Research Article

International Journal of Research in Organic Agriculture
Volume 1, Issue 1, Year 2025

Isolation and prevalence of microplastic contamination in Seabass (*Lates calcarifer*) from the Bay of Bengal

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Received: 25-06-2025 Manuscript No. JQR/IJROA/13; Editor Assigned: 26-06-2025, Manuscript No. JQR/IJROA/13; Reviewed: 25-07-2025, Manuscript No. JQR/IJROA/13; Published: 31-08-2025

Abstract:

The detrimental effects that microplastics (MPs) have on marine life have made them a serious issue as aquatic pollutants. This study looks at the presence and attributes of MPs in the muscle and digestive systems of 25 sea bass (Lates calcarifer) from the Bay of Bengal. MPs were detected in 88% of the fish that were analyzed, indicating that the area is heavily polluted. Several nets were used to catch sea bass weighing between 170 and 310.8 g. MPs counts rose with fish size, from 11.2±4.6 and 0.4±0.5 (T1:122-200 g) to 64.2±3.8 and 20.6±1.1 (T5:299-328 g) particles per individual for sea bass muscle and gastrointestinal tracts (GIT), respectively. Of the six colors of MPs found, the most common in the seabass's GIT and muscle were green (34.1% and 33%) and blue (31.6% and 27%). In the sea bass's muscle (91%) and GIT (94.9%), fibers were the most common form of microplastic. The majority of MPs were less than 500 µm, accounting for 76% of the fish's GIT and 79% of its muscle, respectively. Polyamide and polyethylene made up the majority of MPs, according to FTIR studies, with minor levels of polyethylene terephthalate and polyvinyl pyrrolidone. The significant concentration of MPs discovered in a fish species that is essential to the fisheries emphasizes the need for environmental and health remedies. According to the study's findings, MPs are common in fish, and further research is needed to identify the factors influencing the incidence and risk assessment of MPs in fish in the Bay of Bengal.

Keywords: Muscle; Gastrointestinal Tract; Microplastics; Potential Human Health Impact.

1. Introduction

The manufacturing of plastic has increased over the past few decades because of inadequate waste management procedures in many regions of the world (Lusher et al., 2017a). Particularly, coastal nations produced over 275 million tons of plastic waste, of which it is believed that 2-5% ended up in the oceans (Jambeck et al., 2015). Because of their extreme persistence, marine plastic debris is expected to negatively affect not only the organismal level but also a variety of ecosystem products and services, such as fisheries, tourism, and navigation, which could have a negative influence on the economy and society (CBD, 2016). Bangladesh has a coastal area of 2.30 million ha and a coastline of 714 km along the Bay of Bengal, which supports a large artisanal and coastal fishery. Furthermore, the nation enjoys 166,000 km^2 EEZ in the Bay of Bengal. Bangladesh's fisheries industry has a wide variety of species and resource kinds. Bangladesh is home to 475 marine and 260 freshwater fish species. In the nation, about twelve foreign species are being cultivated (DoF, 2024).

Plastic particles having a diameter of 5 mm or less are referred to as MPs because they gradually break down into smaller pieces in the marine environment due to weathering processes such as photo-degradation, oxidation, and mechanical abrasion (Andrady, 2011). The environment is also exposed to MPs from a variety of sources, including personal care and cosmetics items including lotion, shampoo, toothpaste, shower gel, lipstick, hair dye, shaving cream, and eye shadow (Fendall and Sewell, 2009), and from doing laundry

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(Browne et al., 2011). MPs, which can spread through food webs are dangerous to organisms (Ivar do Sul and Costa, 2014), and are detected in zooplankton, bivalves, crustaceans, corals, fishes and seabirds (Li et al., 2018; Zhao et al., 2016) from different regions of the world.

MPs are hence active elements in trophic interactions that could accumulate in greater concentrations in top predators like marine mammals (Deudero and Alomar, 2015) and humans (Crawford and Quinn, 2017). MPs harm living things in a variety of ways, including by causing physical harm and inflammation, obstructing the digestive tract, changing feeding and reproductive behavior, lowering progeny survival rates, and reducing immune response (Savoca et al., 2019).

One of the most well-known tourist destinations worldwide is Cox's Bazar, which boasts the longest seashore in the world. Due to inadequate waste management, a significant number of plastic bottles brought by visitors are discarded into the Bay of Bengal each year. Once these bottles break down, the plastic particles find their way into the marine species' food web. To improve our understanding, more research is required on the routes that MPs take within marine food webs. High-specific density MPs are more likely to assemble on the ocean floor, while low-density items are dominant at the sea surface (Andrady, 2011). Fish regularly absorb MPs because of feeding in the water column (Boerger et al., 2010). As an illustration, mesopelagic fishes in the North Pacific Subtropical Gyre consumed plastic fibers, filaments, and films. Commonly referred to as Asian seabass or giant sea perch, *L. calcarifer* (Bloch, 1790) is a food fish of economic significance in the tropical and subtropical regions of the Asia-Pacific region. They are medium- to large-sized fish that live on the bottom and can be found in coastal seas, lagoons, and estuaries that range in depth from 10 to 50 meters. These fishes, which are carnivorous and demersal, eat mollusks, crustaceans, zooplankton, and smaller fish. Artisanal fishermen are the primary catchers of these highly prized sport and culinary fish. It has become a desirable commodity for both large and small aquaculture firms due to its comparatively high market value. It is significant as a game fish as well as a commercial and subsistence food fish (Mathew, 2009). This study was conducted to identify, abundance and morphotype of MPs in muscle and Gastrointestinal tract (GIT) of seabass (*L. calcarifer*) from the Bay of Bengal.

2. Materials and Methods

Fish sample collection and processing

For the purpose of this research, *L. calcarifer* samples were obtained from Cox's Bazar Fishery Ghat during January and March 2023. These samples were caught by using different fishing gears e.g., gill nets, set beg nets, and cast nets from Cox's bazar area of the Bay of Bengal. A total of 25 specimens were used for the experiment. The collected fish were preserved in an icebox and then transported to the Marine and Coastal Resources Management Laboratory of the Department of Coastal and Marine Fisheries under Sylhet Agricultural University, Sylhet for examination. This analysis was conducted within two weeks of collection, and the specimens were stored at -20°C for MPs analysis. In the laboratory, Fish were washed with tap and double distilled water and basic measurements such as total length and body weight were taken for each specimen and then prepared by eviscerating and beheading. To investigate the prevalence of MPs ingestion in fish, the fishes were treated in five treatments based on weight: T_1, T_2, T_3, T_4 and T_5. Five specimens (× 5 replicates) of similar sizes to the selected species were combined. Subsequently, the specimens were dissected individually in a metal tray using appropriate tools like scissors, scalpel, and forceps, and GIT was carefully extracted, weighed, and placed individually into the glass beakers. (Hossain et al., 2019) Following that, a knife was used to fillet the samples, and a previously cleaned meat grinder was used to homogenize the muscles. After being dried at room temperature, the homogenized fish muscles were crushed in a blender.

Hydrogen peroxide treatment

MPs were extracted from fish muscles using the method recommended by Karami et al. (2017). Therefore, 10 g of homogenized fish were submerged in 100 ml of 10% (w/v) KOH for 48 hours at 40°C. The residue was cleaned with pure distilled water to get rid of any leftover KOH after it had passed through grade 589/3 blue ribbon S&S quantitative filter paper (pore size <2 mm). Before being analyzed further, the filter paper that contained the MPs was allowed to air dry. The goal of this process was to maintain all forms of MPs with varying densities; hence density separation was not performed because fishbone remnants on filter paper are minimal. Depending on the weight of the soft tissue in each beaker, 100 mL of 30% H_2O_2 was added to each beaker to break down the organic matter in order to extract MPs from the GIT (Li et al., 2015). The beakers were covered, heated to 80°C for 6 hours, and then allowed to rest at room temperature for a further 24 to 48 hours, depending on how well the soft tissue digested.

Saline (NaCl) solution floatation and filtration

According to earlier research (HidalgoRuz et al., 2012; Li et al., 2015), MPs were extracted from the dissolved liquid of soft tissues by flotation using a saline solution (1.2 g/mL NaCl). About 200 mL of filtered NaCl solution was added to each beaker, and the samples were allowed to sit at room temperature for at least two weeks. By allowing undissolved organic residues and inorganic materials to sink to

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the bottom of the beaker and less dense particles, including MPs, to float to the top, the saturated salt solution made the separation process easier (Lusher et al., 2017b). The samples were then passed through a 45 µm stainless steel filter, and the residue that was left behind was back-washed with double-distilled water onto a sterile petri dish and stored in aluminium foil paper for additional examination (Li et al., 2018). Every experimental technique was carried out between 4 and 6 weeks after the samples were first processed.

Microplastic identification and characterization

For precision and repeatability, MPs were extracted from GIT contents at the Bangladesh Oceanographic Research Institute (BORI), Cox's Bazar. A few pre-treatment procedures, such as filtering, density separation, and digesting, were applied to the contents in order to separate the microplastic particles from the organic material and waste. Microscopy methods like stereomicroscopy and microscopy were used to identify and measure the presence of MPs in separated particles. Size, shape, color, and texture were among the morphological traits recorded for every microplastic particle. The polymer composition of MPs was determined using micro-Fourier transform infrared spectroscopy (µ-FTIR). Understanding the kinds of plastics that sea bass consume, and their possible origins were made possible by FTIR analysis. An ocular scale calibrated, and a stage micrometer scale were used to measure the MPs' diameters.

Statistical analysis

Statistical methods were used to examine the data gathered from fish biometric assessments, microplastic abundance, and polymer composition. ANOVA and other parametric statistical analysis were used because the data showed a normal distribution. The abundance of MPs throughout the five treatments of each species was compared using one-way ANOVA, and the association between microplastic abundance and fish characteristics (e.g., body weight and GIT weight) was investigated using linear regression analysis. Additionally, the Pearson test of independence of this species was used to examine the form, color pattern, kind, and size of MPs. The IBM SPSS 27 program was used for all statistical analyses.

Contamination control

Throughout the investigation, strict quality control procedures were put in place to guarantee the precision and dependability of the findings. Samples were collected, prepared, and analyzed in accordance with standard operating procedures (SOPs). Cross-contamination was avoided at every stage of the study, including the collection of fish samples, transportation, preservation, thawing, washing, dissection, removal of the gastrointestinal tract, alkali digestion, NaCl floatation, and identification of MPs. Before being used, the workstation was cleaned, and sample containers were covered as needed. Samples were immediately covered while not in use, and all glassware was washed three times with filtered water. To evaluate and reduce any contamination concerns, blank samples and procedural blanks were used.

Ethical considerations

The study followed ethical standards for acceptable research practices and the humane treatment of animals. The appropriate permissions were obtained for the collection and experimentation of fish, and all procedures involving live animals were carried out in compliance with applicable laws and ethical standards.

3. Results and Discussions

For this study, 25 seabass fish from the Bay of Bengle's Cox's Bazar region were examined. All fish's digestive tracts and 22 fish's muscles contained MPs. MPs are widely distributed throughout the area, as evidenced by the study's findings that they were discovered in 100% of the GIT and 88% of the muscle of the 25 fish analyzed.

Table 1 Total length, standard length, body weight, GIT weight, no. of MPs in GIT and muscle (mean±sd) of *L. calcarifer* in different treatments.

Particulars	Treatments				
Lates calcarifer	T1(122-200g)	T2(201-226g)	T3 (227-263g)	T4 (264-298g)	T5 (299-328g)
Total length (cm)	26.7±.6^{d}	28.2±0.4^{c}	29.2±0.2^{c}	30.6±0.5^{b}	31.9±0.9^{a}
Standard length (cm)	22.9±0.6^{d}	24.2±0.4^{c}	25.2±.2^{b}	26.0 ±0.3^{b}	27.2±0.6^{a}

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Body weight (g)	170±30.2^{c}	217.8±7.8^{b}	246.2±15.8^{b}	293±4.8^{a}	310.8±12.5^{a}
GIT weight (g)	1.1±.2^{c}	1.5±0.1^{bc}	1.7±.1^{bc}	2.2±0.2^{b}	3.4±0.8^{a}
No. of MPs in GIT	11.2±4.6^{e}	25.4±4.2^{d}	37.0±3.5^{c}	51.2±4.9^{b}	64.2±3.8^{a}
No. of MPs in muscle	0.4±0.5^{e}	3.4±1.1^{d}	9.8±2.4^{c}	16.0±1.6^{b}	20.6±1.1^{a}

(Values sharing the same superscript letters within a given column in the table are considered not statistically significant ($P > 0.05$).)

MPs abundance in fish

This study provides a thorough examination of MPs in sea bass, the most often captured commercial fish species. Microplastic pieces of 945 and 251 were discovered in the sea bass's GIT and muscle, respectively. Individual sea bass had varying numbers of particles in their GIT and muscle: 11.2±4.6 and 0.40±0.5 in T1, 25.4±4.2 and 3.4±1.1 in T2, 37.0±3.5 and 9.8±2.4 in T3, 51.2±4.9 and 16.0±1.6 in T4, and 64.2±3.8 and 20.6±1.1 in T5 (Table 1).

Plastic particles were found in the seabass's muscle and GIT during this investigation. Our findings supported those of a few other studies (Thushari et al., 2017; Klangnurak and Chunniyom, 2020; Wang et al., 2020) by confirming the presence of MPs in seabass and identifying the Bay of Bengal's marine environment as the most likely source of contaminants. The average number of plastic bits in the GIT and muscle of each fish in our investigation was 37.8±4.23 and 10.04±7.81 pieces, respectively. According to Srisiri et al. (2024), plastics were identified in the gastrointestinal system of 46.9% of the marine food fishes examined from the upper Gulf of Thailand (GoT). Azad et al. (2018) found a high contamination ratio of 54% in Songkhla province, however Klangnurak and Chunniyom (2020) reported a very low ratio of 7.76–9.92% from three coastal provinces of the GoT. According to the majority of previously published research conducted worldwide, the average number of microplastic particles per individual fish was between one and two pieces or fewer (Lusher et al., 2017a).

Amount of MPs by color

As shown in Fig. 1, six distinct colors of MPs were discovered in the muscular and digestive tracts of sea bass that were tested. Transparent, black, blue, green, red, and yellow are some of these colors. Green (34.1% and 33%) MPs were most common in the GIT and muscle of sea bass, followed by blue (31.6% and 27%), red (27.3% and 15%), transparent (4.7% and 5%), yellow (1.4% and 0%), and black (0.7% and 20%), in that order. It was shown by Bissen and Chawchai (2020) that the majority of the coastal MPs that contaminated the eastern coastline regions of Thailand were blue and white. According to Hossain et al. (2019), the tested Bombay ducks obtained from the Northern Bay of Bengal in Bangladesh had a preponderance of irregular (37–43%), white/transparent (26–68%), and fiber (50–55%) MPs among other shapes, colors, and types.

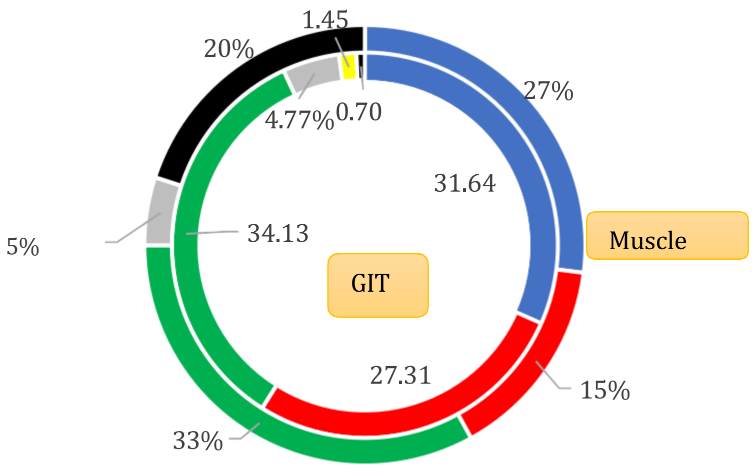

Fig. 1 Color distribution of microplastics of sea bass

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Composition of microplastic type (morphotype)

Fibers were the most common type of microplastic, making up 94.9% and 91% of the total in the seabass's GIT and muscle, respectively, according to the general distribution and characterization of the MPs in the sea bass under study (Fig. 2). The study found that other types of MPs were far less prevalent, with fragments making up 2.0% and 3% of the GIT and muscle of seabass, respectively, and films making up 3% and 5% (Fig. 3). The high fiber content is likely due to their proximity to coastal villages where fishing with various equipment, such as nets and rods, is common.

MPs are many kinds, forms, and colors of plastic pellets and components that are employed in a variety of products that are produced by runoff and widespread usage of plastic materials. The GIT and muscle of sea bass from Cox's Bazar were found to have a total of 945 and 251 MPs, respectively, in the current investigation. The study indicated that the most common type of MPs were fibers. Ryan (2013) found that plastic pieces comprised 95.5% of the floating plastic debris, whereas non-plastic elements such as wood, paper, glass, and tin comprised 4.5%. Boating and fishing, plastic components, packaging, and user items accounted for 54.6%, 30.5%, 6.3%, and 4.1% of the total.

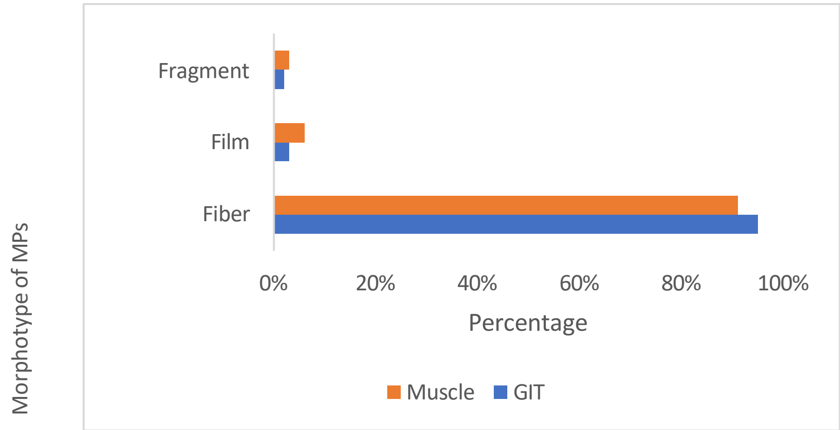

Fig. 2 Composition of MPs type in sea bass

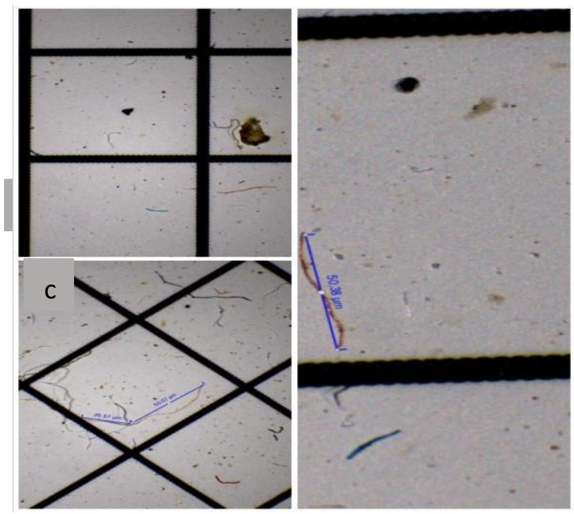

Fig. 3 Optical microscope images of MPs fragments used in this investigation (bar = 200 µm). MPs of the fragment type (a); fiber-type MPs (b and c).

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Size of MPs

Three size groups were identified in this investigation based on the dimensions of MPs (MPs) in muscle and GIT: smaller than 500µm (76% and 79%), between 500µm and 1mm (11% and 9%), and between 1mm and 5mm (13% and 12%), respectively. The variation in MP sizes across multiple species samples is depicted in Fig. 4. The smallest size class (less than 500µm) had the largest share across all samples. In line with the findings of Gago et al. (2018) and Naidoo and Glassom (2019), the fish species included in this study exhibited a high frequency of MPs < 500 µm, 500 µm-1 mm, and 1 mm-5 mm. In fish, microscopic MPs, particularly fibers, can tangle and form agglomerates that block organs and limit or stop food intake (Ziajahromi et al., 2017). By offering a greater surface area, smaller MPs with the same mass as larger MPs can also absorb more hazardous substances from the surroundings, potentially leading to synergistic hazards (Wang et al., 2019). The form and size characteristics found in the study suggest that MPs can present a range of dangers, with the prevalence of smaller MPs being associated with a higher risk.

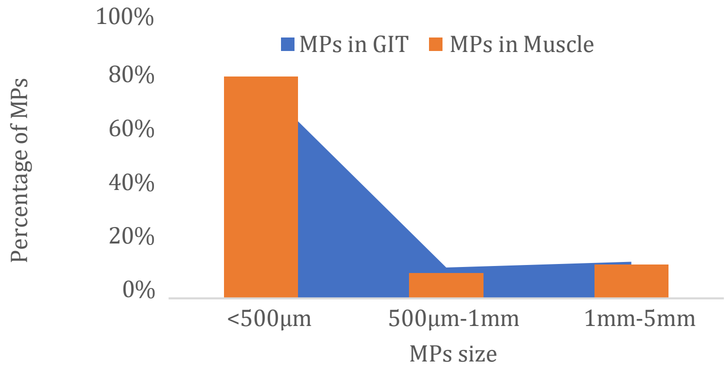
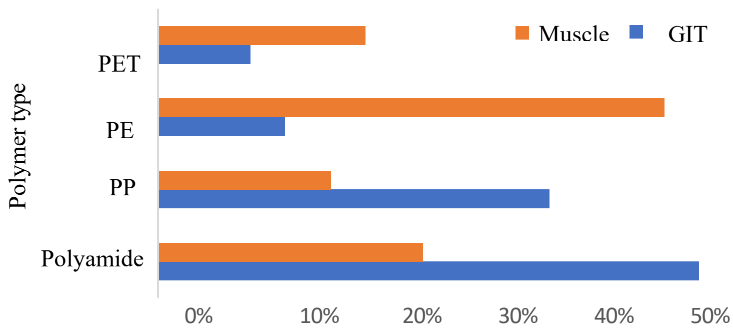


Fig. 4 Size composition of MPs in sea bass

Identification of MPs using µ-FTIR

A total of 105 particles, consisting of four different types of polymers, were found in the fish's GIT using µ-FTIR: 48 polyamide particles (47%), 36 polyvinyl pyrrolidone particles (34%), 12 polyethylene particles (11%), and 8 polyethylene terephthalate particles (8%). However, muscle included a total of 56 particles, which included four different types of polymers: 8 particles of polyvinyl pyrrolidone (15%), 10 particles of polyethylene terephthalate (18%), 13 particles of polyamide (23%), and 25 particles of polyethylene (44%) (Fig.5 & 6).

FTIR analysis revealed four distinct polymer types of MPs particles: polyamide, Polyethylene Terephthalate (PET), Polyethylene (PE), and Polypropylene (PP). PET, PP, and polyamide were the three most common forms of polymers. Organic polymer cellulose is widely used as a releasing agent in the production of fiberglass rubber and in food packaging. According to research by Gago et al. (2018), PP, PE, and PES are the most prevalent MPs in fish and the marine environment. Neves et al. (2015) found that the two most prevalent polymers in fish are PE and PP.



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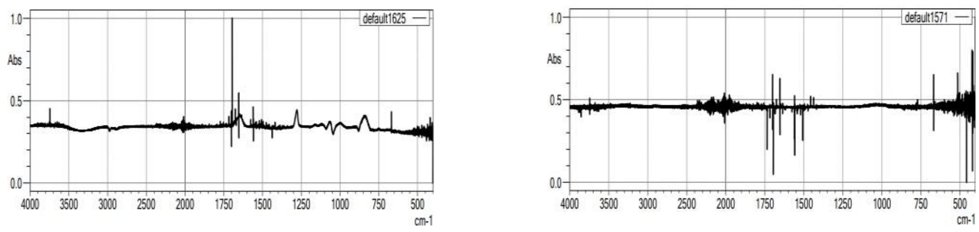
Percentage

Fig. 5 Polymer types of MPs in different samples of sea bass



(a) (b)

Fig. 6 MPs polymer μ-FTIR spectra of representative samples (a = polyamide, b = polyethylene terephthalate).

Potential Human Health Impact of MPS

Potential mechanisms involving damage consequences have been proposed (Prata, 2018; Wright and Kelly, 2017), despite the fact that there is a great deal of uncertainty surrounding the accumulation and effects of MPs consumption in human bodies (Barboza et al., 2018; Toussaint et al., 2019). MPs can damage the human body through both chemical and physical means. MPs may oversee the release of additives, absorbing toxins and constituent monomers once they are in the gut. These substances can result in oxidative stress, inflammation, cell apoptosis, necrosis, fibrosis, genotoxicity, localized cell and tissue damage, and possibly carcinogenesis (Deng et al., 2017).

There is a serious risk to human health when MPs are present in seafood. A vital component of the human diet is seafood. There is a significant chance that MPs contamination of the digestive tract will extend to other parts of the body. Two of the most popular ways that MPs enter the human body are endocytosis and persorption (Bhuyan, 2022). Depending on the level of exposure and the individual's vulnerability, MPs may be detrimental to organisms. They can, in fact, result in cytotoxicity, oxidative stress, and tissue translocation (Prata et al., 2020).

MPs can either affect locally in the colon or travel through the circulatory system to other organs after exposure. Micro- and nanoplastics can induce pulmonary hypertension, flogosis, vascular occlusions, internalization-induced blood cell cytotoxicity, and a systemic inflammatory response once they are in blood vessels (Canesi et al., 2015; Wright and Kelly, 2017).

Humans are not immune to the effects of microplastic pollution since they rely heavily on fish from the ocean and surrounding ecosystems for vitamins and protein (Sana et al., 2020; Yang et al., 2022). According to Cox et al. (2019), one of the main ways that plastics enter the human body is through the ingestion of seafood. Potential exposure routes should be looked into to help manage plastic particles and reduce human exposures, even if research on internal exposure assessments of plastic particles in bodily fluids and tissues and their effects is still in its early stages.

4. Conclusion

Bangladesh is currently dealing with the worst possible marine pollution from plastic, one of the main pollutants impacting oceans and seas worldwide, as a member of the South Asian Sea. In all fish species examined, MPs were detected in the GITs. MPs are commonly found in sediment, water, and fish, especially as films, fibers, and shards. Most likely, these particles are the result of human activities in the area. The findings of the samples collected from marine and coastal waterways suggest that MPs may originate in these regions. The presence of MPs in fish suggests that they could go up the food chain to higher trophic levels in the ocean or on land. Such evidence also includes indicators of human-induced environmental impacts that can be used to address threats to human and environmental health, including the possibility of bioaccumulation in humans and other animals. The government must, however, strengthen waste management systems and enforce stringent laws and regulations to address the issue of plastic pollution. To reduce plastic trash, we must adhere to national regulations such as the "3 R": "Reduce," "Reuse," and "Recycle."

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CITE THIS ARTICLE: Suravi, I. N., Islam, M. S. ., Akter, M., Das, T. R., Rahman, L. ., Mia, M. S. ., & Aktaruzzaman, M. . (2025). Isolation and prevalence of microplastic contamination in Seabass (Lates calcarifer) from the Bay of Bengal. International Journal of Research in Organic Agriculture, 1(1), 8-16. https://ijroa.com/ijroa/article/view/13

International Journal of Research in Organic Agriculture
Volume 1, Issue 1, Year 2025

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