

A Comprehensive Investigation of Microplastic Occurrence and Sources in the Gastrointestinal Tracts of Farmed Trout from Kastamonu, Türkiye

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Received

09.09.2025

Accepted

12.06.2026

Published

30.06.2026

DOI

10.47027/duvetfd.1780503

How to cite: Gökmen S, Doğan V, Çalkandere H (2026). A comprehensive investigation of microplastic occurrence and sources in the gastrointestinal tracts of farmed trout from Kastamonu, Türkiye. *Dicle Univ Vet Fak Derg.*, 19(1):122-129.

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Abstract

Microplastic (MPs) pollution in aquatic environments has raised global concerns regarding its hazards to the biota. Few studies have assessed MPs in farmed trout reared in inland waters. This study evaluated MPs in the gastrointestinal tract (GIT) of trout, considering feed and water as potential exposure sources. We assessed the prevalence, burden, color, length, and polymer type of MPs in the GIT of 100 trout (n=20 per location), as well as in feed and water samples from five locations in Kastamonu, Türkiye. The mean MPs abundance in trout GIT contents ranged from 1.15 ± 0.36 to 3.05 ± 1.57 MPs fish⁻¹ (P<0.05). MPs levels in water and feed samples ranged from 11.10 ± 1.25 to 20.65 ± 1.63 MPs L⁻¹ and 3.10 ± 1.30 to 7.15 ± 1.42 MPs kg⁻¹, respectively (P<0.05). Filaments-type MPs were dominant in water (47.6-77.8%) and feed (50-66.7%), followed by fragments. However, fragments had the highest GIT content (44-60%), followed by filaments. The majority of MPs were polyamide and polyethylene in water; polypropylene and polyvinyl stearate in feed; and polyamide and polyethylene terephthalate in GIT content. Significant correlations existed between MPs concentrations in feed and GIT content samples (P<0.001). This study demonstrated MPs presence in water, feed, and GIT content of trout farms in Kastamonu/Türkiye, highlighting ecological risks and the need for mitigation strategies.

Key Words: Feed, GIT content, microplastic, trout farm, water

Kastamonu'da (Türkiye) Yetiştirilen Alabalıkların Gastrointestinal Sisteminde Mikroplastik Varlığı ve Kaynaklarının Kapsamlı İncelenmesi

Öz

Su ortamlarında mikroplastik (MPs) kirliliği, canlılar üzerindeki tehlikeleri nedeniyle dünya çapında endişelere yol açmıştır. İç sularda yetiştirilen kültür alabalıklarında mikroplastiklerin değerlendirilmesiyle ilgili çok az çalışma bulunmaktadır. Bu çalışma, alabalıkların gastrointestinal sistemindeki (GİS) mikroplastikleri, yem ve suyu olası maruz kalma kaynakları olarak değerlendirerek incelemiştir. Kastamonu'daki (Türkiye), beş farklı tesisten alınan yem, su örnekleri ve toplamda 100 alabalıktan (n=20) elde edilen GİS içeriğinde mikroplastiklerin yaygınlığı, yükü, rengi, uzunluğu ve polimer tipi değerlendirildi. Alabalıkların GİS içeriğinde ortalama mikroplastik yaygınlığı, 1.15 ± 0.36 - 3.05 ± 1.57 MPs balık⁻¹ arasında değişmekteydi (P<0.05). Su ve yem örneklerindeki mikroplastik yaygınlığı sırasıyla 11.10 ± 1.25 - 20.65 ± 1.63 MPs L⁻¹ ve 3.10 ± 1.30 - 7.15 ± 1.42 MPs yem⁻¹ arasında bulundu (P<0.05). Su (%47.6-77.8) ve yem (%50-66.7) örneklerinde filament şekli baskın olup bunu fragment izlemiştir. Ancak GİS içeriğinde fragment (%44-60) daha yaygın olup bunu filament şekli takip etti. Su örneklerinde mikroplastiklerin çoğunluğu poliamid ve polietilenden oluşurken; yemde polipropilen ve polivinil stearat, GİS içeriğinde ise poliamid ve polietilen tereftalat tespit edildi. Yem ve GİS içeriği örneklerindeki MP konsantrasyonları arasında anlamlı korelasyonlar saptandı (P<0.001). Bu çalışma, Kastamonu'daki alabalık yetiştiriciliği yapılan tesislerde su, yem ve balık GİS içeriğinde mikroplastik varlığını ortaya koymuş; ekolojik risklere ve azaltım stratejilerinin gerekliliğine dikkat çekmiştir.

Anahtar Kelimeler: Alabalık, GİS içeriği, mikroplastik, su, yem

INTRODUCTION

Plastic products have brought convenience to modern life due to their properties and low costs. Although global annual plastic production was only 1.5 million tons in the 1950s, it reached approximately 359 million tons in 2018 (1,2). However, plastic pollution has become one of the most serious ecological concerns worldwide, due to poor waste management and low biodegradability. According to reports, only 9% of plastic waste is recycled, 12% is incinerated, and 79% pollutes the environment (3). Plastic waste exists in nearly all parts of the environment, and plastic products are used across various industries. Through sunlight exposure, microbial activity, and mechanical degradation, plastics break down into smaller pieces, resulting in microplastics (MPs) and nanoplastics, which pose significant environmental and health risks (4).

Plastics are classified into four categories based on their sizes: macroplastics (>25 mm), mesoplastics (5-25 mm), MPs (1 µm-5 mm), and nanoplastics (<1 µm) (5). MPs have been detected in various environments worldwide, including aquatic systems (e.g., seas, rivers, lakes, reservoirs, and ponds), terrestrial ecosystems (e.g., soil, islands, cities, and beaches), and the atmosphere (1). The most frequently identified polymers in these environments are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyamide (PA), polyvinyl stearate (PVS) and polyethylene terephthalate (PET) (6). Owing to their small size and widespread distribution, MPs can be easily ingested or inhaled by organisms and accumulate in different tissues, thereby posing potential health risks. Consequently, MPs have received increasing attention worldwide, and studies in this field have been growing steadily (1).

MPs in aquaculture environments originate from five sources: (a) land-based plastic waste, (b) tourism-related plastic waste, (c) maritime transportation, (d) fishing and aquaculture activities, and (e) atmospheric deposition. Land-based plastic waste is the primary source of MPs in aquatic systems (7). Plastic materials are extensively used in fishing and aquaculture (8). These materials release MPs into aquatic environments through the degradation of fishing gear, especially ropes and nets, thereby contributing to elevated MPs concentrations in water bodies. MPs contamination can also occur via the fish feed used in aquaculture systems (8,9).

Numerous studies have shown that marine waters are heavily contaminated with MPs, and a substantial number of marine fish species have been found to contain MPs particles (10-13). Fish feed, a central component of aquaculture systems, is a significant source of MPs pollution (9). MPs have been detected in different types of fish feeds, with an average particle size of $452 \pm 161 \mu\text{m}$ (14). Moreover, a positive correlation has been reported between MPs concentrations in feed and their accumulation in farmed fish species such as cultured common carp (9,15).

Fish are important components of the human diet across diverse sociocultural groups because of their high-quality proteins, essential fatty acids (particularly omega-3), and other vital nutrients (16). Rainbow trout (*Oncorhynchus mykiss*), a benthopelagic species, has been commercially farmed in Türkiye since the 1970s, representing 28% of its

national aquaculture production, with 134 174 tons produced in 2021 (17,18). While they feed on aquatic invertebrates and small fish in natural habitats, farmed trout are typically fed commercial diets containing fishmeal derived from wild-caught fish or processing by-products (19).

This study aims to investigate the presence of MPs in the gastrointestinal tract of farmed rainbow trout reared in freshwater aquaculture systems and comprehensively assess the relationship between MPs contamination in fish, water, and feed.

MATERIAL AND METHODS

Production Facilities and Sample Collection

A total of 100 market-sized rainbow trout (*Oncorhynchus mykiss*), each weighing between 160 and 250 g, were collected from five distinct production sites in Kastamonu Province and its district, Türkiye. Twenty fish were obtained from each location, including those from the concrete rearing units and net enclosures within the aquatic system.

Each fish was individually encased in aluminum foil and labeled (K1-K5) before being transported from the facilities to the laboratory. They were kept at a temperature of -86°C until the gastrointestinal tract (GIT) contents were extracted. Once thawed, the abdominal cavities of the fish were opened, and the section from the upper esophagus to the anal opening was removed using surgical scissors. The GITs were dissected, and all contents were placed in aluminum drying vessels. The vessels were placed in an incubator (Nüve, EC 160) and dried at 80°C for 24 h.

Water samples were collected from 20 points within the fish-farming ponds, with 2 L taken from each pond using glass jars. Fish feed samples (50 g) were obtained from 20 commercial feed bags used in aquaculture facilities. Upon arrival at the laboratory, feed samples were dried at 80°C for 24 h and ground into powder for analysis.

Microplastic Extraction

MPs extraction from the GIT contents was performed as described by Dogan et al. (20) (20). In brief, 3 g of dried GIT content was digested with 100 mL of 10% KOH at 60°C for 24 h, centrifuged (1000 rpm, 5 min), and the supernatant was vacuum-filtered through glass fiber filters (25 mm GF7F, Whatman, Sigma-Aldrich, Germany). The feed samples were ground and homogenized, after which 3 g aliquots were combined with 10 mL of distilled water. The mixture was shaken at 200 rpm for 24 h, centrifuged at 1 000 rpm for 10 min, and the resulting supernatant was filtered. Water samples were filtered immediately upon arrival at the laboratory.

Avoiding Extra Contamination

During extraction and filtration, all liquid solutions (e.g., distilled water and KOH) were pre-filtered to prevent MPs contamination. All glassware was rinsed with filtered distilled water, ethanol, acetone, and distilled water prior to use. All filters were preheated at 300°C for 5 h prior to use. All the filtration processes were conducted in a laminar flow cabinet (MetiSafe, Türkiye).

Light Microscopy Examination

After filtration and drying, the filters were examined under a light microscope (Leica DM500, Germany). Suspected MPs particles were classified as filaments, fragments, or films, and all the particles were counted individually. Each observed particle was photographed and measured using a Leica ICC50W camera. Fourier-transform infrared (FTIR) spectroscopy was performed to further characterize MPs.

Fourier Transform Infrared Spectroscopy

Polymer identification in the GIT content, water, and feed samples was conducted using a Fourier-transform infrared (FTIR) spectrophotometer with a single-reflection attenuated total reflectance (ATR) accessory (ATR-FTIR) (Perkin Elmer, Spectrum-two, USA). Analyses were performed in the 600-4000 cm^{-1} range with 4.0 cm^{-1} resolution and 32 scans in absorption mode. A background scan was conducted for each sample batch. The obtained data were compared with the library (Fiveash Data Management, Inc. 2006-2008) database for validation. A match rate of $\geq 70\%$ was used for polymer identification. Each measurement was performed in triplicates.

Microplastic Ingestion Through Water and Fish Feed

A quadratic regression model was used to assess the relationship between MPs contamination in the water and fish feed. The estimated model equation is as follows:

$$\text{Water MP} = 5.455 + (2.858 \times \text{Feed MP}) - (0.166 \times \text{Feed MP}^2)$$

Multiple regression analysis examined the factors contributing to MPs accumulation in the GIT content of fish. The estimated model equation is as follows:

$$\text{GIT content MP} = -0.355 + (0.099 \times \text{Water MP}) + (0.182 \times \text{Feed MP})$$

The ingestion of MPs by farmed rainbow trout can be estimated using feed consumption and MPs contamination equations. Equations 1 and 2 from Muhib and Rahman (2023) (9) calculated MPs in feed ingested by *O. mykiss*. The total feed consumed (Fc) and estimated MPs intake (Pe) were calculated using the following equations.

Equation (1):

$$F_c = \text{Average weight of cultured fish} \times \text{FCR}$$

Equation (2):

$$P_e = F_c \times \% \text{IFM} \times \text{MPFF}$$

Fc is the total amount of feed (kg) consumed by the fish,

FCR is the feed conversion rate,

%IFM is the percentage of feed intake relative to body weight,

MPFF is the number of MP particles per kg of fish feed.

Data Analysis

Statistical analyses were performed in SPSS (IBM SPSS Statistics 23). Descriptive statistics were calculated for MPs in water, feed, and GIT content. Normality was tested using Kolmogorov-Smirnov and Shapiro-Wilk. Normally distributed data (water MPs) were analyzed with one-way ANOVA and Tukey/Bonferroni post-hoc tests, while non-normal data

(feed and fecal MPs) were analyzed with Kruskal-Wallis and Mann-Whitney U tests (Bonferroni correction). Spearman's correlation was used to assess relationships among sample types, and multiple regression was applied to predict fecal MPs from water and feed concentrations. Significance was set at $p < 0.05$.

RESULTS

Fish Sampling Results

A total of 100 rainbow trout (*O. mykiss*) were analyzed in this study, with individual weights ranging from 161.23 to 249.65 g and a mean weight of 205.23 ± 21.53 g. The weights of the fish varied among the sampled establishments, with averages of 219.31 ± 17.2 g in K1, 196.48 ± 19.41 g in K2, 205.51 ± 19.4 g in K3, 190.53 ± 19.25 g in K4, and 214.32 ± 19.78 g in K5. However, no significant differences in fish size were observed between the establishments ($P > 0.05$).

Microplastic Abundance in GIT Content Samples

MPs polymers of varying shapes and sizes were detected in all GIT samples from fish across establishments. Fragments were the most abundant type of MPs, representing 44–60%. The filament-type MPs varied, with the highest proportion in Establishment K4 (41%). Film-type MPs were found at lower percentages (9–21%) and were highest in Establishment K3 (Figure 1a). The GIT contents of rainbow trout from all Kastamonu establishments showed that MPs were mainly fragments (54%), filaments (37%), and films (9%). Significant differences in MPs particle length were observed among the establishments (Table 1, Figure 1 and 2). The MPs lengths ranged from 51 to 3651 μm , with a mean of 784 ± 866 μm .

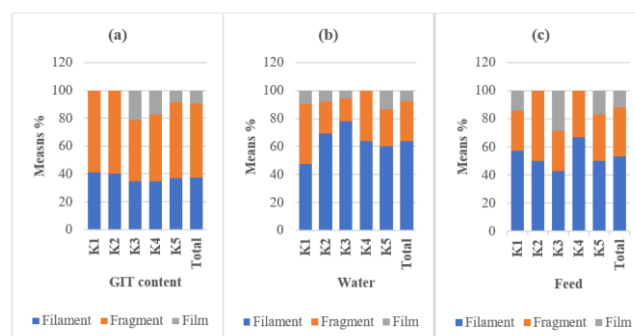


Figure 1. Percentage distribution of MPs morphologies identified in the GIT content (a), water (b), and feed (c) samples

Table 1. Descriptive statistics of MP particle sizes (μm) detected in water, feed, and GIT content samples (mean $\pm$ SD)

Establishment	Microplastics Mean Length (Min-Max)		
	Water	Feed	GIT content
K1	635 $\pm$ 654 (105-2400)	1016 $\pm$ 767 (380-2241)	770 $\pm$ 923 (110-3478)
K2	445 $\pm$ 307 (132-963)	1153 $\pm$ 771 (541-2214)	609 $\pm$ 804 (51-3369)
K3	658 $\pm$ 515 (70-1744)	885 $\pm$ 666 (297-2098)	826 $\pm$ 847 (90-2879)
K4	983 $\pm$ 932 (155-3258)	1149 $\pm$ 503 (774-1874)	773 $\pm$ 771 (73-2654)
K5	554 $\pm$ 343 (162-1202)	1279 $\pm$ 855 (413-2478)	950 $\pm$ 1029 (162-3651)
Total	656 $\pm$ 600 (70-3258)	1083 $\pm$ 686 (297-2478)	784 $\pm$ 866 (51-3651)

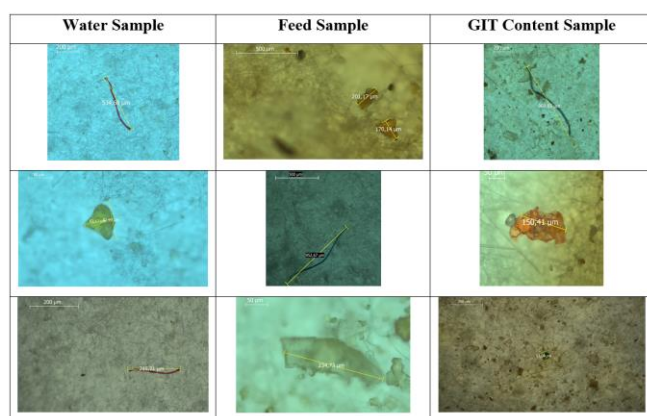


Figure 2. Examples of extracted MPs from water, feed, and GIT content samples

After selecting 25 particles from each group, the MPs polymer types varied across establishments. PA was frequently detected, with the highest levels in Establishment K1 (44%) and notable amounts in K3 (29%) and K5 (24%). PET was most prevalent in Establishment K2 (38%), ranging from 7% to 21% elsewhere. PP was predominant in Establishment K4 (29%). PVS showed a balanced distribution of 8–24%, the highest in K4 (21%) and K5 (24%). PE varied considerably, with the highest levels in Establishments K3 (25%) and K4 (29%), but lower elsewhere. In the GIT contents of rainbow trout from all Kastamonu establishments, PA was the predominant polymer (24%), followed by PET (20%), PE (18%), PP (18%), and PVS (16%) (Figure 3a, Figure 4a-b).

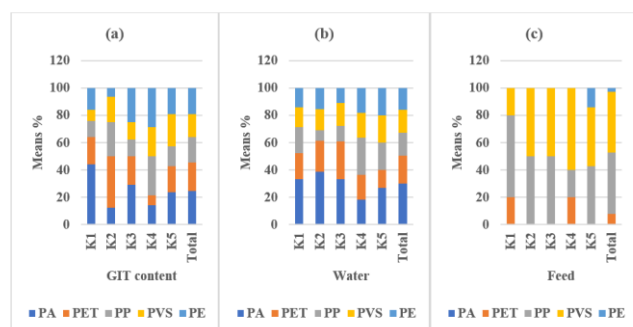


Figure 3. Percentage distribution of MPs polymer types detected in GIT content (a), water (b) and feed (c) samples

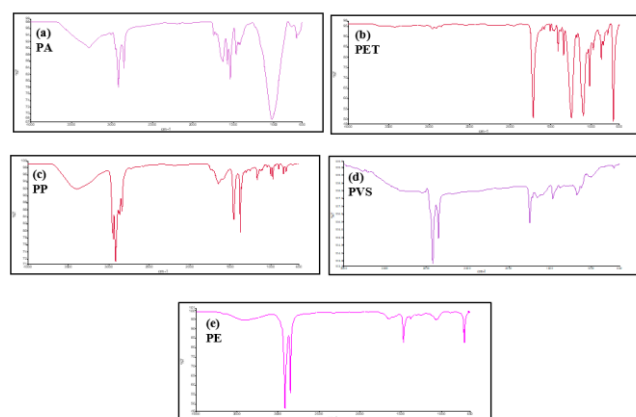


Figure 4. Representative FTIR spectra of selected polymer types. (a–b) Polyamide (PA) and polyethylene terephthalate (PET) identified in GIT content; (c–d) polyvinyl stearate (PVS) and polypropylene (PP) identified in water samples; (e) polyethylene (PE) identified in feed samples.

Descriptive statistics for MPs amount in fish GIT from various establishments are presented, showing significant differences in MPs concentrations ($P < 0.001$; Table 2). The mean rank values for GIT contents MPs contamination were $K1 > K3 > K5 > K2 > K4$, reflecting MPs levels in fish feed samples. Rainbow trout from all Kastamonu aquaculture farms averaged 2.17 ± 1.02 MPs fish⁻¹ in their GIT contents.

Table 2. Average MPs concentrations in water, feed, and GIT content for each establishment (mean $\pm$ SD).

Establishment	n	MPs/Water	MPs/Feed	MPs/GIT content
K1	20	20.65 $\pm$ 1.63 ^a	7.15 $\pm$ 1.42 ^a	3.05 $\pm$ 1.57 ^a
K2	20	12.45 $\pm$ 1.23 ^b	4.45 $\pm$ 1.42 ^c	1.75 $\pm$ 0.97 ^b
K3	20	17.70 $\pm$ 1.84 ^a	6.95 $\pm$ 1.91 ^a	2.60 $\pm$ 1.39 ^a
K4	20	11.10 $\pm$ 1.25 ^b	3.10 $\pm$ 1.30 ^d	1.15 $\pm$ 0.36 ^c
K5	20	15.00 $\pm$ 1.78 ^a	6.05 $\pm$ 2.12 ^b	2.30 $\pm$ 1.30 ^{ab}
Total	100	15.38 $\pm$ 5.54	5.54 $\pm$ 2.89	2.17 $\pm$ 1.02

^{a,b,c,d} Different letters in the same column indicate statistically significant differences ($p \leq 0.05$) based on one-way ANOVA followed by Tukey/Bonferroni post-hoc tests for normally distributed data (water MPs), and Kruskal–Wallis followed by Mann–Whitney U tests with Bonferroni correction for non-normally distributed data (feed and GIT MPs). MPs, Microplastics; GIT, gastrointestinal tract.

Establishment K1 showed the highest GIT contents MPs contamination, which was significantly higher than that of K4 ($P < 0.001$). K3 had relatively high contamination ($\chi^2 = 60.18$), showing a significant difference from K4 ($P < 0.001$) but no difference from K1 ($P = 0.398$). K5 showed moderate contamination ($\chi^2 = 54.90$), which was significantly higher than K4 ($P < 0.001$) and not different from K2 ($P = 0.225$). K2 ($\chi^2 = 44.30$) exhibited moderate contamination, which was lower than K1 and higher than K4. K4 showed the lowest GIT MPs contamination ($\chi^2 = 26.30$), which was significantly different from that of most establishments ($P < 0.001$). Statistical analysis revealed that the distribution of polymer types and amounts did not differ significantly among the establishments ($P = 0.640$ and $P = 0.343$, respectively). These results suggest that, although different polymer types were present across establishments, their occurrence was relatively consistent, implying potential similarities in pollution sources or exposure routes (Figure 5a).

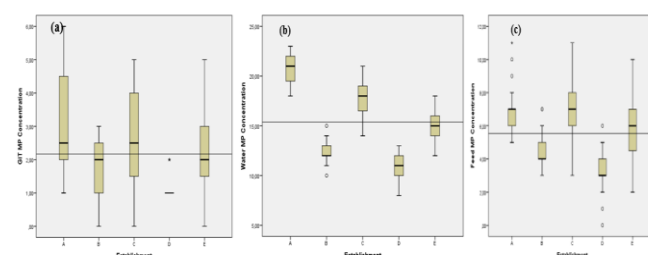


Figure 5. a) The boxplot for Establishment K4 shows minimal interquartile range, indicating low variance in GIT MPs concentrations. However, two extreme outliers suggest some individuals accumulated higher MPs levels. b) Box plot showing MPs concentration in water samples across establishments. K1 exhibited the highest concentration, while D had the lowest. The horizontal line shows overall mean MPs concentration. c) Box plot showing MPs concentration in feed samples across establishments. K1 and C had the highest contamination levels, while D showed the lowest. The horizontal line indicates mean MPs concentration. Outliers are shown by circles, and extreme values by asterisk (*).

Microplastic Abundance in Water Samples

Water samples (2 L each) were collected from rearing pools across the establishments and examined for contamination of MPs. The results showed significant differences in MPs morphologies. Filaments-type MPs were dominant across all establishments, reaching 78% in K3. Fragment-type MPs varied, with 43% in K1, whereas film-type MPs remained low, reaching a maximum of 13% in K3. Most MPs were filaments (64%) in the fish farms of the Kastamonu region, followed by fragments (29%), and films (7%) (Figure 1b).

Significant differences in MPs particle length were observed among the establishments in the water samples (Table 1, Figure 1 and 2). The particle lengths ranged from 70 μm to 3258 μm , with a mean of $656 \pm 600 \mu\text{m}$.

Analysis of 25 particles per group identified PA, PET, PP, PVS, and PE in the water samples. PA was most abundant in Establishments K1, K2, and K3 (33%, 38%, and 33%, respectively), whereas PE and PET were less common. In the water samples from all aquaculture establishments in Kastamonu, the main polymers were PA (30%), PET (20%), PVS (17%), PP (17%), and PE (16%) (Figure 3b and Figure 4c-d).

No significant differences in polymer type or amount were found among the establishments ($P=0.114$ and $P=0.178$, respectively). Post-hoc tests showed significant differences in water MPs among the establishments ($P=0.000$; Table 2). Establishment K1 had the highest contamination (20.65 MP/L), significantly exceeding that of all other establishments. K3 (17.70 MP/L) had higher MPs levels than did K5, K2, and K4. K2 and K4 had the lowest concentrations, with no significant difference between them ($P=0.058$) (Figure 5b).

Microplastic Abundance in Feed Samples

Twenty fish feed samples (50 g each) were collected from different establishments and were examined for MPs contamination. Various types of MPs polymers were detected in all the samples. Filament-type MPs were dominant (67%). Fragment-type MPs varied among the samples, with 50% in the feed from Establishment K2. Film-type MPs were found at low percentages, with the highest being 14% in the feed from Establishment K1 (Figure 1c). In the Kastamonu region, MPs particles were predominantly filaments (53%), fragments (35%), and films (12%). Significant differences were observed in MPs particle lengths among the establishments (Table 1, Figure 1 and 2). The minimum MPs particle length was 297 μm and the maximum was 2478 μm , with a mean length of $1083 \pm 686 \mu\text{m}$.

After selecting 25 particles from each group, the PET, PP, PVS, and PE polymers were identified in the feed samples. The polymer distribution differed significantly among establishments ($P=0.001$). PP and PVS were the most frequently detected, ranging from 20 to 60%. PE appeared only once, whereas PA was not detected in any sample. In fish feed from the Kastamonu region aquaculture establishments, PP (45%) and PVS (45%) were predominant, followed by PET (8%) and PE (3%) (Figure 3c and Figure 4e). Significant differences were observed between K2 and K3, K2 and K4, K3 and K4, and K3 and K5 ($P<0.005$). No significant differences were found between B and E ($P=0.007$) or between K3 and K5 ($P=0.108$). The results suggest that MPs contamination varies across establishments, with D having the lowest

contamination, and K3 and K5 having the highest contamination (Figure 5c). Establishment K5 showed the highest diversity among PP, PVS, and PE polymers. Establishments K1 and K4 contained all polymer types except PE, whereas K2 and K3 showed a limited range. These findings indicated that MPs contamination in fish feed varies across establishments with different polymers. The descriptive statistics for feed MPs amount across establishments showed significant differences ($\chi^2=52.878$, $P<0.001$), indicating that at least one establishment had different MPs concentrations (Table 2). Pairwise Mann-Whitney U tests were used to identify differences between establishments. The mean rank values for feed MPs content were in the order $K1 > K3 > K5 > K2 > K4$ (Table 2).

MP Intake Through Feed in Farmed Fish

Regression analysis showed a significant positive relationship between MPs concentrations in the water and fish feed samples ($p < 0.01$), suggesting that water contamination may be influenced by feed quality in aquaculture systems. A quadratic regression model assessed MPs contamination in water relative to feed across establishments. The model showed a significant association between MPs concentrations in feed and water ($P<0.001$) (Table 2), accounting for 39% of the variance in water MPs levels. The positive linear coefficient ($B=2.858$, $P<0.001$) indicated that increased MPs concentration in the feed was associated with higher MPs levels in water. The negative quadratic term ($B=-0.166$, $P=0.001$) shows a non-linear relationship, where water MPs concentration increases diminish at higher feed MPs levels (Figure 6). These results suggest that MPs transfer from feed to water is not purely linear, indicating that factors such as feeding rates may influence MPs dispersion. These findings highlight that feed is a major contributor to MPs contamination. The multiple regression model for MPs concentration in fish GIT content was significant ($P<0.001$), explaining 26% of the variance. Both feed ($B=0.182$, $P=0.005$) and water ($B=0.099$, $P=0.009$) showed significant positive effects on MPs concentrations in the GIT, indicating that MPs accumulation is influenced by dietary and environmental exposure.

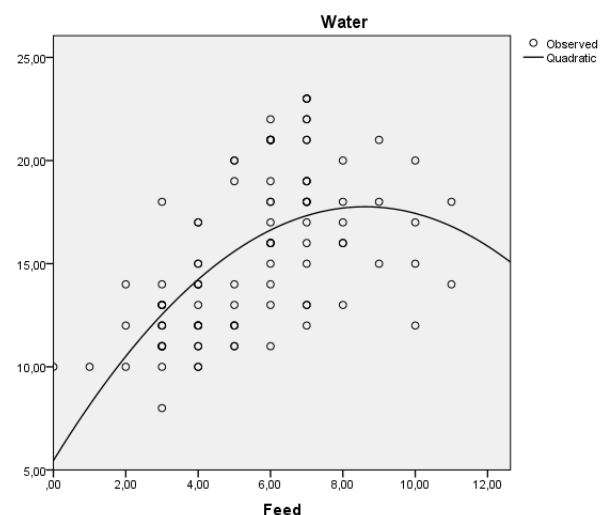


Figure 6. Relationship between MPs contamination in water and feed: Quadratic regression model

In this study, we adapted equations to estimate MPs ingestion by rainbow trout at various growth stages. The average fish weight was 205.23 ± 21.53 g. The corresponding FCR for rainbow trout was 1.5, while the %IFM was assumed to be 1.4 for fish with body weight ≥ 200 g (20-22). By applying these values to MPs contamination data from feed samples, we estimated the potential MPs ingestion rates of rainbow trout at various life stages. Based on the average fish weights observed across the five aquaculture facilities in Kastamonu.

$$F_c = 0.205 \times 1.5 = 0.308$$

$P_e = 0.308 \times 1.4 \times 1847 \text{ particle/kg of fish feed} = 796 \text{ MP particle ingested by fish}$

The MPFF value was indexed to kg based on 5.54 MPs particles detected in 3 g of dry matter feed. This value corresponds to approximately 3.38 MPs particles for the fish feed sample analyzed. This agrees with our study results, where a concentration of 2.17 MP GIT^{-1} was recorded (Table 2).

DISCUSSION AND CONCLUSION

MPs are persistent pollutants and their interactions with aquatic organisms have been extensively investigated (24,25). However, data on freshwater ecosystems are limited. This study is the first to assess MPs abundance in the gastrointestinal tract, water, and feed of commercially farmed rainbow trout in Kastamonu, Türkiye and to investigate the relationships among these components. This study revealed significant variations in MPs contamination among water, fish feed, and GIT content across aquaculture facilities away from major industrial or populated areas. This suggests that MPs sources in these inland waters may include atmospheric deposition, groundwater infiltration, or other diffuse pathways (3). These findings underscore the need to investigate the MPs entry pathways in aquatic systems, particularly in regions with minimal direct anthropogenic influence.

MPs exert harmful effects on fish health by affecting various physiological and biochemical functions. They cause structural damage to the digestive tract, liver, and muscle tissues, and impair growth performance and locomotor activity. MPs can disrupt the oxidative balance, modulate genes involved in immune responses, and compromise antioxidant defense systems. Neurological toxicity, developmental delays, and abnormal behavioral patterns occur in fish exposed to MPs (26). The detection of different MPs polymers, such as PA, PET, PP, PVS, and PE, in all water samples obtained from different establishments suggests that water is a critical medium for MPs exposure in aquaculture systems (27,28). These polymers are commonly associated with plastic-based materials used in aquaculture such as ropes, nets, pipes, and containers (8,9).

Kilic et al. (2022) demonstrated that MPs in trout GITs were predominantly fibers or filaments (89%), followed by fragments (11%) (29). Similarly, our results revealed that filaments were the most frequently observed MPs type (54%), with fragments (37%) and films (9%) occurring in lower proportions.

The absence of PA in feed samples, despite its detection in water samples and GIT content, suggests that feed is not the primary source of MPs ingestion in fish. Instead, PA in the GIT content likely originates from the surrounding

aquatic environment. Because PA was consistently detected in water samples across establishments, fish likely ingested PA through passive filtration or incidental uptake during feeding. Although statistical analysis showed no significant difference in the polymer type distribution among establishments, significant variations were observed in MPs concentrations. Establishment K1 exhibited the highest MPs contamination, which was significantly different from that of the other establishments. This may be attributed to differences in water sources, locations, proximity to pollution sources, management practices, or the plastic materials used in fish farming (30). This analysis revealed that MPs levels in feed could influence MPs concentrations in water, suggesting a link between feed contamination and aquatic MPs accumulation (31). The significant relationship between the MPs concentrations in water and fish feed suggests that contaminated feed may be a key source of MPs pollution in aquaculture systems. The quadratic regression model showed a non-linear pattern, where MPs levels initially increased with feed contamination but plateaued at higher concentrations, possibly because of feeding behavior or environmental factors. The model explained 39% of the water MPs variance, indicating that while feed is important, other sources, such as equipment or environmental inputs, contribute. These findings highlight the need to improve feed quality and management practices in order to reduce MPs contamination. Additionally, plastic materials used in fish farming, such as pipes, tanks, and feeding equipment, can contribute to water contamination (8). The correlation between these variables emphasizes the need to monitor MPs pollution throughout aquaculture to reduce the risks to aquatic organisms and human health.

The MPs in all fish feed samples confirmed that commercial feed serves as a direct source of MPs exposure in fish (9). The polymers identified (PET, PP, PVS, and PE) are commonly found in food packaging and processing materials (32). PA was not detected in feed samples despite its presence in water and GIT content, suggesting that PA contamination in fish is derived from water exposure rather than feed.

In terms of polymer composition, FTIR analysis in our study identified the dominant types of trout GITs content as polyamide (PA) (24%), PET (20%), PE (18%), PP (18%), and PVS (16%). These findings are consistent with those of Ece (2022), who reported that polyester (20%), polyamide (10%), and PE (25%) were the most common polymers in the total MPs particles extracted from the GITs of rainbow trout, gilt-head seabream, and European seabass. However, their study did not provide polymer-specific data exclusively for rainbow trout (29).

Statistical analysis identified significant variations in MPs concentrations across establishments, with K1 and K3 exhibiting the highest levels of contamination. These variations may be due to the differences in feed sourcing, processing, or storage. Feed contamination can occur during manufacturing or transportation, particularly during plastic-based packaging or handling. The detection of PVS and PP as common polymer types suggests that MPs may originate from synthetic additives or from pelletized feed processing. Given the critical role of feed in MPs exposure, further research should explore strategies to minimize MPs contamination in commercial fish feeds.

MPs in the GIT of the examined fish confirmed direct ingestion through contaminated feed and water. The most frequent polymer types in the GIT content samples were PA, PET, and PP, which is consistent with the findings from water and feed samples. While PE was detected in water and feed, it appeared in lower amounts in the GIT content, possibly indicating selective retention or differential digestion of polymers in fish. Statistical analysis revealed significant differences in MPs concentrations among the establishments. Establishment K1 had the highest MPs contamination in the GIT content, which was significantly higher than that of Establishment K4. This pattern resembled the contamination trends in the feed samples, suggesting a link between feed quality and MPs accumulation. Establishments K3 and K5 showed high MPs contamination levels, whereas K4 had the lowest. Variations in MPs accumulation may be influenced by feeding behavior, digestive efficiency, and fish size. The presence of outliers in Establishment K4 suggests that certain individuals accumulated higher MPs levels, possibly because of behavioral differences or selective feeding.

Multiple regression analysis showed that both fish feed and water significantly contributed to MPs accumulation in the farmed rainbow trout GIT content. Feed had a stronger influence ($B=0.182$, $P=0.005$) than water ($B=0.099$, $P=0.009$), suggesting that dietary intake is a primary MPs exposure pathway in aquaculture systems. The model explained 26% of the GIT content MPs concentration variance, indicating that while feed and water are important sources, other factors such as fish size, feeding behavior, and retention capacity may also play a role.

Studies worldwide have documented MPs in the GIT of freshwater fish species. According to our review, MPs abundance in fish GITs varies widely, ranging from 1.07 ± 1.76 22 ± 14.6 MP fish⁻¹. In Türkiye, studies have reported MPs contamination in fish tissues, including the GIT content, gills, and muscle. Kilic et al. (2022) investigated MPs in commercially important fish species from the Asi River, including the Prussian carp (*Carassius gibelio*), Abu mullet (*Planiliza abu*), Common carp (*Cyprinus*), European eel (*Anguilla anguilla*), African catfish (*Clarias gariepinus*), and goldfish (*Carassius auratus*). They reported an average of 5.1 ± 2 MPs fish⁻¹ (a range of 3 to 6.5 MPs fish⁻¹). Based on FTIR analysis, the researchers reported that the predominant polymer type was polyester (50%), followed by PE (10%), PP (8%), and PET (5%). Nonetheless, there is a scarcity of research concentrating on the occurrence of MPs in rainbow trout, especially in those cultivated in inland aquaculture systems in Türkiye. To date, the only study documenting MPs contamination in rainbow trout in Türkiye was conducted by Kilic (2022), who analyzed samples from the Hatay region. In that research, the mean number of MPs in the GIT of *O. mykiss* was documented as 1.2 ± 1.3 MPs fish⁻¹. In contrast, our investigation revealed a broader spectrum, with MPs quantities in the GIT samples of rainbow trout ranging from 1.15 ± 0.36 to 3.05 ± 1.27 MPs fish⁻¹, averaging at 2.17 ± 1.02 , which is generally lower. Estimated ingestion values, calculated using established feed consumption equations, showed that a fish weighing 205 g ingested approximately 796 MP particles, based on feed contamination levels observed in this study. These estimates were consistent with our measured GIT concentrations, with an average 2.17 MPs GIT contents, providing quantitative evidence that feed-derived MPs are directly

transferred to fish. These findings emphasize the need for stricter MPs contamination monitoring in feed and highlight feed management's role in minimizing MPs exposure in aquaculture. Further research should explore polymer retention and excretion dynamics to understand MPs' fate within fish. Using Pe values, we estimated MPs ingestion rates by rainbow trout. Given significant correlations between MPs in feed and GIT content ($P<0.001$), feed contamination is key to MPs accumulation in fish. The regression analysis showed MPs in GIT content were influenced by feed concentrations, confirming dietary exposure as the primary pathway for MPs ingestion. These findings align with previous studies (9) showing feed quality affects MPs retention.

This study demonstrates a link between MPs in aquaculture feed and their presence in farmed fish GIT, highlighting the need for feed monitoring to protect fish and human health. Further research on fish growth, digestive efficiency, and MPs excretion is needed to evaluate bioaccumulation risks. MPs in aquaculture may compromise fish physiology through stress, oxidative damage, and impaired nutrient absorption, while also transporting pollutants and pathogens. Contamination variations among facilities emphasize the need for targeted mitigation. Future research should identify MPs sources, optimize feed processing, and assess long-term health impacts. Implementing filtration systems and best practices could reduce MPs exposure in aquaculture.

ACKNOWLEDGMENT

The authors gratefully acknowledge support from the TUBITAK 2209-A program (Project No. 1919B012306081).

FINANCIAL SUPPORT

This research was part of a project funded by the TUBITAK 2209-A program (Project No. 1919B012306081).

CONFLICT OF INTEREST

There is no conflict of interest to be declared by the authors.

AUTHOR CONTRIBUTIONS

SG: Writing, review and editing, Supervision, Data curation, Conceptualization, Resources, Investigation, Methodology. VD: Writing-review and editing, Formal analysis, Conceptualization, Visualization. HC: Data curation and methodology.

ETHICAL STATEMENT

As the fish material was obtained from dead fish sold at farms and no live human or animal subjects were used, ethics committee approval was not required.

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