A study of microplastics in marine organisms across trophic levels on the west coast of India

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by

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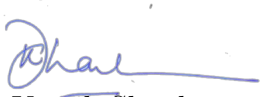
Certificate

This is to certify that this dissertation entitled "A study of microplastics in marine organisms across trophic levels on the west coast of India " towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study carried out by Monali Vasant Patre at CES, IISc Bangalore under the supervision of Kartik Shanker, CES, IISc Bangalore, during the academic year 2017-2023.



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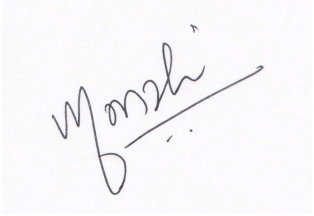
This thesis is dedicated to my family and friends

Declaration

I hereby declare that the matter embodied in the report entitled "A study of microplastics in marine organisms across trophic levels on the west coast of India " are the results of the work carried out by me at the CES, IISc Bangalore under the supervision of Kartik Shanker and the same has not been submitted elsewhere for any other degree.



Monali Vasant Patre

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Abstract

Plastic pollution is rapidly increasing globally, posing a threat to marine organisms. Microplastics negatively impact the biological processes of marine organisms when ingested. It is still unclear how microplastics accumulate in the food web and how microplastic biomagnification varies at different trophic levels. There is a general lack of research on this subject, particularly along the Indian coast. This study aimed to compare the abundance and diversity of microplastics (MPs) across benthic and pelagic species, between two locations with varying anthropogenic pressure (Kochi, Kerala, and Malvan, Maharashtra), and between different tissues. I also investigated whether biomagnification of MPs occurs across trophic levels at both locations. A total of 125 individuals from both locations were sampled and analyzed for microplastics. Additionally, six seawater samples were collected from Malvan for microplastic analysis. Three types of microplastics (fibers, fragments, and films) were observed in the sampled species and water samples. Transparent fibers, mainly derived from degraded fishing lines and nets, were the most abundant type of microplastics observed. Pelagic feeders showed a significantly higher microplastic concentration than benthic feeding species. There was no difference in microplastic concentration between samples from Malvan and Kochi. Microplastics were observed in both the gut and liver of the sampled individuals and were significantly higher in the liver than in the gut of primary consumers (TL1). On the contrary, microplastic concentration was significantly higher in the gut than in the liver of secondary consumers (TL2). No evidence for biomagnification was observed in samples from both locations.

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Contributions

Contributor name	Contributor role
Monali Patre, Kartik Shanker, Shawn Dsouza	Conceptualization Ideas
Monali Patre, Kartik Shanker	Methodology
RStudio 4.2.2	Software
Kartik Shanker, Shawn Dsouza	Validation
Monali Patre, Garima Bora, Shawn Dsouza	Formal analysis
Monali Patre	Investigation
Kartik Shanker, IISc Bangalore	Resources
Monali Patre	Data Curation
Monali Patre	Writing - original draft preparation
Kartik Shanker, Shawn Dsouza	Writing - review and editing
Monali Patre, Garima Bora	Visualization
Kartik Shanker, Garima Bora	Supervision
Garima Bora	Project administration
Kartik Shanker	Funding acquisition

Chapter 1

Introduction

1.1 Background

Plastic pollution has been persistent since the 1950s. The annual rate of plastic production is expected to escalate from 9.2 million tons per year in 2017 to 34 billion tons by 2050 (Geyer, 2020). Plastics' versatility and long-term durability have led to their substantial increase in demand recently. The application of plastics involves many aspects of modern life, like electronics, packaging, construction, electronic appliances, agriculture, clothing, etc. The accumulation of plastic waste in the marine environment has become a major global concern over the years. The exponential increase in plastic production can threaten social-ecological systems and processes in the long run. Marine plastic debris is expected to reach 250 million metric tons by 2025 if we fail to find a long-term solution (Jambeck et al., 2015). A significant portion of marine plastic is derived from human litter, which is transferred from land to the ocean (Sheavly and Register, 2007), (Geyer et al., 2017). Most of this plastic waste gets piled up in the marine environment in large quantities. Plastics resist biodegradation in the environment but disintegrate into fragments through thermal and mechanical processes (Hernandez et al., 2017). The plastics in oceans can undergo physical and chemical fragmentation due to temperature fluctuations, exposure to UV radiation, ocean circulation, and oxygen (Andrady, 2011). The most common sources of marine plastic pollution could be accidental loss or abandonment of fishing gear, sewage effluents, tire covers, and overboard disposal.

1.2 Microplastic pollution

Plastics are subjected to various physical and chemical degradation, which causes them to break into fragments. These degraded plastics with varying sizes, shapes and colors are categorized as microplastics (MPs) (Jahnke et al., 2017), (Galloway et al., 2017). In 2009, the National Oceanic and Atmospheric Administration (NOAA) defined MPs as smaller-sized plastic fragments of size less than 5mm (Barboza and Gimenez, 2015). These particles are smaller than zooplanktons and cannot be detected by the naked eye. However, the lower size limit of the MP particles remains under debate. The recognition of MPs as an emerging marine environmental contaminant has grown in recent years (GESAMP, 2015).

MPs can be classified into two types depending on their source and origin - primary and secondary MPs (Li et al., 2018). Extrusion and grinding of plastics produce primary MPs. They are already in their final form when released into any environment. The major source of primary MPs is the raw material used to create plastic products. Primary microplastics (MPs) that contribute to marine pollution include molded plastic powders, plastic nanoparticles from industries, pre-production resin pellets lost during transportation, microbeads used in personal care and cosmetic products, and 'scrubbers' used for surface blast cleaning (Cole et al., 2011). These particles make their way to the ocean via sewage effluents (Browne et al., 2011). They have a spherical or fibrous shape with a uniform surface. The unintentional degradation or fragmentation of larger plastic items gives rise to secondary MPs which do not have a definite shape.

In the ocean, large plastic items like plastic bags, ropes, boxes, and nets break down into smaller particles and release various chemicals after being subjected to mechanical, UV, and microbial degradation. Secondary microplastics (MPs) enter the environment through three mechanisms: natural degradation of MPs through weathering and microbial activity, the breakdown of macroplastics into smaller MPs through direct organismal activity, and the resuspension of previous MP pollution in soil or sediment (Cverenkárová et al., 2021). The properties of both primary and secondary MPs can alter when subjected to weathering (Crawford and Quinn, 2017). MPs with high density tend to sink in the water column, while those with low density float on the surface until they become heavier through adsorption, after which they sink into the water.

The MPs' shape, size, and color can vary in the aqueous environment. Commonly they

exist in the form of pellets, fragments, fibers, films, ropes, filaments, foams, rubber, and microbeads. In the context of this study, fibers are strand or filament-like structures that can be solitary or in a bundle. Fragments are irregularly shaped, broken, or separated from a larger plastic item. Films are flexible sheet-like structures. Apart from various sizes and shapes, MPs are also found in multiple colors—the most common ones are black, blue, white, transparent, red, and green. Transparent fibers are primarily derived from broken fishing lines or nets (Stolte et al., 2015), and colored fibers are derived from the fragmentation and abrasion of plastic commodities (Wang et al., 2017), (Abidli et al., 2018). Color is an essential factor when considering MP ingestion by aquatic organisms, as some organisms might show color-based preference behavior (Wright et al., 2013). The color of MPs may also serve as an indicator of the level of contamination with pollutants.

MPs may contain chemical additives due to different plastic manufacturing processes, which use chemicals to enhance specific properties. Additionally, the large surface-to-volume ratio and hydrophobic properties of microplastics (MPs) enable them to adsorb chemical contaminants from the surrounding environment. In addition to adsorbing and binding toxic pollutants from the environment, MPs often release those contaminants. As a result, microplastics (MPs) can function as a vector or carrier for the transfer of contaminants to marine organisms. (Mato et al., 2000). Accumulated contaminants on MPs can result in toxicity in aquatic food webs (Andrady, 2011).

1.3 MPs in the marine food web

MPs contaminate the environment, and their interaction with aquatic organisms has also been demonstrated in the food chain. Since MPs are relatively abundant in different habitats and geographical areas, they can be easily ingested by many fish species (Neves et al., 2015), (Bellas et al., 2016), (Karlsson et al., 2017). The small size makes them bioavailable for marine fauna. Aquatic organisms like zooplankton, and small fishes, accidentally ingest MPs via unselective passive ingestion (Rummel et al., 2016), (Tanaka and Takada, 2016) or by confusing MPs with prey species (due to their small size) (Derraik, 2002), (Rummel et al., 2016). MP ingestion has been demonstrated in lugworms, mussels, barnacles (Thompson et al., 2004), mussels (Browne et al., 2008), amphipods, and fish. Reported bite marks on plastic surfaces indicate selective feeding on plastic particles (Carson, 2013). Given the simi-

lar size and appearance of microplastics (MPs) to planktonic species, it is likely that aquatic organisms consume them. Such behavior indicates the facilitation of secondary ingestion in fishes (via contaminated prey) (van Drooge and Grimalt, 2012). In marine ecosystems, zooplankton serve a crucial function as the primary consumers in the food chain. Being situated at a lower trophic level, MP accumulation in primary consumers may transfer MPs to higher trophic levels in the food web (Wright et al., 2013). The widespread distribution of MP across the ocean increases the possibility of interaction between them and zooplankton. Large fish prey on zooplankton and other small fishes, which might form pathway for MPs entering the aquatic food webs.

Upon incidental or intentional ingestion, the MPs travel through the gastrointestinal tract, which may be retained inside the tract or egested through the feces. MP retention in the gastrointestinal tract adversely affects the organism. The particles can penetrate the intestinal lining of fish, causing abrasions and perforations, altering their metabolic profile, reducing their nutritional uptake, and decreasing their feeding activity by inducing false satiation (Walkinshaw et al., 2020). In addition, the accumulation of larger microplastic particles in the gut of fish can cause clogging of the digestive tract and result in lesions (Cole et al., 2013), (Wright et al., 2013). Such factors have a negative impact on an organism's body condition, health, and fitness (de Sá et al., 2015). Furthermore, it was noted that MP particles get entrapped between the appendages of zooplankton, which obstruct their ability to swim and find their prey (Cole et al., 2013).

Given the importance of understanding the effects of microplastic pollution, I aimed to examine the abundance and diversity of various types of MPs in commercially caught fish at two major fishing harbors on the west coast of India. I examined microplastic concentration across feeding habits, tissues, and trophic levels.

1.4 Objectives

1. To compare the microplastic concentration between benthic and pelagic species.
2. To examine differences in MP concentration between two locations with varying anthropogenic pressure, namely Kochi, Kerala, and Malvan, Maharashtra.
3. To compare the MP concentration between gut and liver tissue.
4. To determine whether biomagnification of MPs occurs across trophic levels at both locations.

Chapter 2

Methodology

2.1 Study site

The study was conducted in Malvan (16.0519° N, 73.4680° E), one of the biggest fishing centers in Sindhudurg district, and Kochi (9.9669° N, 76.2394° E), the industrial capital of Kerala and the biggest fishing harbor in India (Figure 2.1). Various fishing gears used in Malvan include gill nets, trawlers, shore seine (Rampan), purse seine, hook, and line and cast nets. In addition to the fishing gears used in Malvan, Chinese fishing nets are prominent in Kochi. These regions are distinct in their industrial development and anthropogenic activities near the coast. Human activities like industries, fishing landing centers, and urban centers prevail more in Kochi than in Malvan. Kochi has major polluting industries such as fertilizer and chemical industries, oil refineries, seafood, cashew, coir, and mineral and metal industries predominantly located near the coasts (Naidu et al., 2018). Many release polluting products in the nearby water bodies (Sreekanth et al., 2015). The release of various heavy metals, industrial wastes, and sewage discharge suggests heavy anthropogenic contamination. Malvan, on the other hand, has low anthropogenic pressure compared to Kochi. With almost negligible industrial activity near Malvan, most waste is released from the tourism industry and fisheries.

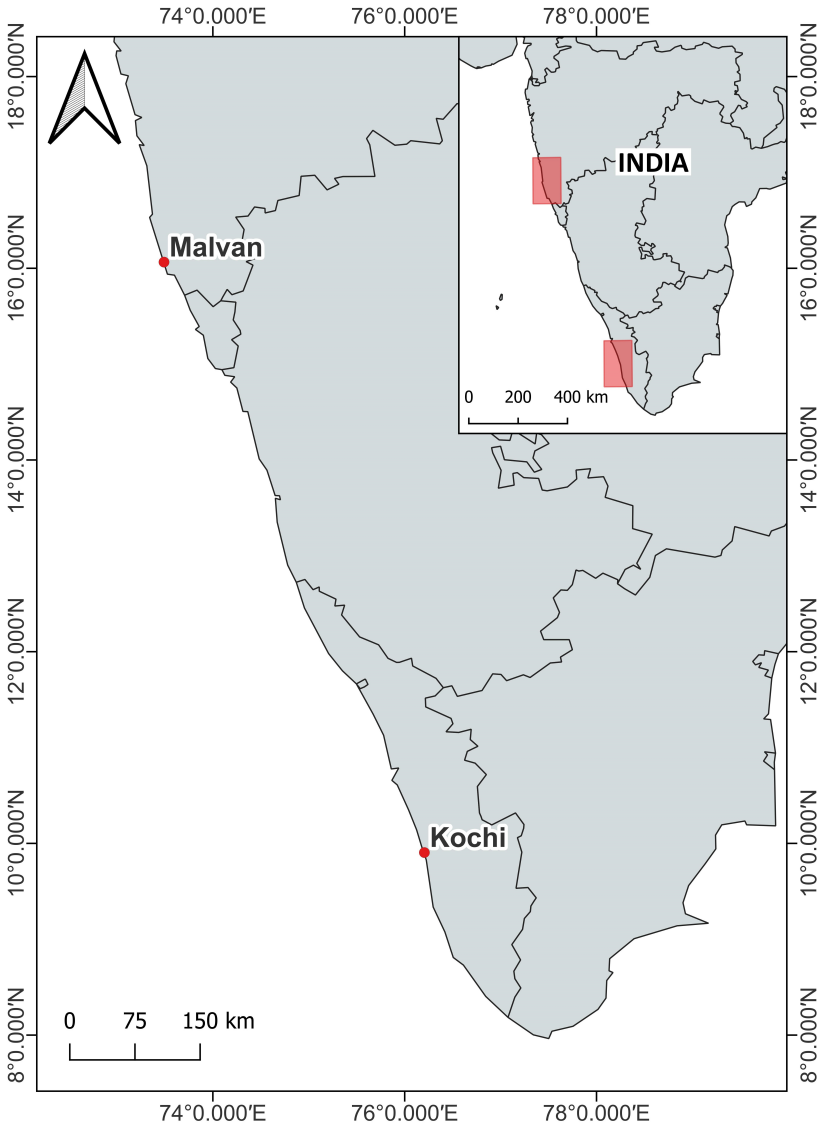

Figure 2.1: A map showing the study locations; Kochi, Kerala and Malvan, Maharashtra

2.2 Field Sampling

Based on the feeding habits, fish species were selected for each of the three trophic levels – primary and secondary consumers. These species were chosen across two locations on the west coast – Malvan, Maharashtra, and Kochi, Kerala. Gut and liver were sampled for this study.

The individuals sampled from the Trophic level 1 (TL1) were Prawns (*Metapanaeus sp.*), Indian anchovy (*Stolephorous indicus*), Goldstripe sardinella (*Sardinella gibbosa*), Tonguefish (*Cynoglossidae sp.*), Indian mackerel (*Rastrelliger kanagurta*), and Indian oil sardines (*Sardinella longiceps*) (Table 2.1). The diet of these species consisted of polychaetes, copepods, diatoms, dinoflagellates, phytoplankton, and zooplankton (Froese and Pauly, 2023). The diet of Indian mackerel and Indian oil sardines consisted of shrimp/prawns, bony fish, polychaetes, fish eggs, diatoms, and other benthic invertebrates. For Trophic level 2 (TL2), the species selected were spadenose sharks (*Scoliodon laticaudus*), bamboo sharks (*Chiloscyllium sp.*), silky sharks (*Carcharhinus falciformis*), and seer fish (*Scomberomoros guttatus*) (Table 2.1). Their diet included mackerel, sardines, bony fish, shrimp/prawns, squids, cuttlefish, mollusks, and benthic crustaceans. There was a lot of overlap between the feeding habits of all the species considered for this study. Based on their habitat, the species were categorized as benthic (organisms that feed on or near the ocean floor) and pelagic feeders (organisms that feed in the water column, away from the ocean floor) (Table 2.1).

2.2.1 Sample Processing

Each sampled individual was recorded for its weight and length at the market. The individuals' gut and liver were obtained from the market cutting stations and stored in a sterile bag at the sampling site. After returning to the base, the gut and liver were weighed and stored in a tarson tube containing 25 ml of 70 percent ethanol (70 percent is lethal to a broad range of microorganisms). If the gut was too large (for example, in sharks and seer fish), it was cut, and the largest part that could fit in the tarson vial was weighed and stored in the tarson tubes. For Prawns (*Metapanaeus sp.*), Indian anchovy (*Stolephorous indicus*), Goldstripe sardinella (*Sardinella gibbosa*), Tonguefish (*Cynoglossidae sp.*), the whole body was stored for analysis as their gut and liver could not be separated. For Indian mackerel

TL	Species name	Common name	Sample size	Location	Habitat
1	*Metapenaeus sp.*	Prawns	5	Malvan	Benthic
	Stolephorus indicus	Indian anchovy	5		Pelagic
	Cynoglossidae sp.	Tonguefish	5		Benthic
	Sardinella gibbosa	Goldstripe sardinella	5		Pelagic
	Rastrelliger kanagurta	Indian mackerel	10		Pelagic
	Sardinella longiceps	Indian oil sardine	10		Pelagic
2	*Chiloscyllium sp.*	Bamboo shark	10	Malvan	Benthic
	Scoliodon laticaudus	Spadenose shark	10		Pelagic
	Scomberomorus guttatus	Seer fish	5		Pelagic
1	*Metapenaeus sp.*	Prawns	10	Kochi	Benthic
	Stolephorus indicus	Indian anchovy	10		Pelagic
	Rastrelliger kanagurta	Indian mackerel	10		Pelagic
	Sardinella longiceps	Indian oil sardine	10		Pelagic
2	*Carcharhinus faciformis*	Silky shark	20	Kochi	Pelagic

Table 2.1: Sampled species in different trophic level (TL) from Malvan, Maharashtra and Kochi, Kerala.

(*Rastrelliger kanagurta*), and Indian oil sardines (*Sardinella longiceps*) in TL1 and spadenose sharks (*Scoliodon laticaudus*), bamboo sharks (*Chiloscyllium sp.*), silky sharks (*Carcharhinus falciformis*), and seer fish (*Scomberomoros guttatus*) in TL2, gut, and liver were separated for analysis. The tarson tubes were stored at -20 degrees Celsius before analysis.

2.3 Digestion

The frozen samples were brought back to the wet lab. The stored fish's gut and liver were weighed again before digestion. The bodies of the species of primary consumers were digested as a whole because their segregation was not possible. The gut of sharks (secondary consumers) was separated into its content and tissue to allow quicker digestion. The gut was squeezed, and the contents were emptied into a beaker and weighed. The tissue was washed with Milli-Q water, weighed, and put in the beaker. All the glassware was first rinsed with Milli Q water to avoid contamination. The gastrointestinal tract and liver were transferred to a 25ml beaker separately. Based on the weight of samples, approximately three times 10% KOH solution was added using a measuring cylinder to digest the organic matter. The organic matter was cut into smaller pieces to increase the surface area for effective digestion. A control solution was prepared with 10% KOH for every batch of beakers to test for any

additional contamination. The beakers were labeled and covered with aluminum foil and placed in an oscillation incubator at 60 degrees Celsius to incubate overnight (Rochman et al., 2015). The seawater samples were not treated with KOH. They were filtered as is.

2.4 Filtration

2.4.1 Assembly

All the glassware used in filtration was rinsed and wiped with Milli-Q water before the filtration. A conical borosilicate glass flask was used, onto which a filter paper holder funnel was affixed with the help of a silicone stopper. The base conical flask had an integral vacuum connection placed above the filtrate drip to avoid contaminating the vacuum line from the droplets. An oil-free vacuum pump was at the end of the vacuum connection. A millipore membrane filter of 0.45-µm pore size and diameter 47mm was placed on the filter with the help of forceps to collect the MPs. A scaled funnel was placed on the filter and secured with an anodized aluminum spring clamp to facilitate vacuum pressure (Figure 2.2).

2.4.2 Processing

The vacuum pump was turned on, and Milli-Q water was poured on the filter paper initially to create a vacuum. Then the digested solution was poured onto the wet filter paper. After emptying the solution, the beaker was rinsed twice to ensure that no MPs were retained inside the beaker. Also, water was poured through the walls of the scaled funnel. The scaled funnel was covered with aluminum foil to avoid contamination through the air. A glass petri plate was cleaned, labeled, and kept aside. When the filtration was done, the filter paper was placed inside the petri plate with the help of forceps. The petri plate was placed in the oven at 50 degrees Celsius for 2 hours to dry the filter paper. The dried petri plates were stored in a box for microscopic analysis. For two species of each trophic level, the filtrate was collected and again poured through a millipore membrane filter of 0.22-µm pore size and diameter of 47mm. This was done to check if MPs smaller than the size 0.45-µm were present in the solution. The other sample solutions, control solutions, and seawater samples were filtered using the same process. After every batch of sample solutions, the apparatus

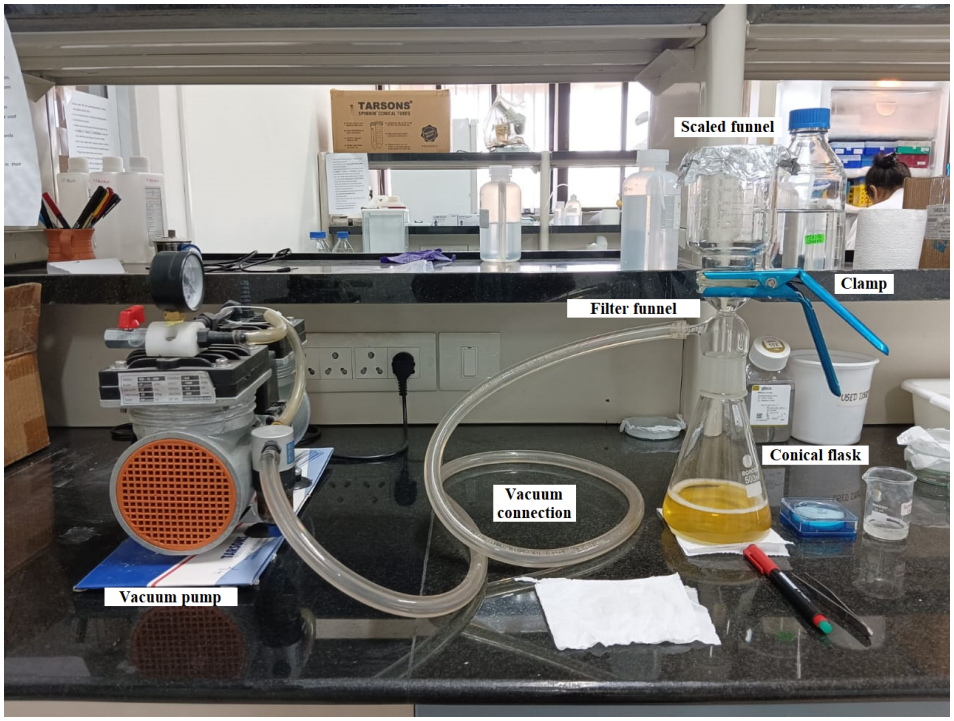

Figure 2.2: Filtration apparatus setup

was thoroughly cleaned, wiped, and stored.

2.5 Microscopic analysis

The dried filter papers were examined using a stereo microscope (Leica EC3) at 10x and 40x resolutions to detect MPs. A visual assessment was done to identify the color and type of MPs. If the MPs were observed, they were counted and categorized according to their color (Red, Black, Blue, Transparent, etc.) and type (Fibers, Fragments, and Films). The total number of MPs was noted for every sample of gut and liver. The control petri plates were also visualized by the same process.

2.6 Statistical Analysis

The type of tissue (gut, liver, and whole body), tissue weight, the individual's trophic level (TL), sampling location, type, and number of MPs was compiled into a dataset. The total number of MP particles was noted for individuals at each trophic level. Since there was a significant difference between the gut and liver weight, the total number of MP particles per individual per gram was calculated for standardization. MP particles per individual per gram is the sampling unit used for further analysis. Similarly, for the seawater samples, the total number of MPs per volume was calculated for standardization.

I used a negative binomial generalized linear model (GLM) to model the abundance of MPs in the gut and liver of TL1 and TL2 across both locations (for all objectives). The coefficient values were reported after being back-transformed using a tidy function from the broom package in R (Silge and Robinson, 2016). For the comparison of the gut and liver of TL1 and TL2, the whole body data were excluded. The liver data were only used for bioaccumulation analysis (Figure 3.6). For the rest of the analyses, only gut data from sampled species were used. All analyses were conducted in the R (Thomas, 2015) statistical software (version 4.2.2).

Chapter 3

Results

3.1 Diversity of MPs at the study sites

A total of 125 individuals from Malvan, Maharashtra, and Kochi, Kerala, were processed. The abundance and diversity of MPs in the gut and liver of the sampled individuals across different trophic levels were examined for both sites. Broadly, three types of MP - fibers, fragments, and films were observed at both sites. The most common type was fibers, followed by fragments and then films (Figure 3.1 and Figure 3.2). Fibers were the most diverse, observed in various colors, such as black, transparent, red, blue, green, grey, black transparent mix, and blue transparent mix (Figure 3.1).

Transparent fibers were the most common, followed by black, irrespective of the trophic level or location (Table 3.1)

3.1.1 Concentration of MPs in seawater samples near Malvan

The overall abundance of MPs in seawater samples was 0.46 MP/ml, including sixty-four fibers (0.42 MP/ml), four fragments (0.026 MP/ml), and one film (0.006 MP/ml). Transparent fibers were most abundant in the seawater samples, followed by black (Table 3.2).

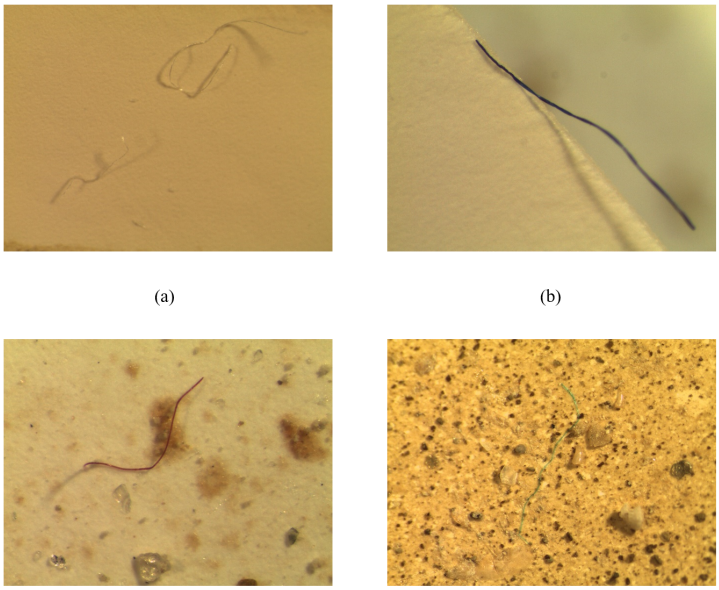

Figure 3.1: Representative images of types of fibers [(a) Transparent fibers, (b) Black fiber, (c) Red fiber, (d) Green fiber] observed on the filter papers.

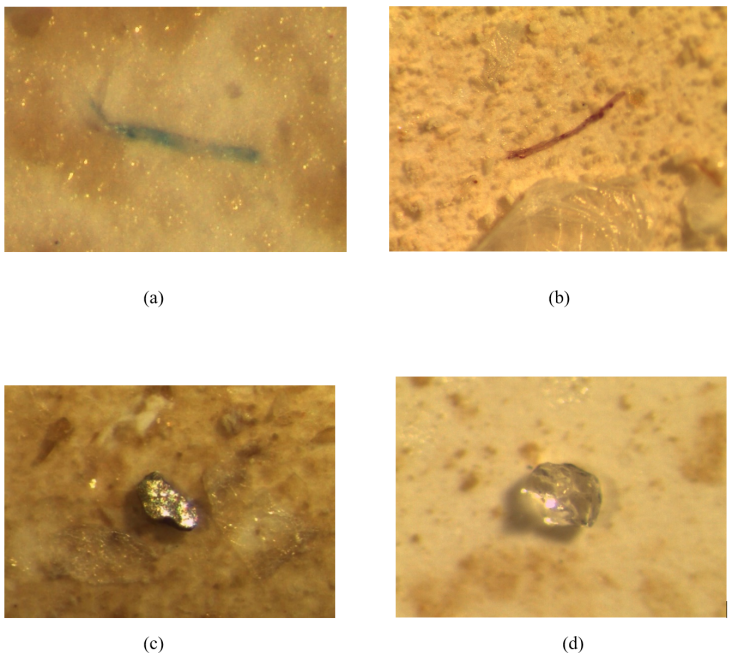

Figure 3.2: Representative images of types of fragments and films [(a) Blue fragment, (b) Red fragment, (c) Black-shiny film, (d) Transparent film] observed on the filter papers

	TL1		TL2	
	Malvan	Kochi	Malvan	Kochi
Black	101	90	72	92
Transparent	189	190	312	208
Red	64	9	13	14
Blue	13	4	2	7
Green	6	0	1	0
Grey	8	4	2	4
Black+Transparent	0	3	4	4
Blue+Transparent	1	2	4	5

Table 3.1: Types of MPs in each trophic level (TL) observed at two locations

Types of fibers	Black	Transparent	Red	Blue
Total Number	11	45	7	1

Table 3.2: Types of MP fibers in seawater samples from Malvan, Maharashtra

3.2 Benthic v/s pelagic feeders

Indian anchovy showed the maximum MP concentration, whereas Tonguefish and Goldstripe sardinella showed almost no MP occurrences. Among the pelagic feeders, Indian anchovy and Indian oil sardines showed a maximum MP concentration than other species (Figure 3.3). Prawns and Bamboo sharks showed minimal MP occurrence in benthic feeders, with no traces of MP in Tonguefish. For the habitat comparison, the pelagic feeders (8.37 ± 10.96 MP/gram, $p = 0.0352$, $Z_{119} = 2.11$, $\beta = 1.65 \pm 0.238$) showed a significantly higher MP concentration than the benthic feeders (5.07 ± 5.6 MP/gram, Figure 3.4).

3.3 Concentration of MPs across locations

For TL1, the difference in MP concentration between Kochi (10.03 ± 13.31 MP/gram) and Malvan (8.69 ± 9.82 MP/gram, $p = 0.628$, $Z_{75} = -0.485$, $\beta = 0.867 \pm 0.295$) was not significant. Similarly, for TL2, the difference in MP concentration between Kochi (4.71 ± 3.40 MP/gram) and Malvan (3.47 ± 2.70 MP/gram, $p = 0.16$, $Z_{42} = -1.41$, $\beta = 0.736 \pm 0.218$, Figure 3.5) was not significant.

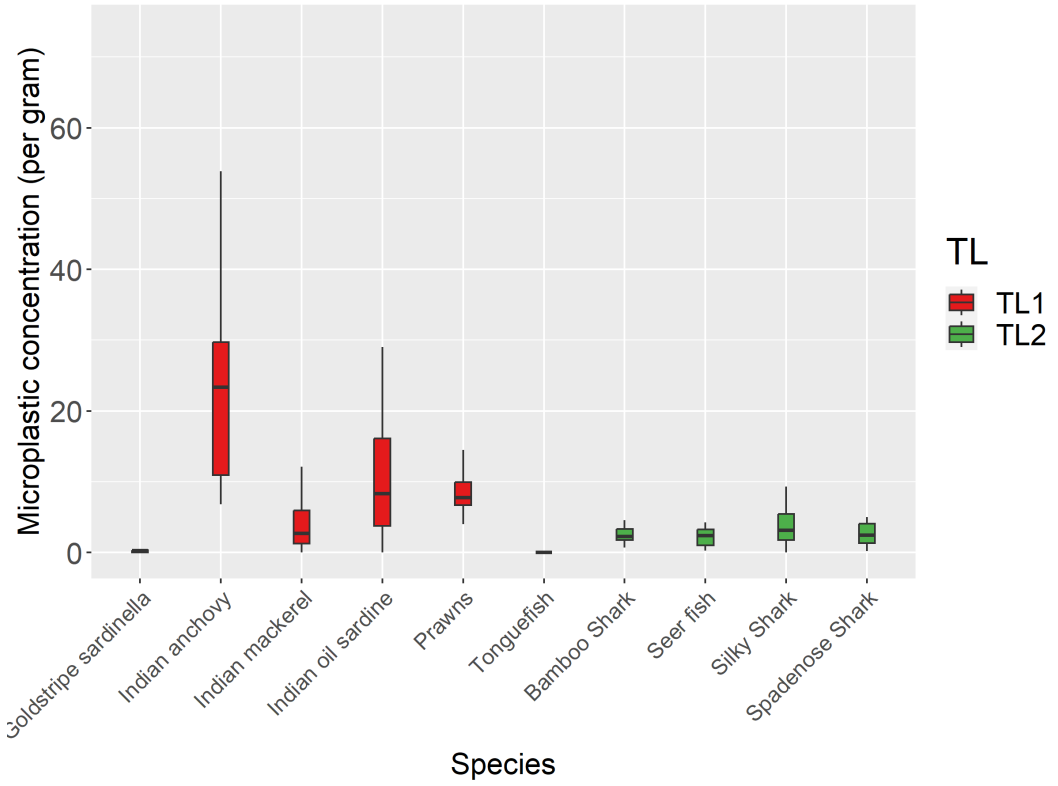

Figure 3.3: Comparison of MP concentration in all sampled species

3.4 Concentration of MPs across gut and liver tissue

Gut and liver tissues were sampled from two species of TL1 (Indian oil sardines and Indian mackerel) and TL2 (spadenose sharks, bamboo sharks, silky sharks, and seer fish). The data for Malvan and Kochi were combined to examine the MP concentration differences between TL and tissue type (Figure 3.6). In TL1, the liver ($p = 0.005$, $Z_{72} = 2.81$, $\beta = 2.08 \pm 0.261$) showed a significantly higher MP concentration than gut. In TL2, the MP concentration was significantly higher for the gut than the liver ($p = 0.00611$, $Z_{86} = -2.74$, $\beta = 0.652 \pm 0.156$).

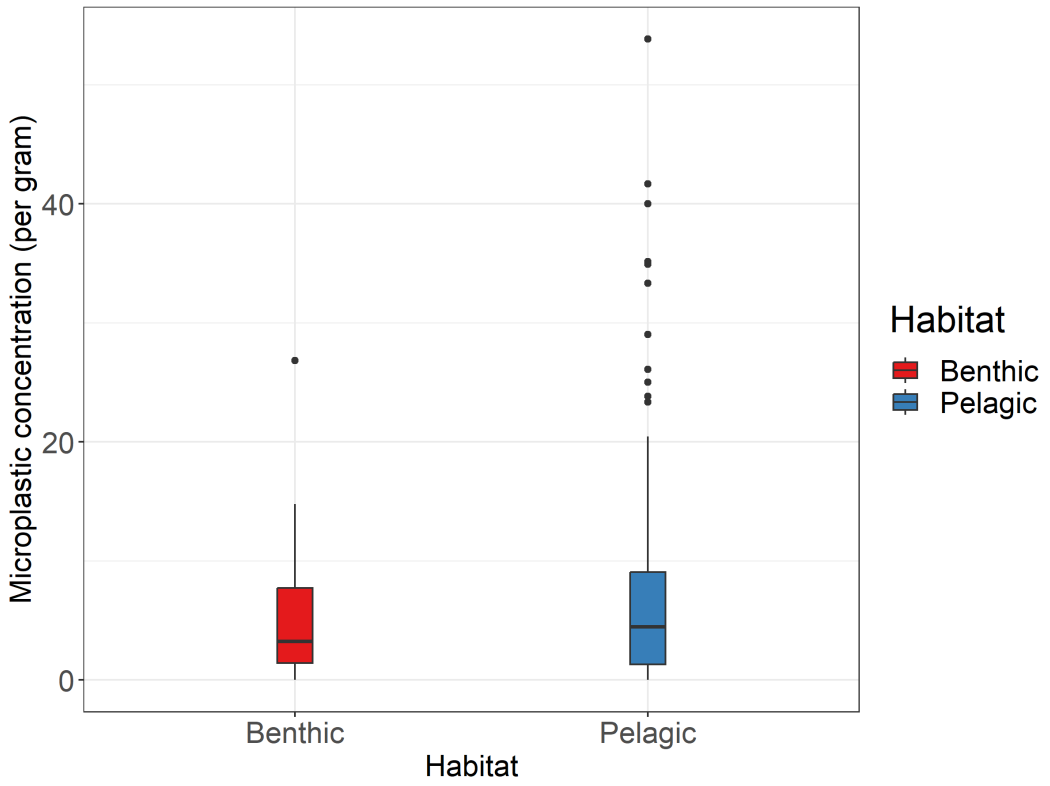

Figure 3.4: Comparison of MP concentration between benthic and pelagic feeding species

3.5 Biomagnification

In Malvan, TL1 had the highest MP concentration (8.69 ± 9.82 MP/gram), followed by TL2 (3.47 ± 2.70 MP/gram) (Figure 7). The MP concentration was significantly higher in TL1 than TL2 ($p = 0.00168$, $Z_{59} = -3.14$, $\beta = 0.399 \pm 0.292$). In Kochi, TL1 had the highest MP concentration (10.03 ± 13.31 MP/gram), followed by TL2 (4.71 ± 3.40 MP/gram) (Figure 3.5). The MP concentration was significantly higher in TL1 than TL2 ($p = 0.0173$, $Z_{58} = -2.38$, $\beta = 0.470 \pm 0.317$).

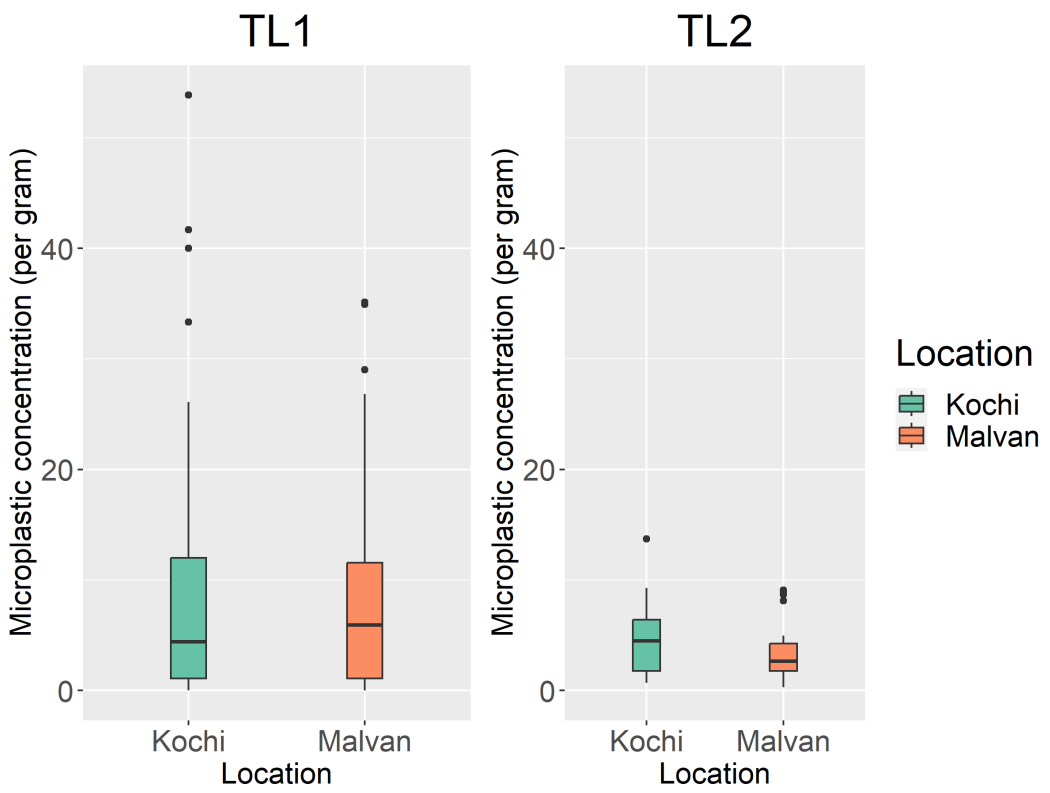

Figure 3.5: Geographical comparison of MPs in trophic levels

3.6 Filtration through 0.22-µm pore-size filter

A 0.22-µm pore-sized membrane was used to filter the filtrate to study the MP's size variation to check the presence of MPs smaller than 0.45-µm. From Malvan, two samples from TL2 were filtered. The MP concentration per gram was 2.35 and 5.96 MP/gram for the gut and 0.60 and 2.73 MP/gram for the liver. Four individuals from each trophic level from Kochi were filtered. The MP concentration per gram for TL1 was 15, 19.05, 10.96, and 4.35 MP/gram. The MP concentration per gram for Indian mackerel and Indian oil sardine of TL1 was 1.06, 5.11, 3.27, 0.26 MP/gram for the gut and 9.52, 5.45, 1.95, and 6.06 MP/gram for the liver. The MP concentration per gram for TL2 was 4.96, 5.21, 3.61, 1.48 MP/gram for the gut and 1.86, 2.55, 3.47, and 0 MP/gram for the liver. Fibers were the predominant type of MP from the gut and liver from both locations.

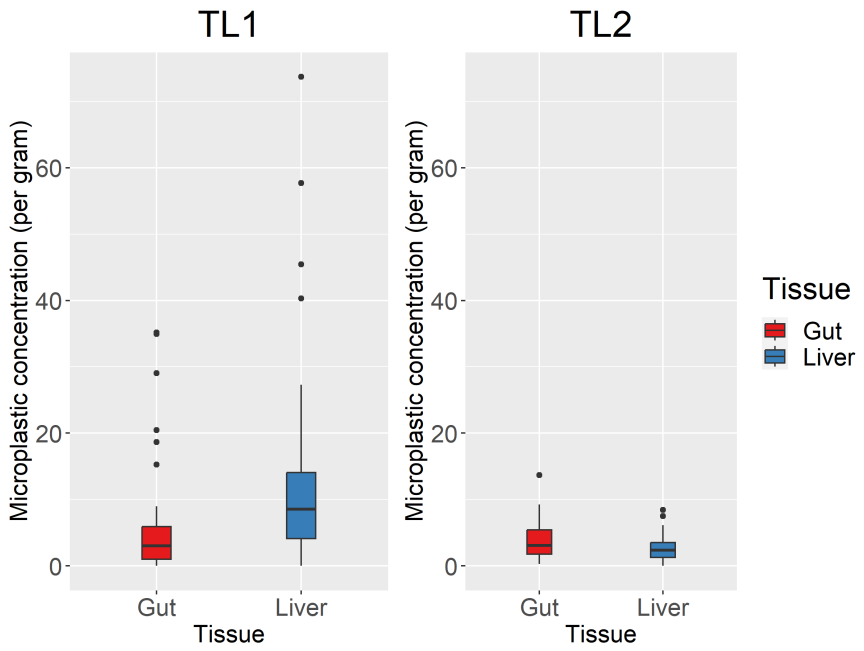

Figure 3.6: Comparison of MP concentration between gut and liver of sampled species.

Chapter 4

Discussion

Plastic production has become a worldwide concern due to its accumulation in water bodies, posing a threat to marine organisms. Physical and chemical stress on plastics breaks them down into smaller particles, known as microplastics, which accumulate in the water column and get ingested by zooplankton (Rummel et al., 2016), (Tanaka and Takada, 2016), (Derraik, 2002), the primary consumers in the marine food chain. MPs then transfer to higher trophic levels, causing damage to fish gastrointestinal tracts and potentially altering their feeding behaviors. The study aimed to investigate the bioaccumulation of MPs in marine organisms across different locations over their lifetimes.

Three types of MPs, namely fibers, fragments, and films, were present in both the water samples and the sampled species. The most abundant type of MP observed was transparent fibers, which are mainly derived from degraded fishing lines and nets, indicating that fishing activity is a significant contributor to MPs in the sampled locations. Pelagic feeders showed a significantly higher concentration of MPs than benthic feeders. There was no significant difference in MP concentration between the samples collected from Malvan and Kochi. MPs were found in the gut and liver of the sampled individuals, and the liver showed a significantly higher concentration of MPs than the gut in primary consumers (TL1). In contrast, secondary consumers (TL2) showed a significantly higher concentration of MPs in the gut than in the liver. The study did not find any evidence of biomagnification in samples from either location.

4.1 Diversity and abundance of MPs

After microscopic analysis, three types of MPs were observed in both tissues: fibers – filament-like structures, fragments – irregularly shaped and generally separated from a larger plastic item, and films – flexible sheet-like structures. Fibers were the most abundant in both tissues across the locations. One of the possible reasons behind this could be that fibers have the highest surface area-to-volume ratio among the observed types of MPs. A higher surface area to volume ratio implies a higher density (Kooi and Koelmans, 2019). As a result, fibers could be suspended in the lower column of seawater, closer to the benthic surface. This vertical distribution of MPs in the water column would increase the chances of fibers getting consumed by the deposit and benthic feeders. These feeding behaviors are generally observed in prawns/shrimps, crabs, polychaetes, and mollusks. These species are typically consumed by the fish species at a higher trophic level. Hence, the transfer of fibers would occur through trophic levels, and fibers would be more common than the other MPs. Another reason could be the smaller size of fibers compared to other MPs. Due to their small size, they can easily pass through the tissue membranes of marine organisms. Hence, fibers could enter the bloodstream through the gut and accumulate in various tissues over the lifetime. These accumulated fibers would then transfer across trophic levels through predation.

The fibers exhibited the widest variety in colors and appearance from the observed MPs, as shown in Table 3.1. It can be seen that transparent fibers were most abundant in the sampled individuals. Transparent fibers are primarily derived from broken fishing nets, whereas colored fibers are derived from the fragmentation of plastic commodities (Stolte et al., 2015). From this observation, it could be inferred that the primary source of MPs near the sampling locations could be fishing lines and nets.

Table 3.2 represents the number of MP particles in the seawater sample collected near Malvan. It was observed that transparent fibers were the most abundant in the water sample, followed by black, red, and blue fibers. This abundance trend matches the pattern observed for fibers in sampled individuals' gut and liver, irrespective of the location. From this observation, it could be implied that the introduction of fibers in the food chain would most probably be non-selective based on their color. The MP introduction could be accidental, or their appearance and color could be irrelevant for ingestion.

Similar to this study, (Horton et al., 2018) found fibers to be the most abundant type of

MP, followed by fragments and films. (Bellas et al., 2016) also detected fibers as the most abundant type and black as the most abundant color of MPs. On the contrary, (Adika et al., 2020) reported high occurrences of microbeads, pellets, and burnt films compared to fibers.

4.2 MP variation with feeding habitat

The depth at which MPs are suspended in the ocean depends on various factors such as the surface area-to-volume ratio, shape, size, and density of the MP particle. The suspension depth could be linked to MP ingestion based on the feeding depth of marine organisms. In this study, the species were categorized as benthic and pelagic feeders based on their feeding habitats. Table 2.1 represents the feeding habitats of sampled species. I observed significantly higher MP concentration in pelagic feeders than in benthic feeding species (Figure 3.3). The difference in MP concentration between benthic and pelagic could be associated with their different feeding habits and residing habitats. The presence of MPs in pelagic species could be due to their filter-feeding behavior, which results in the ingestion of floating MPs (Rummel et al., 2016). The reason for this could be attributed to the fact that MPs are more available for ingesting due to their buoyancy and widespread distribution in water columns. On the other hand, benthic species can ingest MPs unintentionally by ingesting sediment particles or by consuming organisms that have already ingested MPs. Benthic species may not be exposed to MPs because of the lower density of MP particles.

(Jovanović, 2017) reported higher MP ingestion by pelagic fish than benthic fish, which matches the result of this study. Many studies contrasted with the results of this study. (Bessa et al., 2018) observed higher occurrences of MPs in benthic fish compared to pelagic fish, while (Neves et al., 2015), (Filgueiras et al., 2020), and (Lusher et al., 2013) found no significant correlation between MP ingestion and fish habitat.

4.3 Geographical comparison of MP concentration

Anthropogenic activities highly influence MP concentration in the ocean (Browne et al., 2011), (Cole et al., 2011), (Barboza and Gimenez, 2015). MP concentration could differ between geographical locations due to the difference in anthropogenic activity in the ocean.

MPs would most likely be in high concentration near sites with high fishing activity, plastic waste, and industrial waste. For this study, samples were collected from two distant locations on India's west coast: Malvan and Kochi. In both trophic levels, the overall observed MP concentration was not significantly different between Kochi and Malvan samples (Figure 3.5). The possible explanation behind this could be a similar concentration of MP in seawater near both sites. Assuming similar fishing pressure between Kochi and Malvan, a higher industrial activity near Kochi did not contribute significantly to the MP concentration in the samples. An alternate explanation could be a higher fishing activity near Malvan, balanced by the industrial pressure near Kochi, to obtain similar MP concentrations in the samples.

4.4 Bioaccumulation

Once the MPs are ingested by an individual, they could be trapped in the gut, could be absorbed through the gut membrane to enter the bloodstream, or could be excreted out. The MPs in the gut could also be present due to the recent feeding events, and these MPs could have been excreted if the individual had not been captured. Hence, the MPs in the gut are not a reliable indicator of the accumulation of MPs in sampled species. In this study, bioaccumulation refers to the MPs accumulated in an organism's tissue (liver).

For TL1 samples, the MP concentration was significantly higher in the liver than in the gut (Figure 3.6). This result indicates a high degree of bioaccumulation in TL1 individuals. The possible explanation behind this observation could be the potentially high permeability of the gut membrane in these species. While sampling, it was observed that the two sampled species in TL1: Indian oil sardines and Indian mackerel, had thin and delicate gut membranes. A thin gut membrane could facilitate the absorption of MPs through the gut membrane and into the bloodstream. The thin gut membrane would also reduce the likelihood of MP particles getting trapped in the gut. Once the MPs enter the bloodstream, they can travel to different organs via circulation. The liver is vital in detoxifying blood as it acts as a blood filter. The absorbed MPs through the gut could enter the liver and get trapped. These suspended MPs can then accumulate in the liver over the lifetime of the fish. Assuming this implication to be true, the most plausible reason behind higher MP concentration in the liver could be the thin gut membranes of the sampled species in TL1.

For TL2 samples, the MP concentration was significantly higher in the gut than in the

liver (Figure 3.6). This result indicates a low degree of bioaccumulation in TL2 individuals. The possible reason behind this observation could be the thick and rigid gut membranes of sampled sharks and seer fish. The thick gut membrane could inhibit the passage of MPs into the bloodstream. As a result, the accumulation of MPs in various tissues of these species would be limited. In addition, a thick gut membrane would increase the likelihood of MPs getting trapped in the gut. Since most of the gut content gets egested through feces, the overall MP concentration in these individuals could be low.

(Boerger et al., 2010) found that, on average, the larger fish contained more plastic pieces in their gastrointestinal tracts than the smaller fish. (Huang et al., 2020) showed that the majority of MPs accumulated in the fish were found in the intestine (47%), followed by the stomach (30%) and the gills (23%), but none of the MPs were detected in the fish's muscles or liver.

4.5 Biomagnification

Bioaccumulation of MPs could also facilitate the increase in MP concentration at higher trophic levels, as MPs can be transformed across trophic levels through predation. In this study, biomagnification refers to the increasing MP concentration from lower to higher trophic levels. One would expect increasing MP concentration from lower to higher trophic levels in an ecosystem with high MP bioaccumulation at every trophic level.

In this study, MPs in TL1 and TL2 individuals were examined to check for biomagnification at both locations. For both locations, the MP concentration in TL1 individuals was significantly higher than in TL2 individuals (Figure 3.5). This result indicates the absence of biomagnification in samples from both locations.

The most plausible explanation behind this result could be the low degree of bioaccumulation in the individuals at the higher trophic level. TL2 individuals exhibited low MP concentration in the liver than gut (Figure 3.5), which indicates low bioaccumulation. Low bioaccumulation could imply a higher excretion rate of MPs from the gut and a lower rate of MP absorption in the bloodstream. On the contrary, bioaccumulation was observed to be high in TL1 individuals. This decrease in the degree of bioaccumulation from the lower to higher trophic level hints towards a trend opposite to biomagnification.

Numerous research papers have found similar results as in the current study. (Covernton et al., 2021) found that the concentrations of MPs in the guts of fish and their occurrence rates were not significantly affected by their trophic level. This study found that the body size of the fish sampled had a negligible impact on the average MP concentrations in their digestive tracts, which suggests that biomagnification of MPs did not occur in the sampled individuals. (Güven et al., 2017) found no significant correlation between the number of ingested microplastic particles and trophic index, fish length, or fish mass. (Chan et al., 2019), (de Vries et al., 2020) revealed no correlation between the abundance of microplastics found in each fish and the fish's total length or the stomach's weight.

4.6 Study limitations and future prospects

The broad limitations to conducting this study were the unavailability of the same species at both locations, a high overlap in prey composition of sampled species, and the longer time required for filtration due to the small pore-size membrane. The study could be improved by sampling similar species from both locations, as it would give a clearer picture of the difference in MP accumulation between the sites. Since the sampled species were decided opportunistically in Malvan, the same species could not be collected in Kochi in the short sampling period.

The overlap between the diet of the sampled species is also considerably high. This overlap weakens the trophic level structure, so studying the transfer of MPs across the food web becomes challenging. Sampling the species with low overlap between their prey composition would be beneficial to categorize them in different trophic levels.

The MPs are filtered using a membrane that separates them from residuals. If the membrane's pore size is smaller, then the MPs observed after filtration will be finer in size. But there is a trade-off of time required for filtration and the membrane's pore size. For the 0.45 µm filter paper, on average, 30 minutes were required for a single sample, whereas for the 0.22 µm filter paper, around 3-4 hours were required for a single sample. Hence, considering the number of samples and the time constraints of this study, 0.45 µm filter paper was used. The study could be improved using smaller pore-size membranes, given the required time available for filtration.

4.7 Conclusion

Plastic pollution is a rapidly growing global issue that poses a significant threat to marine organisms. In particular, MPs are known to negatively impact the biological processes of these organisms when ingested. Despite that, there is still much to learn about how MPs accumulate in the food web and how they biomagnify at different trophic levels. Research on this topic along the west coast of India remains limited, and the development of baseline data on marine ecosystems in this region is still in its early stages. This is primarily due to a lack of research and records on marine systems, which can be attributed to the significant challenges associated with studying these complex and dynamic ecosystems. Gathering high-quality data that covers a wide range of taxa over time is particularly challenging for marine ecosystem studies. This challenge becomes even more complex when studies need to be conducted across various regions to examine similarities and differences between systems over time. However, it is essential to explore marine ecosystems more deeply, given their impact on the entire globe and the rapid changes they are undergoing due to human interventions. The broad objective of this study was to investigate the effects of human activities on marine food webs along the west coast of India by analyzing the presence of MPs in the tissue of a select few species. Studying the MPs in the tissues of marine organisms is a good way to learn the effects of plastic pollution on these ecosystems. Understanding the presence and distribution of MPs in marine food webs can provide crucial insights into the potential risks they pose to human health through the consumption of contaminated seafood. Thus, investigating the effects of anthropogenic activities on marine ecosystems is essential for developing effective management strategies to mitigate the impacts of plastic pollution on these fragile ecosystems.

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