scatophagus argus*(Spotted scat)

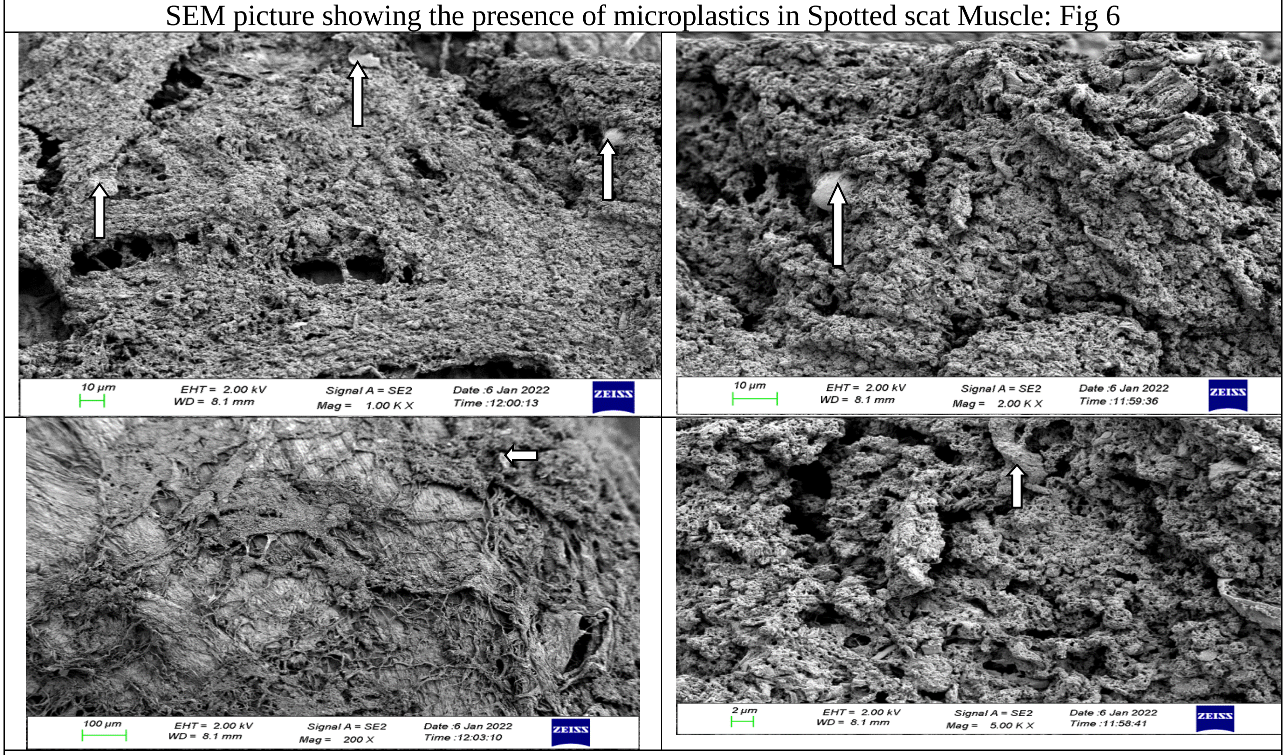


SEM picture showing the presence of microplastics in *Scatophagus argus*(Spotted scat)
Liver: Fig 5

ISSN NO: 2230-5807

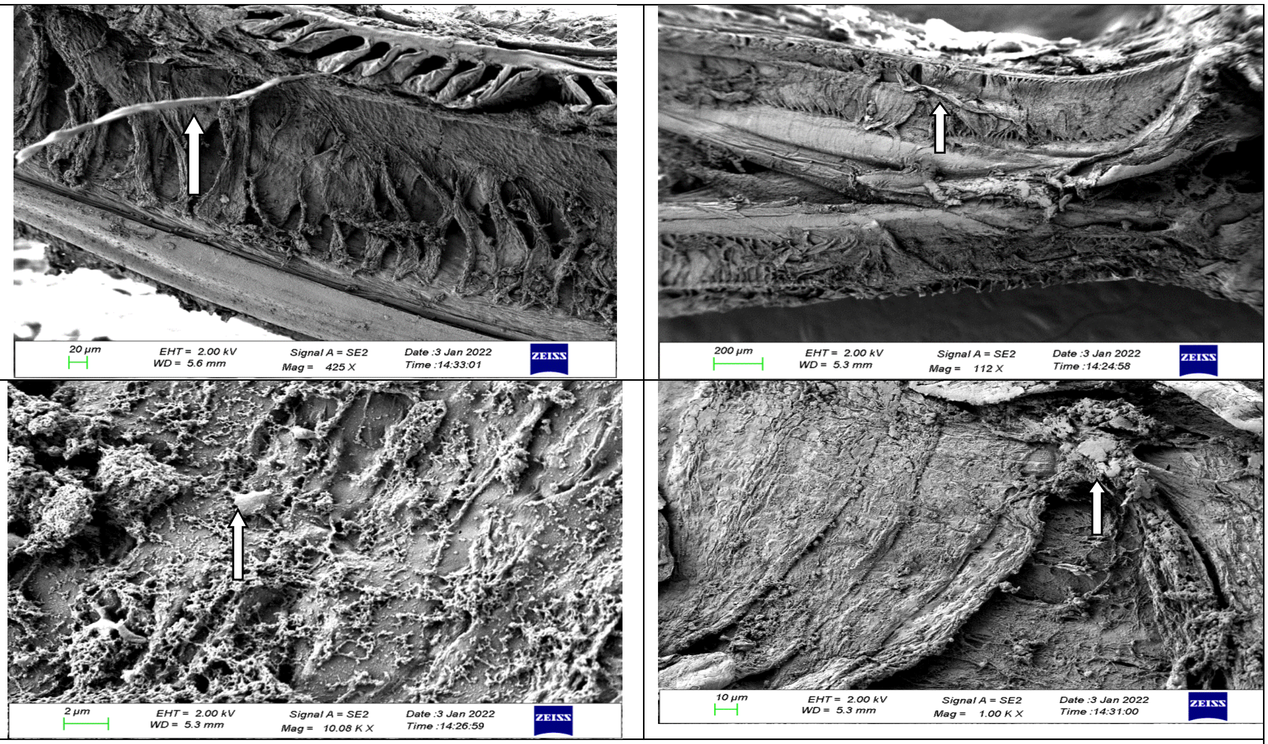


SEM picture showing the presence of microplastics in Spotted scat Muscle: Fig 6



SEM picture showing the presence of microplastics in Spotted scat Intestine: Fig 7

ISSN NO: 2230-5807



SEM picture showing the presence of microplastics in Spotted scat Gill: Fig 8

Discussion

In the present study there was a significant difference in the number of microplastics found among species. In *Scatophagus argus* the deposition is high in gill. They are surface feeders, found in the water column. So microplastic suspended in the water column may enter into gills more easily than bottom forms. *Epinephelus chlorostigma* is a benthivore, so the entry of microplastic was mainly through the ingestion of other organisms with microplastics. So its deposition is high in internal tissues. The chance of entry of microplastic through respiration is very low. So this may be the reason for the absence of microplastic in its gill. Similar results were reported by Brillant and Mac Donald [17], with northern pike having significantly more deposition when compared to other species of fish. They suggested that this difference may be due to differences in the feeding habits of the various species. Benthivores such as white suckers, fathead minnows, and sticklebacks are likely exposed to microplastics that have settled onto the sediments, whereas planktivores such as shiners would be more likely to consume microplastic suspended in the water column [18].

Feroke region of River Chaliyar is an urban area. A lot of previous work reported that urbanization is the main reason for microplastic accumulation in aquatic biota. A comparably high prevalence of microplastics in freshwater fish has been found in several rivers in the mid-western USA [19], Canadian prairie creeks [20], and an urbanized river in Texas [1].

Most studies investigated microplastics in the gastrointestinal tract rather than whole organisms [21]. Evidence of microplastic in the tissue outside the digestive system is slowly emerging. Microplastics have been found in the gill of a number of fish species [22]. In the present study also microplastic were found in the gill of the *Scatophagus argus*. Microplastic has been found in the liver of European anchovies, but not in the liver or muscle of a number of other important commercial species [23]. In the present study also microplastic was found in the liver and muscle in both fish species in varying amounts. They were all in the form of fibers and beads. The prevalence of plastic fibers in freshwater systems, suggests that a reduction of plastic use needs to be more comprehensive for protecting life on land and in water.

Summary and Conclusion

Rivers and estuaries are a major component in the water ecosystem. River Chaliyar, the fourth longest river of Kerala is of greater importance. Most of the aquatic organisms and several other animals including humans depend on this water for various domestic uses. So its purity and management is of great concern. The analysis of microplastic toxicity levels in two different common edible species of estuarine fishes helps us to compare the quality of common edible fishes and also to estimate how far this particular ecosystem has deteriorated. Microplastic deposition varies with respect to species and tissues. In *Epinephelus chlorostigma,* the deposition of microplastic is high in the liver and muscles when compared to *Scatophagus argus*, But the presence of microplastic is very high in the gill of *Scatophagus argus*. They are in the form of large filaments over the gill rackers. In *Epinephelus chlorostigma,* no detectable amount of microplastics was observed in the gill. The natives inhabiting the river banks of Feroke Chaliyar depend largely on fishing as a livelihood means and they do supply these common fishes to nearby local markets. The damaged and abandoned fishing net materials, household debris, and runoff due the improper agricultural waste management give to the accumulation of microplastic fibers and threads. These fibers and threads of nano plastics increase the fatality of fish and other aquatic fauna. This data provides useful background information for further investigations and subsequent development of management policies to monitor and control microplastics in a fragile ecosystem like the backwaters.

Acknowledgments

Authors wish to thank Dr. Manzur Ali P.P., Principal, Prof. O.P. Abdurahiman, Management Committee secretary, Dr. Anoop Das K.S., Head of Department of Zoology, MES Mampad (Autonomous) College for giving us an opportunity, facilities, and support for this work. We also express our sincere thanks to Dr. Jos T. Puthur, Director, of the Central Sophisticated Instrumentation Facility, and Mr. Shamjith Khan of Calicut University for helping us with the analysis.

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