Original Article

Microplastics in Different Tissues of *Oncorhynchus mykiss* Reared in Recirculating Aquaculture Systems in Iran

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ABSTRACT

Background: Microplastics (MPs) have emerged as a growing research focus in aquatic environments, particularly within freshwater aquaculture systems in recent years. However, the presence and characteristics of MPs in Iran's freshwater aquaculture systems remain unexplored.

Objectives: To address this gap, this study aimed to quantify and characterize MPs in freshwater aquaculture systems across two regions: Mazandaran and Golestan provinces in Iran.

Methods: Thirty samples of *Oncorhynchus mykiss* from each area were collected from a pool in the fall of 2024. MPs were extracted from the gastrointestinal tract, gills, and liver and analyzed for abundance, shape, size, and polymer type/compound. Their polymer compositions were determined using a Fourier transform infrared spectroscopy (FTIR).

Results: The digestive tract had the highest abundance of MPs (26±11 particles/g) and the liver had the lowest abundance (2±0.2 particles/g). The average MPs observed in all tissues studied was 83.11±92.5 particles. The highest abundance was fiber MPs (92.96%) and the lowest abundance was fragments (7.04%). The 300-600 µm group had the highest abundance (31.37%), while the 3000-5000 µm group had the lowest abundance (5.88%). Black color had the highest abundance (54.9%) and pheasant color had the lowest abundance (1.96%). The polymer composition of MPs included polyethylene (PE; 57.14%), polyethylene terephthalate (PET; 23.81%), polypropylene (PP; 9.52%), and polyester (PS; 9.52%).

Conclusion: All examined fish contained plastic particles, mainly in the gastrointestinal tract. Common MPs were synthetic fibers from PE and PET. While plastic can bioconcentrate in tissues, it does not bioaccumulate significantly, indicating low biomagnification potential in rainbow trout. Recirculating aquaculture systems (RAS) components contribute minimally to contamination.

Keywords: Microplastics (MPs), Recirculating aquaculture systems (RAS), *Oncorhynchus mykiss*, Plastic polymers, Pollution

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Introduction

Microplastics (MPs), as emerging pollutants, pose significant concerns within the aquaculture industry. These small particles, measuring less than 5000 micrometers, can enter aquaculture systems through various sources and negatively impact fish health and seafood quality, particularly in recirculating aquaculture systems that heavily rely on plastic components (Ali et al., 2024).

Aquaculture, as the fastest-growing sector of food production, participate nearly in half of the fish consumed by humans (Hoseini et al., 2025). This industry contributes to global food security while facing new challenges, such as MPs. MPs can enter aquaculture systems through external sources, including human activities and storage environments (Hoseini et al., 2022a). Additionally, fish feed may become contaminated with MPs due to pollution of raw materials or during production processes (Hoseini et al., 2022b). The absorption of MPs has been observed in various fish species, under both natural and cultured conditions (Oza et al., 2024). These particles can pose additional risks to human health, particularly when they enter the food chain (Liu et al., 2022).

Recirculating aquaculture systems (RAS) are recognized as a sustainable solution for seafood production, relying on nutrient recycling and reduced water consumption (Naylor et al., 2021). However, these systems may face the accumulation of pollutants, including MPs, raising serious concerns within the aquaculture industry (Martins et al., 2009). In response to concerns regarding MPs, efforts are underway to develop non-plastic alternatives, such as natural biodegradable filter materials (Mnyoro et al., 2022). These modifications in the structure and content of the text facilitate a better understanding of the challenges and impacts of MPs in the aquaculture industry.

Exposure to waterborne and diet-borne MPs has been found to induce serious drawbacks to fish, including pathological damage to vital organs. These injuries are primarily associated with immune responses. Furthermore, exposure to MPs can lead to oxidative tissue damage, neurotoxicity, behavioral changes, and growth disruption (Jia et al., 2024; Rashid et al., 2025; Şener et al., 2025).

In 2020, global production of rainbow trout (*Oncorhynchus mykiss*) reached 981,239 tons (FAO, 2022). Of this total, 99% was produced through aquaculture, predominantly comprising rainbow trout, which accounted for 98% of the overall production. Iran, with a production of 197,370 tons (20% of global output), is recognized as the largest producer of rainbow trout in the world, followed by the European Union-27, which produced 187,936 tons in 2020 (19% of global output) (FAO, 2022).

MPs have emerged as a global environmental concern due to their widespread presence in aquatic and terrestrial ecosystems. Although extensive research has been conducted on MPs in marine environments, freshwater systems—particularly aquaculture facilities in Iran—have not been sufficiently investigated. This study aimed to address this knowledge gap by quantifying and identifying MPs in a freshwater aquaculture system in Iran. The focus of this research was on MPs in rainbow trout from key production provinces, namely Mazandaran and Golestan. The objective of this research was to establish baseline data regarding MPs in freshwater aquaculture systems in Iran, which are of significant importance for local livelihoods and regional economies.

Materials and Methods

Fish collection and sample preparation

The RAS facilities studied in this study included various plastic components. The fish tanks, connecting pipes, and filters are made from polyvinyl chloride (PVC), polypropylene (PP), polyethylene (PE), and polyester (PS) and polyethylene terephthalate (PET).

This study examined 60 adult rainbow trout, randomly selected during harvest from growth tanks in commercial RAS facilities located in Mazandaran and Golestan. To comply with ethical guidelines for fish handling and minimize suffering, the fish were euthanized using a buffered solution of 300 mg/L MS-222 with sodium bicarbonate ($NaHCO_3$) (Rairat et al., 2021). Prior to dissection, each fish's total length (cm) and weight (g) were measured. To eliminate surface contaminants, the fish were rinsed with distilled water. Tissue samples from the gut, gills, and liver were collected and subsequently stored at -20 ˚C for MP analysis. The mean sample weights (±SD) are detailed in Table 1.

Dissection was performed to obtain samples from the gut, gills, and liver. The wet weight of each organ was recorded before placing the samples into sterile screw-cap glass vials. These samples were stored at -80 ˚C until further analysis. Fish were transported on ice to the Ecology Laboratory at Gonbad Kavous University for processing.

Table 1. Morphological statistics of rainbow trout

Sampling Province	Number of Fish	Mean±SD			No. (%)				Mean±SD
		Total Length (cm)	Total Weight (g)	GIT Weight (g)	Fiber		Fragment		Mean number of MPs per Fish
Mazandaran	30	26.82±2.9	653±51.03	5.91±2.81	41	90.18	3	6.82	1.01±0.91
Golestan	30	24.91±2.15	601±49.54	5.6±3.61	25	92.59	2	4.55	0.91±0.64

GIT: Gastrointestinal tract.

Separation and visual identification of plastic particles

To isolate and identify plastic particles, different tissues of fish were processed. A solution of KOH (10%) was prepared to digest the samples at a ratio of 3:1 (v:w). The mixtures were incubated at 60 ˚C for 24 hours. Then, the plastic particles were separated by vacuum filtration (1.2 µm membrane). The filters were then dried for 24 hours at 40 ˚C, and the particles retained on the filters—suspected to be of plastic or anthropogenic origin—were examined and measured using a stereomicroscope equipped with a Nikon camera (SMZ1000 with DS-Fi1, Japan). Following the guidelines for plastic pollution monitoring (GESAMP, 2019), particles were categorized based on their shape (fibers or fragments), color (black, blue, brown, gray, transparent, and red), and size (all particles measured were below 3000 µm). The size of each particle (largest cross-sectional dimension) was categorized into the following ranges: 100–300 µm, 300–600 µm, 600–1000 µm, 1000–2000 µm, and 2000–3000 µm. An imaging analysis software program (Olympus Cell, GmbH, Hamburg, Germany) was used to determine particle size.

Polymer quantification

The analysis of samples was conducted in accordance with the methodology outlined by Gholizadeh et al. (2024). Size exclusion chromatography was employed to separate compounds using an Acquity LC system (Waters, Milford, MA, USA) equipped with an advanced polymer chromatography (APC) column. MP polymer identification was performed using Fourier-transform infrared (FTIR) spectroscopy with an ATR attachment, utilizing a Nicolet Nexus 470 spectrometer (Thermo Nicolet, USA) connected to OMNIC software and a ZnSe crystal analyzer. The analyzed spectral range was from 4,000 to 650 cm^{-1}. Calibration of the instrument was conducted prior to each test, and the spectra were evaluated for polymer differentiation and identification based on peak shape and position.

Contamination control

To mitigate the risk of external MP contamination during sample handling, the samples were collected and processed in a controlled environment free from potential airborne plastic contamination. Personnel involved in sampling wore full-cotton white coats and powder-free nitrile gloves during the sampling procedure. Plastic equipment was avoided, as far as possible, during sampling. Cross-contamination between and within samples was avoided by cleaning all surfaces with 70% ethanol and ultrapure water (Karami et al., 2017).

Results

MPs were detected in all fish samples from both Mazandaran and the Golestan provinces. The mean MP abundance was 44 particles/fish in Mazandaran ponds and 27 particles/fish in the Golestan ponds (Figure 1).

From the examined fish samples, 71 particles were initially recovered, with 28% excluded from the final analysis due to contamination from control samples (particularly cellulosic fibers). Only fibers showing clear signs of degradation and discoloration were included. Ultimately, 39 particles recovered from various rainbow trout tissues were selected for analysis.

The total plastic particles per fish ranged from 2 to 4, with a mean of 3.2±1.7 particles/fish. Figure 2 displays representative plastic particles found in different trout tissues. No significant correlation ($P>0.05$) was observed between sample weight and plastic particle count.

Morphological characteristics, size, and color of MPs in rainbow trout

Fibers (92.96%, $P<0.05$) dominated the particle shapes recovered from trout tissues, followed by fragments (7.04%) (Figure 3). Fiber prevalence was highest in the gut (58.54%), gills (34.15%), and liver (7.32%) in Mazandaran and similarly distributed in the Golestan samples (56%, 36%, and 8%, respectively).

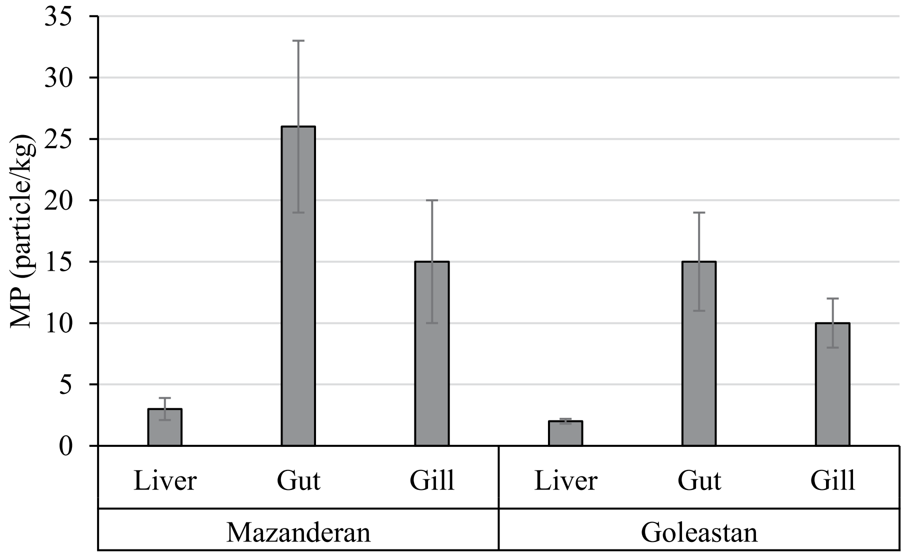

Figure 1. The average frequency of MPs in the sampling sites

Six color categories were identified (Figure 3B), with black (54.9%), gray (15.69%), and brown (13.73%) being most prevalent across all tissues ($P<0.05$). Size distribution analysis revealed significantly higher abundance of 300-600 µm (31.37%) and 100-300 µm (29.41%) particles ($P<0.05$, Figure 3C). Significant correlations were found between particle shape and the predominant color/size categories.

Polymer composition analysis

FTIR analysis successfully identified polymers in 39 of the 71 observed MPs. Four polymer types were detected: PE (57.14%), PET (23.81%), PP (14.29%), and PS (9.52%) (Figure 4). No significant differences were found in polymer composition between sampling sites ($P=0.15$).

Discussion

This study provides the first documented evidence of MP contamination in various tissues of farmed rainbow trout raised in RAS. Our findings corroborate existing data on the presence of MPs in samples from open aquaculture systems. Notably, the majority of the detected particles were classified as MPs (<5000 µm), and their diverse characteristics reflect the variety of particles present in the analyzed RAS.

As the present study showed, trout reared in RAS are readily contaminated by MPs, which might come from the river water and/or RAS facilities. Among all the tissues examined, the gut exhibited the highest levels of MP contamination. This observation suggests that this species originally tend to ingest plastics, as reported before in the fish from wild and aquaculture facilities (Alak et al., 2023).

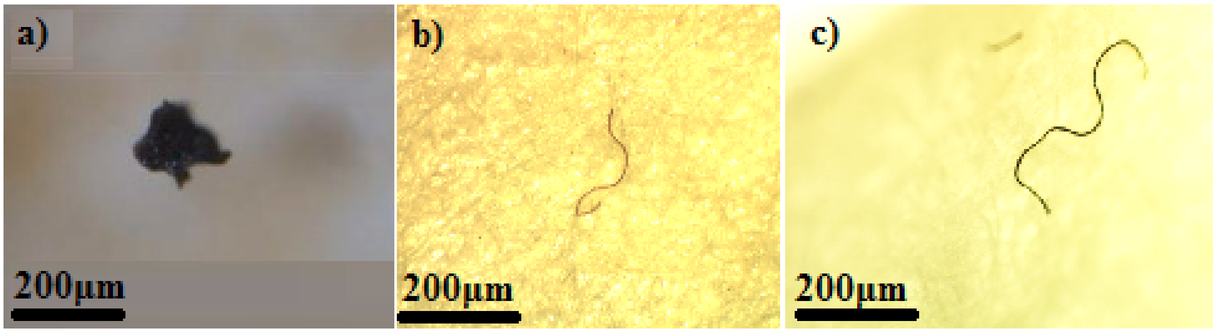

Figure 2. Micrographs of common MPs

a) Fragments, b and c) Fibers

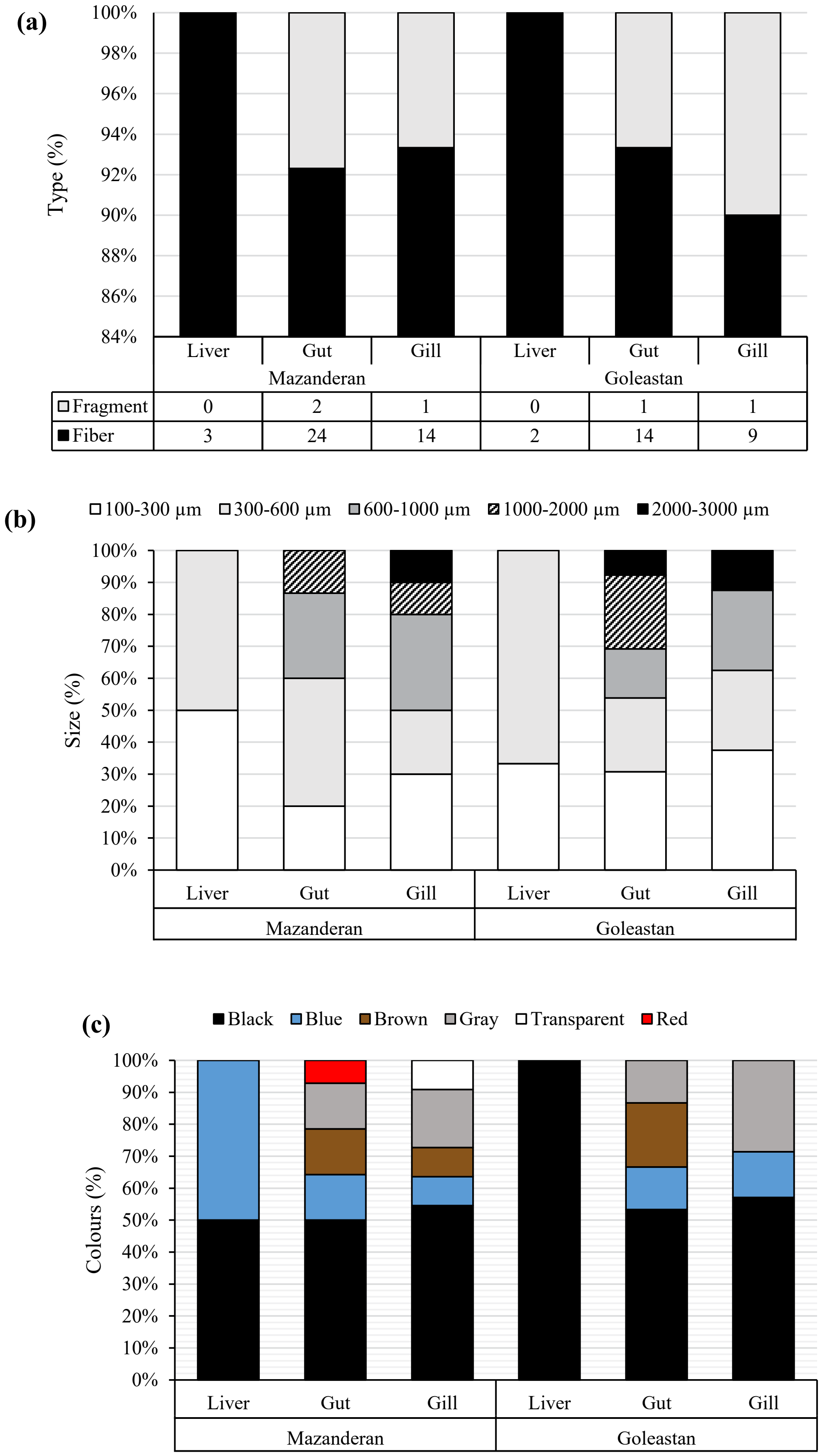

Figure 3. Percentage of extracted MPs categorized by (a) type, (b) size, (c) color in µm

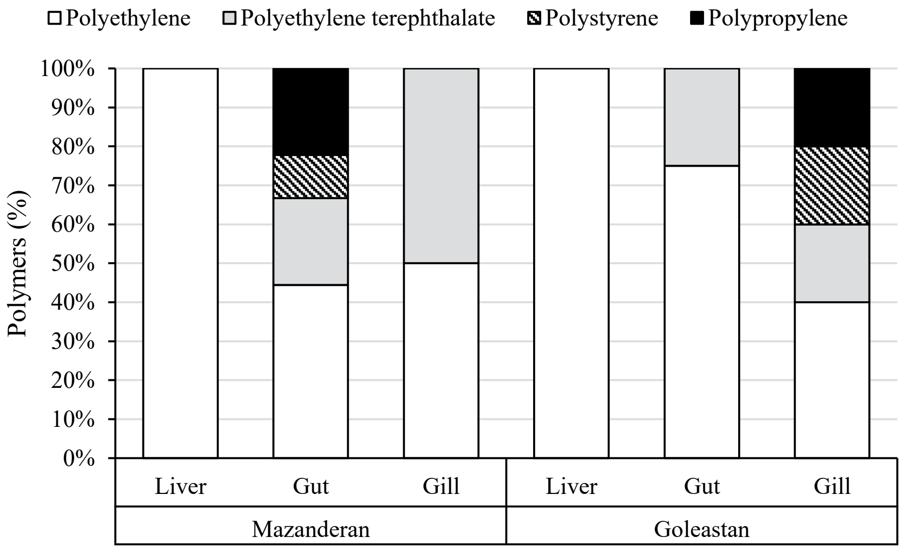

Figure 4. Abundance of polymers identified from study samples

Of the 39 MPs recovered from the gut, black, gray, and blue fibers were the most prevalent in terms of shape and color. The size of MPs found in the gut ranged from 123 to 2511 µm, with overall particle sizes being larger than those found in other body parts. Notably, PE comprised more than half of the MPs detected in the gut. This predominance can be attributed to its high abundance in RAS water, underscoring the significance of this exposure route for fish. As visual predators, trout may inadvertently consume MPs directly from the water, probably by mistaking them for prey (Siddique et al., 2024).

In this study, fish were fasted for 24 hours prior to sampling, during which most ingested MPs were likely excreted. Consequently, all fish presented empty guts at the time of collection, suggesting that a substantial number of MPs may have been passively eliminated in their feces. Therefore, caution is warranted when evaluating fish with gut contents, as this may lead to an overestimation of MP levels.

Similar to the gut, the gills serve as a primary barrier against environmental stressors, potentially retaining more particles through passive filtration as water passes through the gill filaments (Kılıç et al., 2022). In terms of MP characteristics, the gills contained fibers comprising a total of 36% of MPs per body. This prevalence can be attributed to the higher occurrence of fibers in water and their potential retention during respiration (Bour et al., 2020). The colors and size range (57–6672 µm) of plastic particles found in the gills were comparable to those identified in the gut.

Fish gill contamination by MPs is common; for example, Kılıç et al. (2022) found MPs in gill filaments of *Mullus barbatus*, *Mullus surmuletu*s, *Mugil cephalus*, and *Saurida undosquamis*. Despite the potential release of polymers from certain components of RAS, the majority of particles recovered were synthetic polymer fibers, such as PE and PET. This underscores the significant role that both water input and air pollution play in RAS facilities (Parta et al., 2020).

The findings from this study further affirm the critical role of the gut and gills as sentinel organs for monitoring MP pollution in both natural and artificial aquatic environments. Out of the 71 MP particles identified in the gills, fibers constituted the largest proportion. This prevalence can be attributed to two factors: the dominance of fiber-shaped particles in the surrounding water and the gills' high capacity to capture these fibers during respiration.

Although some polymers may originate from RAS system components, the majority of recovered particles were synthetic. This suggests potential contamination sources, including water entering the system and aeration-related pollution within the facility. The presence of MPs in the liver and gut, which exhibited similar characteristics to those found in the gills, indicates that MPs can accumulate in internal organs. Notably, the levels of MPs detected in the muscle tissue of the fish raised in RAS were consistent with those reported in wild-caught fish. The study also revealed that MP uptake was likely restricted to particles smaller than 300 µm, with only 10% of MPs found in the liver and 55% in the gut being absorbed. Furthermore, particles detected in the liver were significantly smaller than those in the gut and gills, with all particles measuring less than 500 µm. This observation can be explained by two factors: the absence of size restrictions for particles in the gut and gills, and the preferential transport of smaller particles to internal organs. These results illustrate the complex mechanisms governing the distribution and accumulation of MPs within aquatic organisms, highlighting the necessity for further research to fully comprehend the transport pathways and long-term impacts of these pollutants.

The study reinforces the importance of gills and the digestive tract as indicator organs for assessing MP pollution levels in aquatic environments. Given their direct exposure to water, natural filtration capabilities, and sensitivity to pollutants, these organs serve as effective tools for evaluating aquatic ecosystem health. Consequently, these findings underscore the need for stringent water quality control and pollution management in aquaculture facilities, as well as the development of continuous monitoring methods.

Conclusion

The aquaculture sector is increasingly transitioning toward sustainable production systems to ensure high-quality and safe seafood products. Among various production systems, RAS represent the most technologically advanced approach for sustainable aquaculture. This study provides the first evidence of plastic particle contamination in various organs of farmed rainbow trout reared in RAS facilities. Every fish sample analyzed contained plastic particles, with the gut exhibiting the highest average particle density per gram. Notably, the particles found in the gut and gills were significantly larger than those detected in the liver and muscle tissues.

The most commonly identified MPs in the RAS were synthetic fibers, primarily made of PE and PET, which were mostly black, gray, or blue, measuring between 123 and 2598 µm. Our research indicates that while plastic particles can accumulate within fish tissues from their aquatic environment (a process known as bioconcentration), they do not appear to bioaccumulate or increase in concentration as they move up the food chain, suggesting a minimal biomagnification risk in rainbow trout. Furthermore, no notable differences in oxidative stress indicators were found between fish with low (<2 particles) and high (≥4 particles) MP loads. The limited presence of RAS-related polymers (e.g. PE and PP) suggests that system components contribute minimally to MP contamination.

Nevertheless, we recommend the following: implementing alternative (non-plastic or more durable) system components; regularly monitoring the lifespan of plastic equipment to minimize MP leaching; and conducting further research on water quality and feed composition to mitigate contamination risks. Our results indicate that RAS-produced fish primarily ingest MPs through water and feed. Future studies should conduct comprehensive MP risk assessments for fish and human health, establish monitoring protocols for production systems, and provide policymakers with data to develop effective mitigation strategies. These findings highlight the need for proactive measures to ensure the sustainability and safety of RAS aquaculture while maintaining its environmental advantages.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization: Ali Taheri Mirghaed, Hosseinali Ebrahimzadeh Mousavi, and Seyed Saeed Mirzargar; Methodology: Sina Vahdatirad; Investigation: Sina Vahdatirad; Formal analysis and project administration: Mohammad Gholizadeh; Mohammad Gholizadeh; Writing the original draft: Sina Vahdatirad and Mohammad Gholizadeh; Review and editing: Ali Taheri Mirghaed, Hosseinali Ebrahimzadeh Mousavi, and Seyed Saeed Mirzargar.

Conflict of interest

The authors declared no conflict of interest.

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