International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 20-06-2026, Accepted: 21-07-2026, Published: 05-08-2026
Volume 11, Issue 3, 2026, Page No. 247-252

A study on microplastic contamination in indigenous fish species of the Krishna River region: Evidence from GC–MS analysis

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Abstract
Microplastics (MPs) and nano plastics (NPs) are worldwide pollutants affecting the environment - marine, freshwater aquatic or terrestrial/ aerial. The plastic generated waste has the potential to pollute the sea, water bodies, rivers and laker and fishes. Therefore, theseresearch aimed to analyse microplastics contamination in three species of fresh water eatable fishes in the Andhra Pradesh and Telangana areas of paleru lake and Krishna river coastal areas. The sampling was carried out for 4 Month from september to december 2025 at three locations, namely paleru reservoir (sample station-1), krishna river in Vijayawada (Sample station-2) and Costal area of Krishna (Sample station-3). The fish sample Catla (*Catla catla*), Rohu (*Labeo rohita*) and Hilsa (*Tenualosa ilisha*). The find the result were four forms of microplastics, namely microbeads, nurdle and microfibers with five types of colours, include block, brown, blue, yellow and read. The dominant shape and colour were nurdle and red, respectively. In Rohu fish, the highest and lowest number of microplastic particles were found in sample in paleru reservoir (3.12 particles/fish) (sample station-1) and Krishna river water (2.90 particles/fishe). Meanwhile, Catla caltla had the highest and lowest abundance in Costal area of krishna (4.32 Particles/fish) and Krishna River Vijayawada Krishna River water (2.80 particles/fishe), respectively. In general, Catla fish had a higher abundance of microplastic than the other two species, and microplastics were highest in fish from Krishna River near urban areas station-2. According to Gas Chromatography and Mass Spectrometry (GC-MS) test on flesh sample, namely nylon and polyproplene. In this study the flesh of species analysed were contaminated with microplastic.

Keywords: Microplastic contamination, rohu, hilsa and catla, nurdle, microfiber and GC-MS

Introduction
With the advents of new technology and tools in the modern world, man has also modified or changed himself in accordance with the availability of the material required for daily needs. (Gandhi G *et al*., 2024) [10] The modern world has become much more dependent on the use of material made from plastic since the later half of the 20th century, be it the daily use of bags for marketing, utensils for various purposes - drinks, eatables, transportation of material or storage replacing the readily degradable packaging or dispensing material, due to their low cost and more persistency (Gandhi G *et al*., 2024) [10]. Plastic does not decompose easily in a short period due to high molecular mass density (Rodrigues *et al*. 2019 and Fachrul 2021) [9, 26]. Most of the plastics released in the environment constitute plastic debris with small-sized particles-microplastics (MPs). The small-sized plastic debris with a diameter of < 5 mm (1 µm to 5 mm) forms MPs, which are considered to be toxic and particles even smaller than 1 µm constitute nanoplastics (NPs) (Gandhi G *et al.*, 2024, Barnes DK *et al*., 1985 and Duis K *et al*., 2016) [8, 10]. Pollution in aquatic environments primarily originates from agricultural runoff, aquaculture practices, and the discharge of industrial and domestic waste. Among these pollutants, plastics pose a significant threat due to their persistence and widespread distribution. In aquatic systems, plastics can enter food chains both directly and indirectly, leading to contamination of various organisms (Herrera, A *et al*., 2022) [14]. Microplastics, in particular, accumulate within the tissues of aquatic biota such as fish, shellfish, and mollusks and cyclopes (Herrera, V *et al.*,2022 and Gandhi, G *et al*., 2023) [11, 14]. This accumulation can result in multiple adverse effects, including damage to digestive systems, disruption of enzyme activity, impairment of reproductive processes, reduction in steroid hormone levels, and, in severe cases, mortality (Witczak, A *et al.*, 2024) [32].

Studies have documented the presence of microplastic contamination in marine environments worldwide. In particular, waters of the Western Pacific Ocean and the South China Sea have shown notable levels of microplastics, with reported concentrations ranging from 187 to 1816 particles per cubic meter (Cui *et al.*, 2022 and Pan *et al*., 2022) [7]. Most of these studies have primarily concentrated on pollution in rivers and lakes, with comparatively limited attention given to its impact on fish. This gap in research is significant, as understanding these effects is essential for safeguarding food security in India as well as worldwide (Shaika, N. A *et al*., 2025) [30]. The fisheries sector plays a vital role in the economies of Andhra Pradesh and Telangana. A large proportion of fishers in India are small-scale, artisanal operators who rely on modest vessels, typically limited to nearshore waters within approximately 3–5 miles of the coastline. In many areas, traditional fishing practices and gear, including various forms of coastal trawling and net hauling, are still widely used. Previous studies have identified several commonly harvested species in the coastal regions influenced by the Krishna River, including rohu (*Labeo rohita*), catla (*Catla catla*), and hilsa (*Tenualosa ilisha*), along with species such as *Leiognathus equulus* (Hine, M., *et al*., 2010). These species are particularly vulnerable to microplastic exposure because they inhabit coastal waters that are influenced by multiple pollution sources, including urban settlements, agricultural runoff, fishing activities, and industrial discharge (Schnurr, R.E *et al*., 2018) [28]. Despite these risks, there remains a lack of comprehensive studies examining microplastic contamination in fish from these coastal regions. In the present study, GC-MS was utilized to identify alkane functional groups, which serve as indicators of microplastic polymers, in fish muscle tissues. This analytical technique has been only rarely applied in studies investigating microplastic contamination in fish. Accordingly, this research focuses on assessing the extent of microplastic contamination in three commonly harvested marine fish species from the coastal waters of Andhra Pradesh and Telangana. The findings are intended to contribute to a better understanding of potential risks to food safety and food security in India (Mandal A, *et al*., 2023) [21].

Material and Methods

Time and Location

This study was conducted between September and December 2025 at three sampling locations in Andhra Pradesh and Telangana, which were also identified and validated using remote sensing and GIS techniques (Gujju Gandhi, *et al*., 2013 and Gujju Gandhi *et al*., 2017) [13, 12]. The selected sites included Paleru Reservoir (Station 1), the Krishna River at Vijayawada (Station 2), and the coastal region of the Krishna estuary (Station 3). The fish species chosen for analysis were rohu (*Labeo rohita*), catla (*Catla catla*), and hilsa (*Tenualosa ilisha*), as they are among the most widely consumed and commercially significant species in the region.

Fish samples were collected monthly from all three locations with the assistance of local fishermen using inland and coastal trawling methods. At each site, approximately 30–50 individuals of each species were randomly sampled (Megan K. *et al*., 2021). Immediately after collection, the specimens were preserved in ice-filled cool boxes at approximately 4°C and transported to the Marine Chemistry and Biotechnology Laboratory, Faculty of Science, Kakatiya University, Warangal, India, for further processing.

Microplastic analysis was conducted in the Chemistry and Zoology laboratories of the Faculty of Science, Kakatiya University. Identification of polymer characteristics was performed using Gas Chromatography and Mass Spectrometry (GC-MS) at the Environmental Quality Assessment Laboratory, Government Degree College, Khammam, India (Sebastian A.S., *et al.*, 2016) [29].

Preparation of sample and analysis

Prior to analysis, each fish specimen was measured for total length and body weight. The abdominal cavity was then carefully opened, and the digestive tract was removed under clean laboratory conditions to minimize contamination (Pasquier, G *et al*., 2022) [23]. The gut contents were transferred into clean glass beakers for further processing. To digest organic matter, a 30% hydrogen peroxide (H_2O_2) solution was used as an alternative to alkaline digestion (Pfeiffer, *et al*., 2020) [24]. The solution was added in sufficient volume to completely immerse the sample. The mixture was then covered with aluminum foil and left to react for 24–48 hours at room temperature, with occasional gentle agitation to enhance digestion efficiency. Following digestion, the samples were vacuum-filtered using Whatman filter paper. The filter papers were carefully placed in sterile Petri dishes, covered to avoid airborne contamination, and dried in an oven at 60°C until completely moisture-free(Huq A *et al.,* 2012) [17].

The dried filters were examined for the presence of microplastics using a stereo microscope under magnifications of 100× and 400× (Mahu E *et al*., 2024) [20]. Microplastics were identified and categorized based on their physical characteristics, including shape, color, size, and type, following established classification approaches reported in previous studies (Ziani K *et al*., 2023) [34]. The abundance of microplastics in the digestive tract of each fish was calculated as the number of particles per individual, using standard quantification methods commonly applied in microplastic research.

Gas Chromatography and Mass Spectrometry (GC-MS)

Polymer characterization of suspected microplastic particles in fish muscle tissue was carried out using Raman spectroscopy in combination with Gas Chromatography–Mass Spectrometry (GC–MS) for enhanced confirmation (Xu J, *et al*., 2023) [33]. For Raman analysis, particles were placed on clean glass slides and examined using a micro-Raman spectrometer to obtain characteristic spectral fingerprints across an appropriate wavelength range. The acquired spectra were processed using specialized software and compared with reference polymer libraries to identify material composition based on spectral similarity (Li YS *et al*., 2024) [19].

To further validate the polymer types, selected samples were subjected to pyrolysis followed by Gas Chromatography–Mass Spectrometry (Py-GC/MS). In this process, microplastic particles were thermally decomposed into smaller molecular fragments under controlled conditions. The resulting compounds were separated using gas chromatography and subsequently analyzed by mass spectrometry (Cho MH *et al*., 2023) [5]. The generated chromatograms and mass spectra were then matched with standard reference databases to confirm the identity of the polymers.

The combined use of Raman spectroscopy and GC-MS provided improved accuracy and reliability in identifying microplastic polymers present in fish tissues.

From each sampling location, three individuals of each fish species were randomly selected for muscle tissue analysis. The edible portions were carefully separated, homogenized, and dried in a hot air oven at 60°C for approximately 48 hours to remove moisture. The dried tissues were then finely ground into a uniform powder and transported to the laboratory at the Faculty of Science, Kakatiya University, for further analysis (Bakhshalizadeh S *et al*., 2022) [3]. For polymer characterization, Gas Chromatography-Mass Spectrometry (GC–MS) was employed as an alternative to conventional spectroscopic methods. A small of the powdered sample was collected using a clean stainless-steel spatula and placed into pyrolysis tubes. The samples were subjected to controlled thermal decomposition (pyrolysis), breaking down complex polymer structures into smaller volatile compounds.

These decomposition products were separated using gas chromatography and subsequently analyzed by mass spectrometry to generate characteristic chromatograms and mass spectra. The obtained spectral data were interpreted using specialized software and compared with standard reference libraries to identify the presence and types of polymeric materials associated with microplastics. In addition, Raman spectroscopy was used as a complementary technique for confirming polymer types without the need for chemical reagents such as potassium bromide (KBr) (Umurhan Y *et al*.,2025) [31] This approach provided a more direct and contamination-minimized analysis of microplastic particles in fish tissue samples.

Result and Discussion

A total of 110 rohu, 108 catla, and 90 hilsa specimens were collected from the Krishna River sampling site. At the Paleru Reservoir, 108 rohu, 65 catla, and 89 hilsa were obtained. In the coastal region of the Krishna estuary, 110 rohu, 118 catla, and 123 hilsa samples were collected (Table1).

Analysis of the digestive tracts revealed variation in microplastic abundance among species and locations. In rohu, the highest mean abundance of microplastics was observed in samples from the Krishna River urban stretch (3.51 particles per fish), while the lowest was recorded in Paleru Reservoir (1.80 particles per fish). For catla, the highest abundance was detected in Krishna River samples (1.83 particles per fish), whereas the lowest was found in Paleru Reservoir (1.30 particles per fish). In hilsa, the highest microplastic load was recorded in coastal samples (1.67 particles per fish), while the lowest was observed in Krishna River samples (1.02 particles per fish).

Table 1: Sample size and microplastic abundance in fish species from different locations

Location	Species	Number of Samples (n)	Microplastic Abundance (particles/fish)
Krishna River	Rohu	110	3.51
	Catla	108	1.83
	Hilsa	90	1.02
Paleru Reservoir	Rohu	108	1.80
	Catla	65	1.30
	Hilsa	89	1.20
Coastal Area	Rohu	110	1.10
	Catla	118	1.05
	Hilsa	123	1.67

Four major types of microplastics were identified in the digestive tracts of the sampled fish: microbeads, fragments, nurdles, and microfibers (Table 1 and Figure 1). Among these, nurdles were the most prevalent form across all three species and sampling locations.

The present study provides clear evidence of microplastic contamination in three commonly consumed fish species rohu (*Labeo rohita*), catla (*Catla catla*), and hilsa (*Tenualosa ilisha*) from the Krishna River system, Paleru Reservoir, and adjacent coastal waters. The detection of microplastics in all sampled locations indicates that plastic pollution is widespread across both freshwater and marine-influenced environments in Andhra Pradesh and Telangana

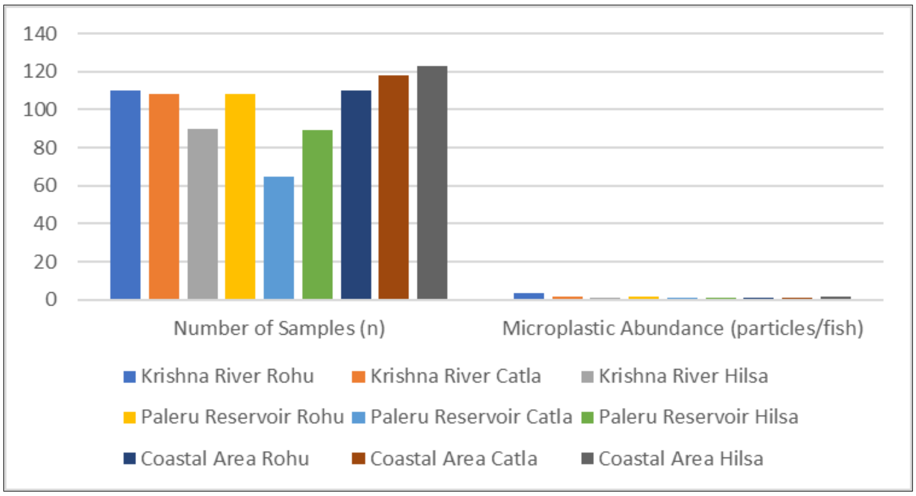

Fig-1: Showing the Sample size and microplastic abundance in fish species from different locations

Table-2: GC–MS identified compounds and corresponding polymer types in fish samples

Location	Species	Major Compounds Detected	Identified Polymer Type
Krishna River	Rohu	n-alkanes (C_{12}–C_{24}), phthalates	Polyethylene (PE), PP
	Catla	Styrene, benzene derivatives	Polystyrene (PS)
	Hilsa	Terephthalic acid, esters	PET
Paleru Reservoir	Rohu	n-alkanes (C_{10}–C_{20})	Polyethylene (PE)
	Catla	Branched hydrocarbons	Polypropylene (PP)
	Hilsa	Trace esters	PET (low levels)
Coastal Area	Rohu	Long-chain alkanes, olefins	PE, PP
	Catla	Phthalates, aromatic compounds	PS, PP
	Hilsa	Terephthalate derivatives	PET

The present study provides clear evidence of microplastic contamination in three commonly consumed fish species rohu (*Labeo rohita*), catla (*Catla catla*), and hilsa (*Tenualosa ilisha*) from the Krishna River system, Paleru Reservoir, and adjacent coastal waters. The detection of microplastics in all sampled locations indicates that plastic pollution is widespread across both freshwater and marine-influenced environments in Andhra Pradesh and Telangana.

The observed variation in microplastic abundance among locations highlights the influence of anthropogenic activities. Fish collected from the Krishna River, particularly near urban areas, exhibited relatively higher microplastic loads. This trend can be attributed to increased inputs from domestic sewage, industrial discharge, urban runoff, and improper waste disposal. In contrast, the comparatively lower abundance recorded in Paleru Reservoir suggests reduced pollution pressure, although the presence of microplastics still indicates contamination even in semi-closed freshwater systems. Coastal samples showed moderate to high contamination levels, likely due to combined influences of riverine discharge, fishing activities, and marine debris accumulation.

Species-specific differences were also evident in this study. Among the three species, catla showed relatively higher microplastic abundance compared to rohu and hilsa in several locations (Hitesh, Chauhan 2025) [16]. This variation may be linked to differences in feeding behavior, habitat preference, and trophic level. Filter-feeding or column-feeding fish such as catla are more likely to ingest suspended microplastic particles, while pelagic species like hilsa may exhibit different exposure pathways (Conowall P *et al*., 2024) [6]. These findings are consistent with earlier studies suggesting that feeding ecology plays a crucial role in microplastic ingestion.

The characterization of microplastics based on physical properties revealed the presence of microbeads, fragments, nurdles, and microfibers (Gandhi G *et al*., 2024) [10]. Among these, nurdles were the dominant form across all sampling sites. Nurdles, being primary industrial plastic pellets, indicate direct input from plastic manufacturing, transportation, or handling processes. The presence of microfibers suggests contributions from domestic wastewater, particularly from textile washing, while fragments and microbeads may originate from the breakdown of larger plastic debris and personal care products. The predominance of red-colored microplastics observed in this study may be due to selective ingestion, as fish often mistake colored particles for food items.

The GC–MS analysis further confirmed the presence of synthetic polymer residues in fish tissues, including polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and nylon (Albignac, M., *et al*., 2023.) [2]. The detection of long-chain alkanes, phthalates, styrene derivatives, and terephthalate compounds supports the identification of these polymers. Polyethylene and polypropylene were the most dominant polymers, which is consistent with their widespread use in packaging materials and fishing gear (S. Yasuda *et al*.,1984). The presence of nylon is particularly indicative of fishing-related sources such as nets and ropes, while PET and PS are commonly associated with consumer products and urban waste (Kumar A *et al*.,2025) [18].

Spatial variation in polymer types also reflects differences in pollution sources (Raab K, *et al. 2025*) [25]. The Krishna River samples showed higher levels of aromatic compounds and plastic additives, suggesting strong urban and industrial influence. Coastal samples were dominated by PE and PP, likely linked to marine-based activities, whereas Paleru Reservoir showed relatively lower polymer diversity, indicating comparatively limited contamination sources.

The detection of microplastics and associated polymers in edible fish tissues raises important concerns regarding food safety and public health (Alberghini L *et al*., 2022) [1]. Microplastics can act as carriers of toxic chemicals, including plastic additives and environmental pollutants, which may accumulate in fish and potentially transfer to humans through consumption. In addition, ingestion of microplastics can adversely affect fish health by causing physical damage, oxidative stress, and disruption of metabolic and reproductive processes.

Overall, the findings of this study emphasize the widespread occurrence of microplastic pollution in the Krishna River ecosystem and its potential implications for aquatic life and human health. The results highlight the urgent need for improved plastic waste management, stricter environmental regulations, and increased public awareness to mitigate plastic pollution. Furthermore, long-term monitoring and advanced analytical studies are essential to better understand the fate, transport, and ecological impacts of microplastics in freshwater and coastal ecosystems.

Conclusion

This study demonstrates the widespread occurrence of microplastic contamination in commonly consumed fish species rohu (*Labeo rohita*), catla (*Catla catla*), and hilsa (*Tenualosa ilisha*) from the Krishna River system, Paleru Reservoir, and adjacent coastal regions of Andhra Pradesh and Telangana. Microplastics were detected in all sampled locations, confirming that both freshwater and coastal environments are affected by plastic pollution. The abundance of microplastics varied across locations and species, with higher levels generally observed in fish collected from the Krishna River, particularly near urban areas. This indicates the significant influence of anthropogenic activities such as domestic waste discharge, industrial effluents, and urban runoff. Among the studied species, catla exhibited comparatively higher levels of microplastic contamination, suggesting that feeding behavior and ecological characteristics play an important role in exposure. Different forms of microplastics, including microbeads, fragments, nurdles, and microfibers, were identified, with nurdles being the most dominant type. The GC–MS analysis confirmed the presence of polymer types such as polyethylene, polypropylene, polystyrene, PET, and nylon, indicating multiple sources of plastic pollution, including packaging materials, fishing gear, and urban waste.

The presence of microplastics in edible fish tissues raises concerns regarding food safety and potential risks to human health. These findings highlight the need for effective plastic waste management, stricter environmental regulations, and continuous monitoring of aquatic ecosystems. Further research is required to better understand the long-term ecological and health impacts of microplastics, particularly in regions where fish form a major component of the human diet.

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