

## Original Article

# Tissue-specific accumulation and species-level variability of microplastics in four freshwater fish from the Dez River, Iran

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**Abstract:** Microplastics (MPs) are emerging contaminants of global concern due to their persistence, bioaccumulation potential, and implications for both aquatic ecosystems and human health. This study explored MP contamination in four economically important freshwater fish species—*Carasobarbus luteus*, *Arabibarbus grypus*, *Luciobarbus barbulus*, and *Oreochromis niloticus*—collected from the Dez River in southwestern Iran. A total of 120 specimens (30 per species) were sampled near the Dez Dam using gill nets. Edible (muscle) and inedible (skin and gastrointestinal tract) tissues were analyzed separately for MP content following alkaline digestion with 10% KOH and vacuum filtration. Visual identification under a stereomicroscope was used to quantify and categorize MPs by color. Microplastics were detected in all species and tissue types, with significant interspecific variation. *Carasobarbus luteus* exhibited the lowest MP burden, significantly lower than *O. niloticus* and *L. barbulus*. No significant differences were found among tissue types, suggesting widespread tissue distribution. Color composition varied across species, with black MPs being the most abundant. Notably, green MPs were only observed in *O. niloticus* and *L. barbulus*. These findings highlight the impact of ecological traits, including feeding behavior and habitat use, on the risk of MP exposure. The detection of MPs in edible tissues highlights potential food safety concerns and underscores the need for routine monitoring of freshwater fish in affected river systems. This study reports the first evidence of MP contamination in fish from the Dez River and contributes to the growing body of data on freshwater plastic pollution in understudied regions.

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## Introduction

Microplastics (MPs), defined as plastic particles smaller than 5 mm, have emerged as a global pollutant of increasing ecological and public health concern (Grattagliano et al., 2025). These particles are manufactured as small-sized plastics (primary MPs) or result from the degradation of larger plastic debris (secondary MPs) through mechanical, chemical, or biological processes (Obeng et al., 2025; Sahai et al., 2025). Because of their small size, persistence, and mobility, MPs are widely distributed across terrestrial, freshwater, and marine environments (Lee et al., 2024).

Rivers serve as key conduits for the transfer of land-based plastic waste into aquatic systems. Sources

such as municipal effluents, stormwater runoff, industrial discharges, and agricultural activities contribute significantly to MP pollution in freshwater bodies (Jaikumar et al., 2024). Once introduced, MPs can be ingested by a broad range of aquatic organisms, including fish, thereby entering food webs (Barboza et al., 2023; Drova et al., 2025). The ingestion of MPs has been associated with adverse effects, such as intestinal obstruction, inflammation, oxidative stress, and disrupted metabolism in fish (Ghosh, 2024; Wang et al., 2024; Zeynali et al., 2025). Moreover, MPs may act as vectors for hydrophobic pollutants and microbial pathogens, further amplifying ecological and human health risks (Teuten et al., 2007; Gu et al., 2020; Sinha et al., 2025).

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Table 1. Average total length and weight ( $\pm$  SE) of fish species used in this study, n=30 each species.

Common name	Species	Family	Total length (cm)	Total weight (g)	Feeding habit and water column distribution
Mesopotamian himri	<i>Carasobarbus luteus</i>	Cyprinidae	22.33 $\pm$ 1.07	174.55 $\pm$ 25.29	Benthic herbivore-detritivore; feeds primarily on periphyton, algae, and plant material in bottom zones
Shirbot	<i>Arabibarbus grypus</i>	Cyprinidae	28.35 $\pm$ 1.01	221.81 $\pm$ 21.85	Omnivore; feeds on aquatic insects, benthic invertebrates, and plant debris in demersal to benthopelagic zones
Heckel's Orontes barbel	<i>Luciobarbus barbulus</i>	Cyprinidae	32.61 $\pm$ 1.10	384.43 $\pm$ 41.60	Benthic invertivore; consumes detritus and macroinvertebrates from sediment surfaces
Nile tilapia	<i>Oreochromis niloticus</i>	Cichlidae	18.52 $\pm$ 0.93	158.19 $\pm$ 18.44	Omnivorous filter feeder; ingests plankton, detritus, and suspended matter in pelagic and littoral zones

Recent studies have highlighted the widespread presence of MPs in wild and farmed fish species (Azizi et al., 2021). Jamal et al. (2025) reported the occurrence of MPs in the gastrointestinal tract, gills, and muscles of three commercial marine fish from the northern Bay of Bengal, identifying fibers and fragments as the dominant types. Shakik et al. (2025) also documented MP accumulation in four freshwater fish species from the Rupsha River, Bangladesh, highlighting its higher prevalence in demersal species and in gastrointestinal tissues than in muscle tissue. These findings highlighting the influence of factors such as feeding behavior, habitat preference, and environmental exposure levels on this variability.

Despite growing global concern, studies on MP pollution in freshwater fish in the Middle East remain scarce. The Dez River in Khuzestan Province, southwestern Iran, is an ecologically and economically important river system that supports extensive agriculture and inland fisheries. It receives considerable anthropogenic input, including urban runoff and agricultural discharge, which are potential sources of plastic waste. However, no published data currently exists on the levels and distribution of MPs in fish from this river.

The present study addresses this gap by evaluating MP contamination in four economically important freshwater fish species—*Oreochromis niloticus* (Nile tilapia), *Carasobarbus luteus* (Mesopotamian himri), *Arabibarbus grypus* (Shirbot), and *Luciobarbus barbulus* (Heckel's Orontes barbel)—caught from the

Dez River. We assessed the occurrence, abundance, and characteristics of MPs in the edible (muscle) and inedible (skin and gastrointestinal tract; GIT) tissues of these fish. This research not only provides the first data on MP contamination in fish from the Dez River, but also contributes to the broader understanding of freshwater MP pollution in underrepresented regions.

## Materials and Methods

**Study area and sample collection:** Fish samples were collected from the Dez River, a major tributary of the Karun River in Khuzestan Province, southwestern Iran. Sampling was conducted at a designated fishing location downstream of the Dez Dam, near Dezful City (Fig. 1). This area is characterized by moderate flow, variable sediment load, and direct exposure to urban, agricultural, and aquaculture-related runoff, making it a suitable site for assessing environmental microplastic contamination in freshwater biota. A total of 120 fish specimens, comprising four species (n = 30 per species), were collected using gill nets during April-May 2024. The selected species included *C. luteus*, *A. grypus*, *L. barbulus*, and *O. niloticus*, all of which are commonly found in the region and represent different ecological feeding niches. Immediately after capture, fish were transported on ice to the laboratory and processed the same day. Each specimen was measured for total length and weight before dissection. The average morphometric values ( $\pm$  standard error) for each species are provided in Table 1.

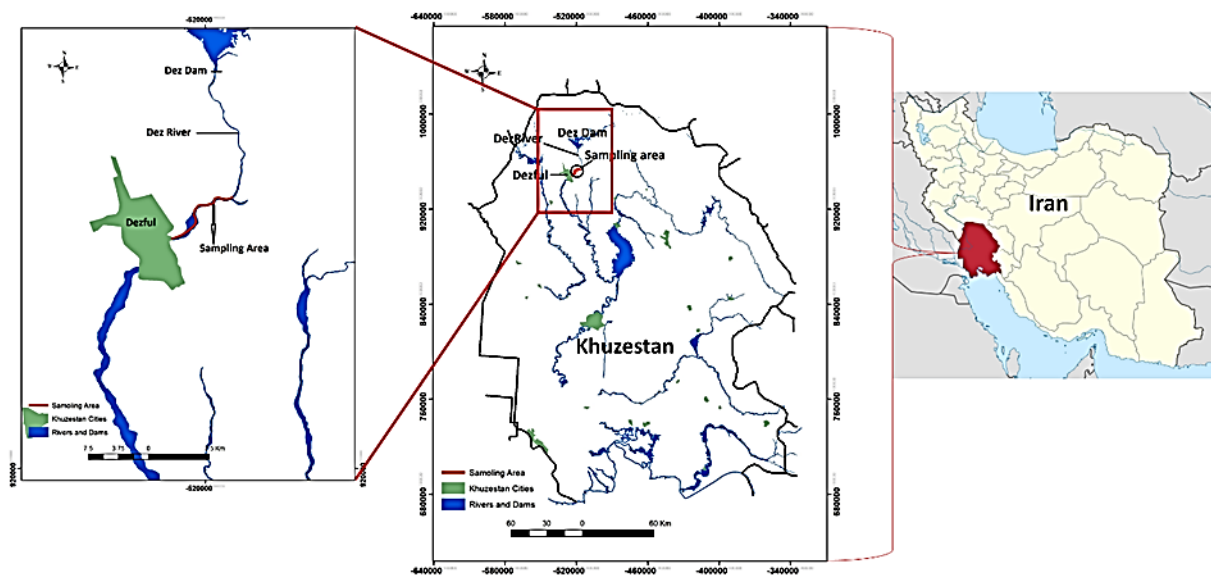


Figure 1. GIS map of the sampling point.

**Sample preparation:** Upon transport to the laboratory, all fish specimens were individually rinsed with distilled water to remove surface debris and potential external contaminants. Each specimen was then blotted dry with clean paper towels and measured for total length (cm) using a measuring board and for total weight (g) using a digital balance with 0.01 g precision. Dissections were performed using sterilized stainless-steel instruments in a contamination-controlled environment. For each fish, the muscle, skin, and gastrointestinal tract (GIT) tissues were carefully separated. Skin was removed using fine forceps and scalpels, ensuring complete isolation from the underlying muscle tissue. All tissue samples were weighed to the nearest 0.01 g and stored in pre-cleaned glass containers covered with aluminum foil. To prevent degradation or microbial interference, dissected tissues were immediately placed in a laboratory freezer at  $-20^{\circ}\text{C}$  and maintained frozen until further processing for microplastic analysis.

**Isolation of microplastics from fish tissues:** Microplastics (MPs) were isolated from fish tissues using a potassium hydroxide (KOH) digestion protocol (Jin et al., 2025), modified to effectively break down organic matter while preserving MPs across a range of densities. For each fish, three tissue types—muscle, skin, and GIT—were carefully dissected and processed individually to allow tissue-

specific quantification of microplastic contamination. Approximately 10 g of each tissue type was placed into clean, foil-covered glass digestion containers. A 10% (w/v) KOH solution was prepared using analytical-grade pellets and ultra-pure water. Digestion conditions were optimized by tissue type: muscle samples were digested at  $60^{\circ}\text{C}$  for 24 hours, whereas skin and GIT samples were digested at  $60^{\circ}\text{C}$  for 48 hours, reflecting their greater resistance to enzymatic digestion. During incubation, containers remained sealed with aluminum foil to reduce airborne contamination. Following digestion, the resulting solutions were vacuum-filtered through 70-mm Whatman cellulose filters (Grade 1, 11  $\mu\text{m}$  pore size). Filters were pre-rinsed, air-dried, and stored in covered glass petri dishes. To retain MPs of all density ranges, density separation was not performed. Filters were subsequently rinsed with ultra-pure water and left to dry at room temperature in a clean environment before microscopic analysis. Plastic materials were excluded throughout the procedure. All instruments and surfaces were cleansed, and samples were handled using only glass or metal tools. To ensure quality control, procedural blanks were processed alongside tissue samples and confirmed the absence of background MP contamination (Shakik et al., 2025).

**Microscopic identification and color classification of microplastics:** Following filtration and drying,

filters containing residual particles were examined under a stereomicroscope (Olympus SZ-ST, Japan) at magnifications of 3× to 6.3×. Microplastic particles were identified visually using established morphological criteria, including shape uniformity, coloration, absence of cellular or organic structures, and resistance to pressure and fragmentation during manipulation. Suspected plastic particles were handled with stainless steel forceps under controlled lighting to minimize misidentification. Only particles meeting at least three of the following criteria were classified as microplastics: (1) unnatural color (e.g., bright or dark synthetic tones), (2) homogeneous texture and lack of biological features, (3) inability to fracture under mechanical stress, and (4) absence of visible cellular or mineral structure under magnification (Jin et al., 2025). To reduce false positives, particles with ambiguous morphology were excluded from quantification. Each confirmed microplastic particle was further categorized by color using a standardized color reference chart under consistent illumination, based on visual comparison. The following color categories were recorded: black, blue, red, and green, corresponding to dominant colors observed in prior environmental MP studies. For each tissue sample, MPs in each color category were counted and recorded for subsequent statistical analysis. To prevent contamination during microscopic analysis, all work was conducted in a laminar-flow cabinet or an enclosed workspace. Cotton lab coats and nitrile gloves were worn throughout, and all tools were pre-cleaned using filtered ethanol and ultra-pure water. Filters were analyzed individually and stored in covered petri dishes before and after examination. The number of recovered particles was normalized and expressed as particles per 100 g of tissue, based on the initial sample weight used for digestion.

**Statistical analysis:** All analyses were performed using R (version 4.2.1; R Core Team, Vienna, Austria). Statistical significance was accepted at a threshold of  $P < 0.05$ . To assess differences in microplastic (MP) contamination among the four studied fish species, an analysis of covariance

(ANCOVA) was employed. In this model, microplastic abundance was used as the dependent variable; species was treated as a fixed categorical factor, and fish length and weight were included as covariates to control for potential confounding. Following a significant ANCOVA result, Duncan's multiple range test was used for post-hoc pairwise comparisons to identify homogeneous groups. The normality of data distributions was tested using the Shapiro–Wilk test. As the data for tissue-specific and color-specific MP concentrations deviated from normality ( $P < 0.05$ ), non-parametric statistical methods were employed. Differences in microplastic (MP) concentrations across tissue types (muscle, skin, and gastrointestinal tract) within each species were analyzed using the Friedman test, a non-parametric method suitable for related samples. The same test was employed to examine intra-species variability in MP color categories. Where significant results were detected, post hoc pairwise comparisons were performed using the Wilcoxon signed-rank test, with the Bonferroni correction applied to control for the risk of type I error due to multiple testing. Data visualization included bar plots with standard error bars, and the ANCOVA-derived means were graphically presented to facilitate comparison among species. Statistically homogeneous groups identified by Duncan's test were annotated using distinct letter codes above each bar.

## Results

**Species-specific differences in microplastic contamination:** The results revealed a significant effect of species on MP load ( $P < 0.05$ ), whereas neither length nor weight was significantly associated with MP abundance (both  $P > 0.05$ ), indicating that species identity is the primary determinant of MP accumulation, independent of body size. Post hoc comparisons using Duncan's multiple range test identified significant differences among species (Fig. 2). *Carasobarbus luteus* exhibited the lowest mean MP count, which was significantly lower than those of *O. niloticus* and *L. barbatus*. The means and standard errors are depicted in bar plots in Figure 2, with

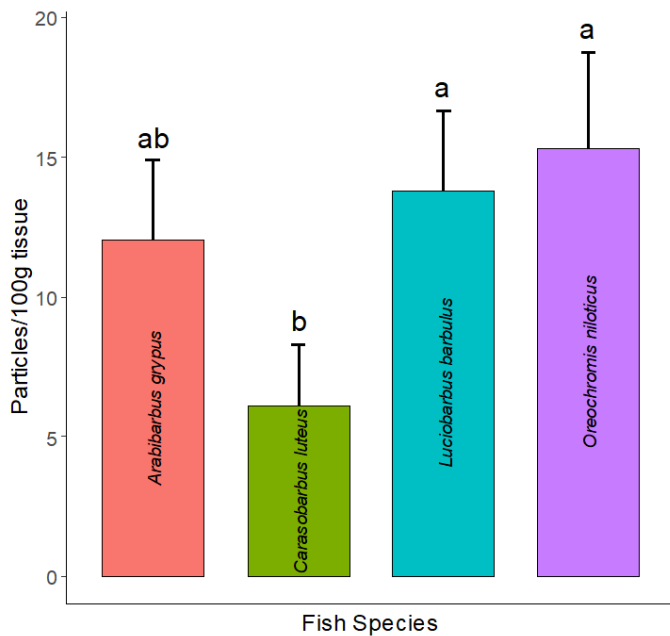


Figure 2. The means of microplastic abundance (particles/100 g tissue) in four freshwater fish species collected from the Dez River, Iran. Microplastic abundance was analyzed using ANCOVA with species as the fixed factor and fish length and weight as covariates. Letters above bars denote statistically homogeneous groups based on Duncan's multiple range test ( $P<0.05$ ). Data are presented as mean  $\pm$  standard error.

different letters indicating statistically significant differences between groups.

#### Tissue-wise distribution of microplastics:

Microplastic concentrations were quantified in the muscle, skin, and GIT tissues of *C. luteus*, *A. grypus*, *O. niloticus*, and *L. barbulus*. As shown in Figure 3, the Shapiro–Wilk test indicated that the data deviated from normality ( $P<0.05$ ), and the Friedman test detected no statistically significant differences in MP concentrations among tissue types within any species ( $P>0.05$ ). For all species, mean MP counts in muscle, skin, and GIT tissues were similar, as confirmed by non-parametric pairwise comparisons.

**Color variability of microplastics:** Significant intra-species differences were observed in the color distribution of recovered microplastics (Fig. 4). The results showed significant differences in color composition within each species ( $P<0.05$ ). Post-hoc pairwise comparisons using the Wilcoxon signed-rank test with Bonferroni correction revealed that in *C. luteus* and *A. grypus*, black microplastics were significantly more prevalent than blue and red

particles. In *O. niloticus* and *L. barbulus*, black microplastics were significantly more abundant than blue, green, and red particles.

#### Discussions

One of the key findings of this study was significantly lower microplastic (MP) contamination in *C. luteus* than in *O. niloticus* and *L. barbulus*. These interspecific differences likely result from species-specific ecological and behavioral traits, such as feeding strategies, habitat preferences, and vertical distributions within the water column (Narwal and Kakakhel, 2025). *Carasobarbus luteus* is a benthopelagic species that predominantly consumes periphyton, algae, and plant material from submerged surfaces (Mohamed and Abood, 2018). Its selective foraging in relatively stable benthic habitats may reduce incidental ingestion of MP. In contrast, *O. niloticus* is an omnivorous filter feeder that consumes a wide range of suspended materials, including plankton and detritus (Tesfahun and Temesgen, 2018), making it more vulnerable to MP ingestion in midwater or surface layers. *Luciobarbus barbulus*, while also a benthic feeder, consumes macroinvertebrates and organic detritus (Zamanpoore and Abbaspour, 2004), increasing the chance of ingesting sediment-bound MPs.

Our results are consistent with global patterns identified by de Araújo et al. (2025), who reported the highest MP prevalence in omnivorous freshwater species, followed by carnivores and herbivores. In the Ravi River, Pakistan, Aslam et al. (2023) found significantly higher MP ingestion in omnivorous fish compared to their herbivorous and carnivorous counterparts. Similar trends have been reported in the Pearl River Basin (Wang et al., 2020) and in South American systems (Mizraji et al., 2017). Conversely, studies such as those by Li et al. (2025) and Buwono et al. (2022) have observed higher MP concentrations in benthic fish, suggesting that sediment contamination and species-specific sediment foraging may sometimes outweigh the protective effect of herbivory. Furthermore, our findings on *O. niloticus* align with previous observations from Lake Victoria,



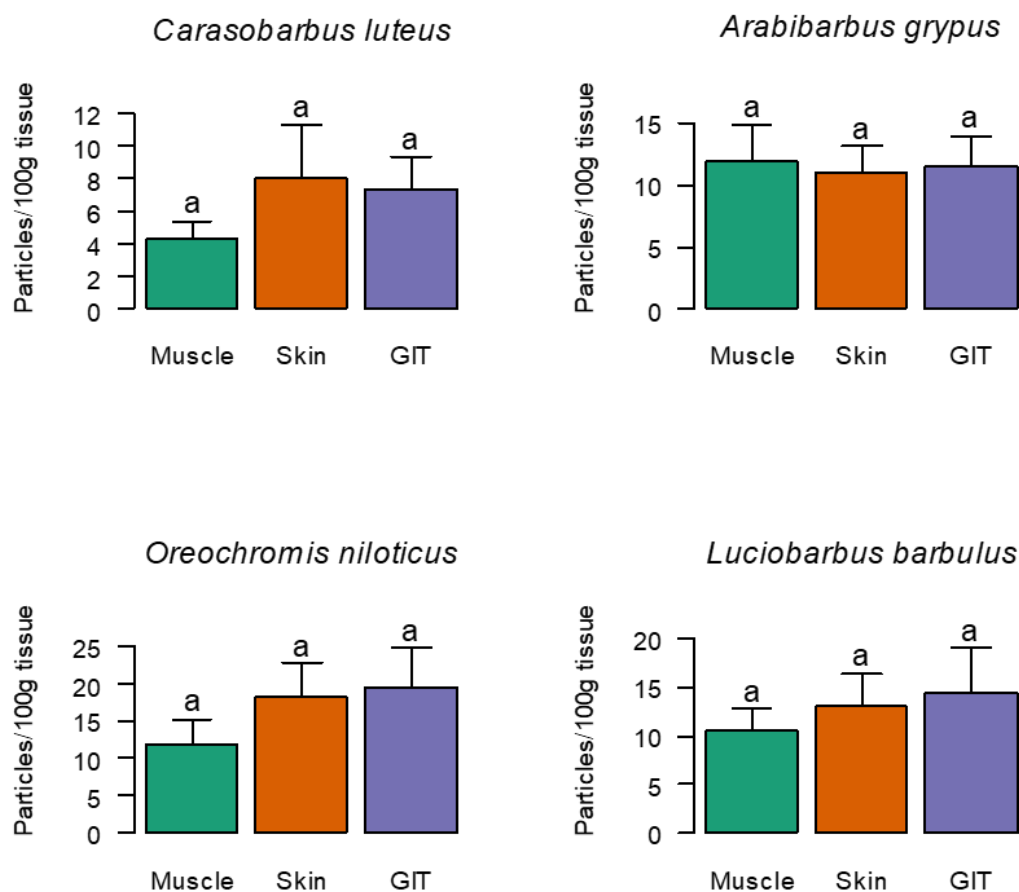


Figure 3. Tissue-wise distribution of microplastics (particles/100 g tissue) in muscle, skin, and gastrointestinal tract (GIT) tissues of four freshwater fish species from the Dez River. No statistically significant differences were observed among tissues within each species by the Friedman test ( $P>0.05$ ). Bars represent mean  $\pm$  standard error. Identical letters indicate no significant differences among tissues within species.

where Biginagwa et al. (2016) documented MP ingestion in 35% of *O. niloticus* specimens. Mercy et al. (2023) similarly reported high MP loads in *O. mossambicus* in urban freshwater systems in Bangladesh, underscoring the susceptibility of tilapia species to MP contamination due to their feeding plasticity and overlap with contaminated zones.

In addition to ecological factors, physiological traits, such as digestive retention time, may contribute to species-specific differences. Fish with longer intestines or slower digestion may retain ingested MPs for longer periods, thereby increasing their accumulation potential (Andrade et al., 2019; Prata et al., 2020). However, comparative physiological data remain limited for these species and warrant further investigation.

No significant differences in MP concentrations were observed among the muscle, skin, and GIT

tissues of any species. This uniformity may result from MP translocation across intestinal barriers into systemic circulation. Experimental studies have shown that small MPs (<5 mm) can migrate from the gut to internal tissues, including the liver and muscle (Liu et al., 2024). Similar findings were reported by Buwono et al. (2021), who detected MPs in the gills and muscles of multiple freshwater species in Indonesia. These results partially contrast with earlier studies, where GIT was the primary site of MP accumulation (e.g., Silva-Cavalcanti et al., 2017; Biginagwa et al., 2016). A global review by de Araújo et al. (2025) indicated that the GIT accounted for more than half of all tissue-specific MP reports, whereas muscle was less frequently studied. However, more recent investigations — such as those by Justino et al. (2021) and Adji et al. (2022) — have reported the presence of MP in edible tissues, raising concerns

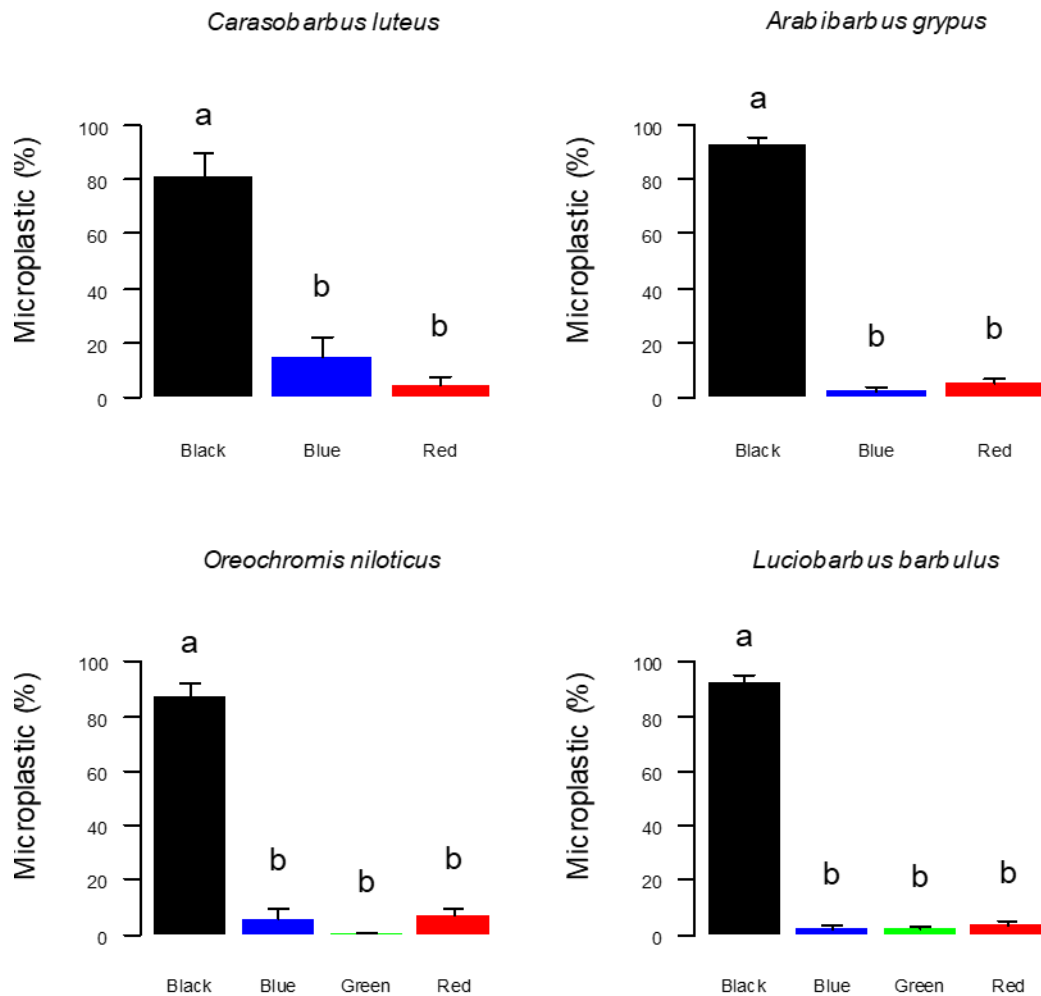


Figure 4. Relative abundance of microplastic color types in four freshwater fish species from the Dez River, Iran. Microplastic particles were categorized by visual color (black, blue, red, and green). The Friedman test revealed significant intra-species differences in MP color distribution ( $P < 0.05$ ), and post-hoc pairwise comparisons using the Wilcoxon signed-rank test with Bonferroni correction identified specific group differences. Letters indicate statistically different groups within each species.

about food safety and supporting our findings of broad MP distribution.

Chronic exposure to low but consistent MP loads may lead to tissue distributions that are equilibrated, especially when translocation mechanisms are active. Moreover, the small size of most particles may allow passive diffusion or endocytotic uptake across epithelial membranes. While methodological contamination was minimized through strict lab protocols, future studies should include controlled blank assessments and digestion validation to ensure procedural integrity.

Significant differences in MP color composition were observed within each species. Black MPs were the most abundant in all species, especially in *C. luteus* and *A. grypus*, where they significantly exceeded blue

and red particles. In *O. niloticus* and *L. barbulus*, black particles were also dominant, followed by statistically lower quantities of blue, red, and green particles. The presence of green MPs in these two species, but not in *C. luteus* and *B. grypus*, suggests exposure to a broader spectrum of MP sources. These results align with previous studies, which have shown the dominance of black and blue MPs in freshwater systems (Azizi et al., 2021). Mercy et al. (2023) found similar color patterns in *O. mossambicus* from polluted lakes in Bangladesh. In global surveys, black and blue MPs are frequently associated with synthetic textiles, tire wear, and industrial runoff (Oza et al., 2024; de Araújo et al., 2025). Loayza et al. (2022) documented more varied MP colors in high-altitude lakes with less urban influence, highlighting the role

of local pollution sources in determining MP color profiles. Color may also influence the likelihood of ingestion through visual foraging. Fish that rely on sight may mistake MPs for prey, especially when particles resemble insect larvae or algae. In turbid or vegetated habitats, darker particles may be more visible or more frequently encountered. These behavioral dynamics could explain both inter- and intra-species variation in color selection (Oza et al., 2024).

Toxicologically, different MP colors may indicate distinct chemical additives or sorbed pollutants. Wang et al. (2018) noted that color-specific MPs could carry different contaminant loads, potentially influencing exposure risk across species. Our findings emphasize the importance of integrating both ecological and toxicological perspectives when evaluating MP profiles.

This study provides new insights into species-specific and tissue-specific MP contamination in freshwater fish from the Dez River, emphasizing the influence of ecological traits on MP exposure. The widespread detection of MPs in edible tissues and the observed variation in MP color profiles highlight potential implications for fish health and human consumption. Nevertheless, some limitations must be acknowledged. The study did not include polymer identification or particle-size analysis, both of which are crucial for understanding source attribution and toxicological risk. This limitation aligns with common gaps reported in global reviews (Khan et al., 2024; Nyaga et al., 2024; de Araújo et al., 2025). Additionally, seasonal dynamics, spatial variation across pollution gradients, and particle morphology were not examined, which could impact MP accumulation patterns. Future work should incorporate polymer-type characterization using techniques such as FTIR or Raman spectroscopy, and should include broader sampling across seasons and river zones. Further assessments of physiological effects, such as histological damage, immune responses, or oxidative stress, would help clarify the biological impacts of MP exposure.

## Conclusion

This study presents the first detailed analysis of microplastic contamination across multiple tissues and species in freshwater fish from the Dez River, Iran. Significant interspecific differences were observed, with *C. luteus* showing notably lower MP loads than *O. niloticus*, and *L. barbulus*, likely due to differences in feeding strategy and habitat use. MPs were uniformly distributed across muscle, skin, and gastrointestinal tissues, suggesting potential translocation mechanisms or equilibrated exposure under chronic environmental contamination. Additionally, distinct intra-species differences in MP color composition were recorded, with black particles consistently dominant and green particles observed exclusively in *O. niloticus* and *L. barbulus*. These findings underscore the role of ecological traits in shaping MP exposure risk in freshwater ecosystems and highlight the presence of MPs in edible tissues, raising potential food safety concerns. Future research should integrate seasonal and spatial variability, particle characterization, and biological response metrics to understand better the ecological and toxicological consequences of MP contamination in freshwater biota.

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**Ethical Approval:** All experimental procedures involving fish were conducted in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. The study protocol was reviewed and approved by the Ethics Committee of Khorramshahr University of Marine Science and Technology (Approval No. 10-1402/07/26).

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