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Detection and analysis of microplastic contamination in *Anodontostoma chacunda* from Kasimedu fish landing centre, Bay of Bengal Coast

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Abstract
The contamination of marine environments by plastic debris presents a significant environmental concern, particularly in coastal regions where plastic waste disposal is prevalent. Over time, these plastics undergo degradation processes, resulting in the formation of microplastics, particles measuring less than 5mm in size. Pelagic fish, such as *Anodontostoma chacunda* from the Clupidae family, are known to ingest microplastics during feeding activities, leading to their accumulation in the gastrointestinal tract. In this study, conducted at the Kasimedu fish landing centre in the Bay of Bengal, 100 fish specimens were collected and subjected to stomach contents digestion using the wet peroxidation method. Subsequent filtration procedures revealed the presence of 200 microplastic particles, with an average of 3±0.33 particles per fish. Morphological analysis using a stereomicroscope identified two primary categories of microplastics: Fibers and fragments, constituting 66% and 34% of the total particles, respectively. Further analysis of the isolated microplastics using Fourier Transform Infrared Radiation-Attenuated Total Reflection (FTIR-ATR) spectroscopy confirmed the presence of Rayon. These findings underscore the extent of plastic pollution in the Bay of Bengal and its integration into the marine biota, highlighting the urgent need for mitigation measures to address this environmental issue.

Keywords: Plastic pollution, microplastics, ingestion, polymers

1. Introduction

Marine debris is the most disruptive solid matter that can remain in aquatic environments for an extended period. Plastics are a significant threat among many marine pollutants since they do not disintegrate for millions of years [1]. Because of their durability, low cost, and rapid accessibility, plastics are widely used and abandoned along coastlines [2]. Since they are deposited near the water, they reach the ocean immediately and have an impact on the aquatic ecosystem. Once within the ocean, various natural processes influence the destiny of the plastics [3]. The monomeric foundations of the polymers found in plastics are derived from oil or natural gas [4]. Sun's UV radiation, wind currents, and hydrodynamic forces break down bigger plastic debris into tiny particles known as microplastics (MPs), which have a diameter of less than 5 mm [5]. There are two principal forms of MPs: Primary MPs, which are microbeads or granules intended for use in cosmetics. Secondary MPs are generated by the physiochemical breakdown of bigger plastic materials from shipping industries, industrial scrapers, and similar sources [6].

MPs possess a multitude of attributes, encompassing diverse colour combinations, dimensions, densities, and compositions [7]. With regard to their density, MPs are transported between different coastal regions [8]. Developing nations such as India have a relatively low plastic consumption; however, reports suggest the existence of MPs along the south eastern coast [9]. This critical circumstance necessitates further investigation in this field.

Human activities in close proximity to coastlines lead to the release of plastics, which subsequently enter the food chain of aquatic organisms [10]. Fish in the oceans inadvertently consume MPs, mistaking them for prey, leading to a substantial accumulation of plastic debris ingestion [11]. Physical, chemical, and hormonal alterations are among the detrimental effects that MPs provoke once they enter the organism.

MPs induce apoptosis, disrupt the immune system, impair the process of reproduction, and hinder dietary intake and gastrointestinal metabolism. They also cause a rapid decline in the organism's growth and reproductive capability [12-14]. MPs have been documented to be ingested by numerous invertebrates, including polychaetes, bivalves, and crustaceans, in addition to fish [15, 16].
Various additional contaminants adhere to the surface of MPs. Plasticizers, surfactants, paint materials, polishes, and insecticides are substances that readily bond to the surface of MPs [17]. Many microorganisms also inhabit MPs. These microbes may be harmful and trigger several anonymous illnesses in fish once they enter the fish's body [18]. This enhances the ecotoxicological impact on the organism exposed to MPs [19].
There is a lack of data reporting the occurrence of MPs in fish from the Bay of Bengal. Approximately 300 million people living in this coastal area rely directly on the bay fisheries for their survival [20]. Thus, it is crucial to assess the pollution levels in fish around the Bay of Bengal. This research aims to assess the presence of MPs in *A. chacunda*, a commercially important fish species known as Chacunda gizzard shad that belongs to the Clupeidae family. Furthermore, to determine the quantity and variety of MPs in the gastrointestinal system of the shad. Also, to characterize the polymer utilizing the FTIR-ATR method to determine the type of bonds present in the polymers composing the MPs.

2. Material and Methods

2.1 Collection of sample

Fish were procured from the Kasimedu fish landing centre in December 2023, during the early morning hours between 5 am and 7 am. Fishing vessels operating from the Kasimedu port are known to gather substantial quantities of fish from the sea, which are subsequently brought ashore for auction and sale. It is estimated that approximately 35% of the catch is exported to other nations. A total of 100 *A. chacunda* (see Fig.1) fish were acquired from local vendors, promptly placed in an icebox, and transported to the laboratory facilities. Upon arrival at the lab, the fish underwent a thorough rinsing with distilled water to remove any dirt, mucus, blood, or other adhering substances. Subsequently, each fish was photographed, and its morphometric measurements were meticulously recorded. Following this, the fish were dissected to extract the entire gastrointestinal system.

Fig 1: *Anodontostoma chacunda* fish sample

2.2 Methodology

The Gastrointestinal tract is preserved in a deep freezer at -20°C. Before the experiment begins, it is thawed to initiate the procedure. The GI tracts of ten fishes are pooled indica to make ten total samples which are kept in oven at 60°C for 12 to 24 hours. The dehydrated GI tract undergoes wet peroxidation for breaking down organic material. A solution containing 60 ml of 0.05 M Iron (II) and 40 ml of 25% Hydrogen peroxide is combined and mixed with the GI tract which is then subjected to heat treatment on a hot plate at 60°C. If more organic matter is found, an additional 20 ml of H_2O_2 may be introduced [21]. The digested solution is filtered using a sieve filter with a mesh size of 1mm. The solution obtained from previous step is filtered using a vacuum filtration device equipped with MF-Millipore 0.45 µm MCE membrane filters from MERCK [22]. The filter paper is collected, kept in a petri dish, covered with aluminium foil, and allowed to air dry. After 24 to 48 hours, the filter papers are inspected for the presence of MP.

2.3 Microscopic examination and characterization

The filter layers undergo analysis utilizing a stereo microscope with magnifications ranging from 10X to 100X. Detailed documentation of the quantity, colour, shape, and dimensions of the microplastics (MPs) is conducted. Photographs of the MPs are taken and tabulated based on the observed characteristics (refer to Table 1). Subsequently, Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) is employed for the characterization of MPs to identify the polymer type, utilizing the JASCO FT/IR-4X model.

3. Results

3.1 Characterization of fish

Out of the 100 specimens, 73 were males and 27 were females. The mean length and weight were determined to be 23.6±0.4 cm and 93±2 g. The mean weight of the GI tract was 0.003±0.001g. The species obtained belongs to the Clupeidae family, which are planktivorous and invertivorous. When *A. Chacunda* stomach was examined, eight groups of food items were found. They were seaweeds, sponge spicules, crabs larvae, diatoms, dinoflagellates, blue - green algae, protozoans, and other zooplankton. The diet mostly included phytoplankton, making up 58.5% of the average stomach contents, followed by zooplankton at 40.5%, with a small percentage of seaweeds at 0.4% and sponge spicules at 0.6%, as seen in Fig. 2.

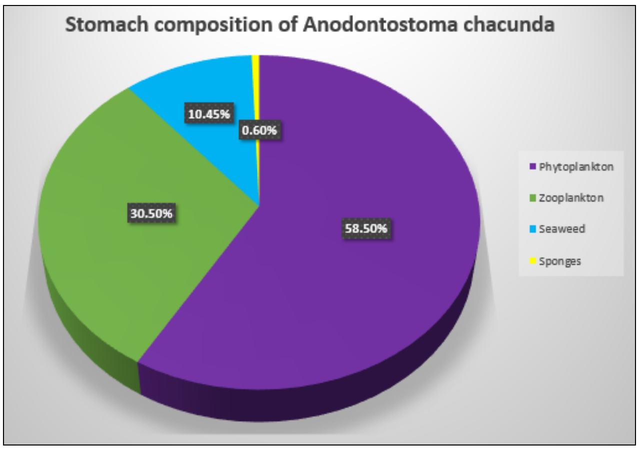

Fig 2: Pie chart depicting the stomach contents of the fish

3.2 Occurrences of MPs in fish

Upon analysis of the samples, it was determined that 60% of the specimens had inadvertently consumed microplastics (MPs). A total of 200 MPs were extracted from the gastrointestinal (GI) tracts of the fish (refer to Table 1), with each fish ingesting an average of 3±0.33 MPs. The isolated particles were categorized as Fibres (66%) and Fragments (34%), exhibiting various color including white, blue, and yellow. White fibers constituted the majority of MPs (30%), followed by blue fibers (25%). Yellow and white fragments accounted for 15% and 19% of the MPs, respectively, while the occurrence rate of yellow fibers was the lowest at 11%.

Table 1: Represents the number of MPs isolated from polled fish GI tracts

S. No	Presence/Absence of MP	White fibres	Yellow fragment	White fragment	Yellow fibre	Blue fibre	Total polymer
1	Present	10	5	8	2	8	33
2	Absent	-	-	-	-	-	-
3	Present	15	2	7	3	10	37
4	Present	5	8	8	5	5	31
5	Absent	-	-	-	-	-	-
6	Present	13	5	10	6	8	42
7	Present	12	6	3	4	12	37
8	Absent	-	-	-	-	-	-
9	Present	5	4	2	2	7	20
10	Absent	-	-	-	-	-	-

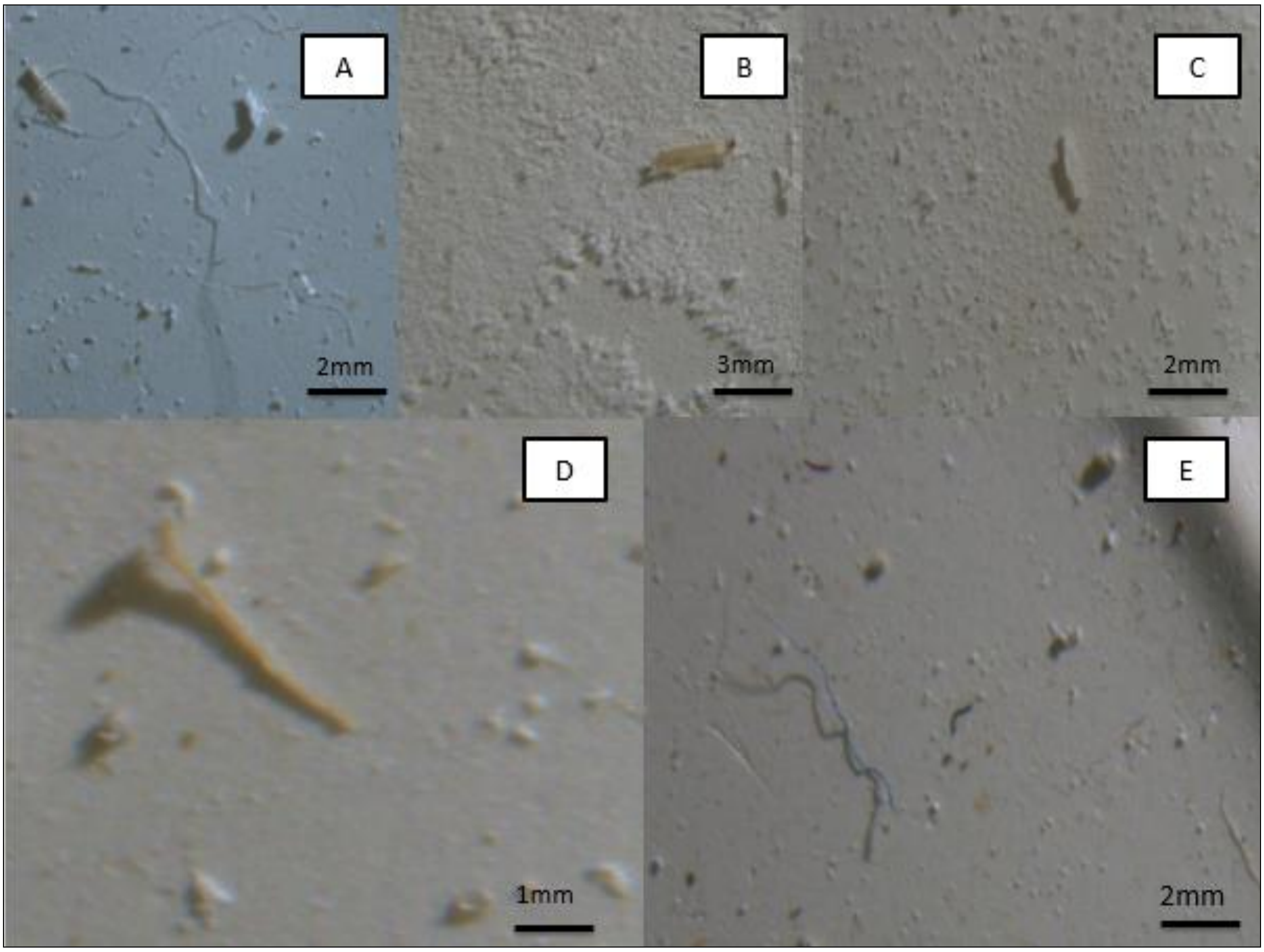

Fig 3: shows stereomicroscopic images of various MPs collected from fish (A) white fibres (B) yellow fragment (C) white fragment (D) yellow fibres (E) Blue fibres

3.3 Characterization of MPs

For the characterization of the polymer, Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) analysis was utilised. The spectral peaks indicate the functional groups and bonding types that comprise the polymeric compound. The peaks observed in the range of 600 cm^{-1} to 3000 cm^{-1} (Fig. 4) are the representative peaks of Rayon polymer. Peaks formed at wavenumbers 2935.27 cm^{-1}, 1572.9 cm^{-1}, 1382.84 cm^{-1}, and 995.19 cm^{-1} indicates C-H stretching, C-H bending, and C-O-C stretching which are the features of Rayon.

This analysis provides compelling evidence supporting the presence of rayon in the gut of the fish *A. chacunda*, shedding light on the sources and pathways of microplastic contamination in aquatic environments.

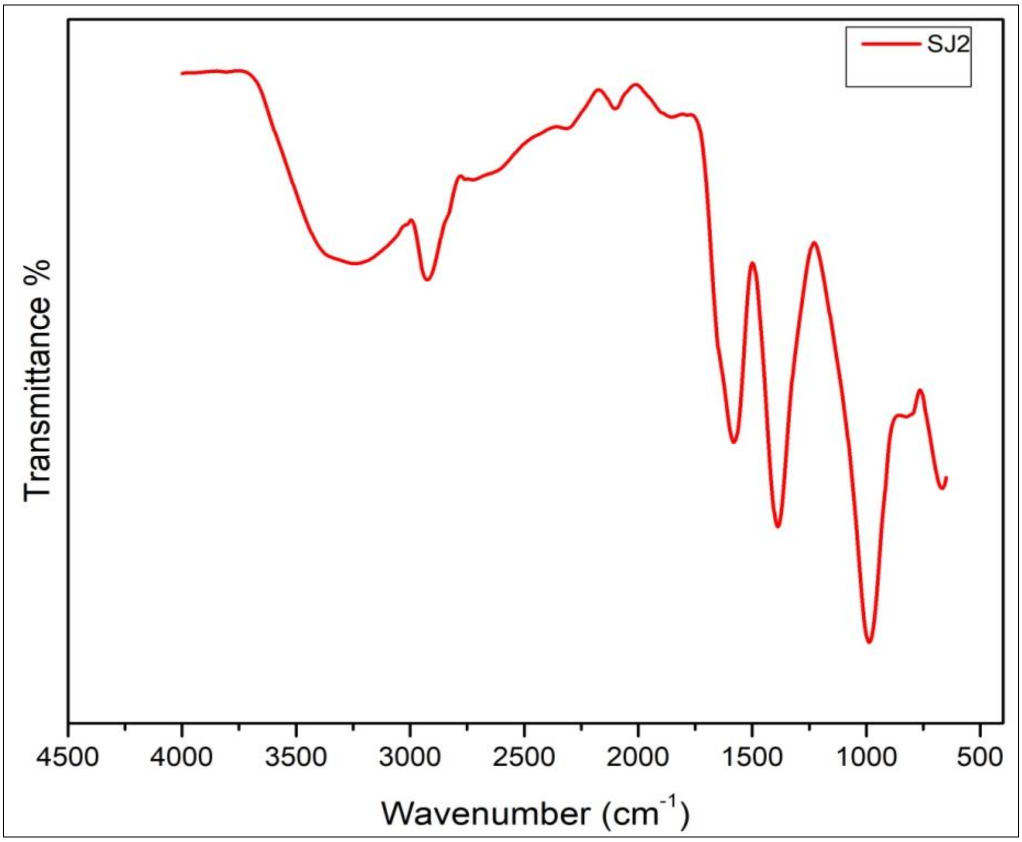

Fig 4: FTIR-ATR peak confirming the presence of rayon

4. Discussion

This study provides clear evidence of the presence of MPs in planktivorous fish *Anodontostoma chacunda* belongs to pelagic fish species it is evident that all the low density MPs has been consumed from the water column. The predominant constituents of the MPs that isolated were fibres, comprising 132 particulates exhibiting three distinct coloration, including white, yellow, and blue in the gut. Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) analysis aids in confirming the presence of rayon in the gut of the fish. FTIR-ATR analysis involves examining spectral peaks corresponding to functional groups and bonding types within the polymeric compound. In this study, peaks observed in the range of 600 cm^{-1} to 3000 cm^{-1} align with characteristic peaks of rayon polymer. The existence of rayon suggests that the discarded plastics originated from the textile industries and also from medical products like bandages, and surgical wraps which are being dumped in larger amounts into the ocean. The contamination level along the Chennai coast was unequivocally demonstrated by the fact that the majority of the examined species contained MP. Prolonged pollution along the Bay of Bengal is a consequence of the substantial volume of products transported by sea in goods ship and the accompanying tourism and extended fishing activities.

The planktivorous dietary behaviour suggests that it may have consumed smaller quantities of these particulates in comparison to carnivorous and omnivorous fish species. Thus, it is evident from this research that feeding and forage also contribute significantly to the accumulation of MP within the bodies of fish. Further an assessment of the abundance of MPs in secondary and tertiary consumers with diverse dietary preferences requires further investigation. An approximation of the quantity of MPs traversing the food chain is also necessary. The mechanism of trophic transfer requires considerable investigation. MP translocation from the GI tract to numerous organs, particularly the muscles, has been confirmed by several studies [23]. Humans commonly ingest muscles, which are an edible component of fish. Thus, there is a high probability that humans could ingest MPs via fish. To ascertain the toxic effects of MPs ingested by humans, a comprehensive investigation must be conducted. Although there is a paucity of literature reviews on the subject, MP consumption can have physical and biochemical effects on humans. Apart from triggering inflammation, microplastics (MPs) have the potential to cause intestinal obstruction and abrasions [24]. Additionally, MPs serve as carriers for other compounds, such as persistent organic pollutants [25]. Understanding the combined toxicity of these pollutants within an organism necessitates further investigation. This underscores the importance of comprehensive research to elucidate the potential health impacts associated with exposure to microplastics and their associated contaminants. By unravelling the intricate interactions between microplastics and other pollutants, we can better comprehend the overall risk posed to organisms and ecosystems alike.

5. Conclusion

In conclusion, this study provides compelling evidence of microplastic (MP) contamination in planktivorous fish. The predominant MPs isolated were fibers, displaying distinct white, yellow, and blue coloration within the gut. The presence of rayon indicates that discarded plastics originate from textile industries and medical products such as bandages and surgical wraps, significantly contributing to ocean pollution. The widespread contamination along the Chennai coast underscores the urgent need for mitigation measures. The persistent pollution in the Bay of Bengal is attributed to extensive maritime transport, tourism, and fishing activities, emphasizing the importance of concerted efforts to address plastic pollution and preserve marine ecosystems.

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