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ORIGINAL ARTICLE

Microplastic contamination in the gastrointestinal tract of Common Kilka (*Clupeonella cultriventris*) caught from Kiashahr coast (southeastern Caspian Sea)

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Abstract
Microplastics, as emerging contaminants in aquatic ecosystems, have the potential to transfer through food webs. This study aimed to determine the relative frequency of shape, size, color, and polymer type of microplastics present in the gastrointestinal tract of common kilka (*Clupeonella cultriventris*) from the Kiashahr coast (southeastern Caspian Sea) during winter and summer seasons. Sampling was conducted in winter at six stations (S1 to S6) and in summer at two stations (S1 and S2). After dissection, the gastrointestinal tract of 80 fish specimens was digested using 30% H_2O_2 and separated via flotation in NaCl solution. Microplastics were categorized by shape (fibers, fragments, films, foams), size (five classes), color (five groups), and polymer type (determined by FTIR spectroscopy). At all stations and in both seasons, fibers were the dominant shape (winter: 65–75%; summer: approximately 63%). The size class of 500–1000 µm exhibited the highest frequency (winter: 40–50%; summer: 46–49%). Black color consistently showed the highest percentage (winter: 45–50%; summer: 45–47%). Polyethylene (PE) was the dominant polymer (winter: >40%; summer: 43–47%), followed by polypropylene (PP). No significant difference was observed among stations regarding the distribution of microplastic characteristics ($P>0.05$). Microplastic contamination in Kiashahr, kilka exhibits a uniform seasonal and spatial pattern, indicating a widespread and persistent source of pollution. Black polyethylene fibers measuring 500–1000µm were identified as the most common contaminant type. Controlling the input of municipal wastewater and fishing-related waste into the Sea appears essential.

Keywords: Microplastics, *Clupeonella cultriventris*, Caspian Sea, Kiashahr, Polyethylene, Fiber

INTRODUCTION

Owing to their low weight, low cost, and high durability, plastics have found widespread application in daily life. However, it is precisely this durability that causes these materials to persist in the environment for decades or even centuries. Estimates indicate that between 8 and 12 million tonnes of plastic waste enter the world's oceans and seas annually. This waste is exposed to sunlight, wave action, and mechanical abrasion, degrading into particles smaller than 5 mm, known as microplastics. What makes these particles among the most concerning environmental contaminants is their high chemical stability, their ability to adsorb toxic pollutants onto their surfaces, and their capacity to cross biological barriers in living organisms (Hedayati et al. 2022; Bagheri et al. 2024; Arkhi et al. 2026).

The Caspian Sea, with an area of approximately 371,000km², is the largest landlocked water body in the world. This enclosed nature implies that, unlike open seas, pollutants entering the Caspian Sea have no outlet and gradually accumulate in bottom sediments, the water column, and the tissues of biota. The southern coasts of the Caspian Sea, comprising the Guilan, Mazandaran, and Golestan provinces of Iran, are recognized as critical hotspots for plastic pollution due to high population density, the presence of tourism and fisheries industries, and a lack of advanced wastewater treatment plants. Within this context, Kiashahr, located in eastern Guilan Province, is particularly exposed to high levels of microplastic inputs owing to the presence of one of the largest kilka fishing piers and the inflow of agricultural and rural rivers (Gholizadeh & Bagheri 2026).

The common kilka, *Clupeonella cultriventris*, accounts for approximately 70% of the total fisheries catch in the southern Caspian Sea and plays a vital economic and livelihood role for coastal communities. Ecologically, kilka is a planktivorous fish that forages in the surface layers of the water and serves as the primary prey for larger fish. These characteristics- high abundance, a key position in the food web, and feeding on suspended fine particles- make kilka an ideal bioindicator for assessing microplastic pollution (Lusher et al. 2017).

Internationally, numerous studies have investigated the presence of microplastics in commercial fish. Masoudi et al. (2021) in the Mediterranean Sea reported that fibers, accounting for 91%, were the dominant microplastic shape in fish. Irnidayanti et al. (2023) reached the same conclusion in Jakarta Bay. Domestically, research has been conducted on mullet and Kutum fish in various parts of the Caspian Sea. However, most of these studies have merely counted the total number of microplastics without concurrently examining the four primary characteristics: shape, size, color, and polymer type. Specifically, for common kilka in the Kiashahr region, no study has yet investigated these characteristics comparatively between winter and summer (Irnidayanti et al. 2023; Mobasheri et al. 2026).

Given this knowledge gap, the present study was designed and conducted with three main objectives: first, to determine the relative frequency of shape, size, color, and polymer type of microplastics ingested by kilka off the Kiashahr coast; second, to compare the distribution of these characteristics among different stations in the cold and warm seasons; and third, to identify the dominant polymer type using FTIR spectroscopy.

MATERIALS AND METHODS

Study area and sampling stations: Sampling was conducted along the coastal strip of Kiashahr (in eastern Guilan Province; coordinates: east longitude 49°56′-50°04′ and north latitude 37°25′-37°28′). Winter sampling was performed in February 2023, and summer sampling in August 2023. In the cold season, six stations approximately 500 m apart (S1–S6) were established. In the warm season, due to access limitations, two stations were selected: S1 (adjacent to a river outlet) and S2 (near the fishing pier).

Fish collection: At each station, ten specimens of common kilka were caught using a specialized kilka net (5 mm mesh size) and immediately transported to the laboratory on ice. The mean total length of the captured fish was 105±12 mm, and the mean weight was 5.8±1.2g.

Extraction and preparation of microplastics: The entire gastrointestinal tract of each fish (from the esophagus to the anus) was dissected using sterile instruments. Samples were then processed according to Masura et al. (2015) with minor modifications. The procedure involved four steps: (a) chemical digestion by adding 30% hydrogen peroxide (1:5 volumetric ratio to tissue weight) and incubating at 50°C for 24 h; (b) flotation by adding saturated sodium chloride solution (density 1.2 g cm^{-3}) and allowing to stand for 24 h; (c) filtration of the supernatant through a cellulose membrane filter (0.45 µm pore size); and (d) examination of the filters under a stereo microscope at 40× magnification (Masura et al. 2015).

Identification and categorization of microplastics:

Shape: Based on the framework proposed by Hidalgo-Ruz et al. (2012), particles were classified into four groups: fibers (thread-like and cylindrical), fragments (irregular pieces with sharp edges), films (thin, flexible sheets), and foams (spongy and porous).

Size: Using ImageJ software, the longest dimension of each particle was measured and grouped into five size classes: 100–500 µm, 500–1000µm, 1000–2000µm, 2000–3000µm, and 3000–5000µm.

Color: Based on visual matching with a color reference card, particles were assigned to five color groups: black, white, blue, green, and red.

Polymer type: Twenty percent of the particles were randomly selected and analyzed using Fourier transform infrared (FTIR) spectroscopy in the range of 4000–400cm^{-1} (Hidalgo-Ruz et al. 2012; Irnidayanti et al. 2023).

Statistical analysis: Percentage frequency data for each station are reported descriptively. Given the non-normal distribution of the data (as confirmed by the Shapiro–Wilk test at the 0.05 significance level), the nonparametric Kruskal–Wallis test was used to compare stations. The significance level was set at 0.05. All calculations were performed using SPSS version 26.

RESULTS

Microplastic characteristics in summer (Stations S1 and S2): A total of 80 specimens of *C. cultriventris* were examined across both seasons. In summer, sampling was conducted at two stations: S1 (adjacent to a river outlet) and S2 (near the fishing pier). At station S1, fibers were the dominant morphological type, accounting for 63.41% of all microplastics, followed by fragments (25.60%), films (8.53%), and foams (2.43%). A nearly identical distribution was observed at station S2, where fibers comprised 63.63%, fragments 22.07%, films 10.38%, and foams 3.89% (Fig. 1).

Regarding particle size, the 500–1000 µm class was the most frequent at both stations, accounting for 48.78% at S1 and 46.75% at S2. The second most abundant size class was 1000–2000 µm (32.92% at S1 and 28.57% at S2). Larger particles (3000–5000 µm) were rarely observed, accounting for only 1.21% at S1 and 2.59% at S2 (Fig. 2).

Black microplastics were predominant in summer, accounting for 45.12% at S1 and 46.75% at S2. White (28.04% at S1, 28.57% at S2), blue (17.07% at S1, 12.98% at S2), green (6.09% at S1, 9.09% at S2), and red (3.65% at S1, 2.59% at S2) followed in descending order (Fig. 3).Polymer analysis by FTIR revealed that polyethylene (PE) was the most abundant polymer at both stations (47.05% at S1, 43.75% at S2), followed by polypropylene (PP) (17.64% at S1, 25.00% at S2). Other detected polymers included polyamide (PA) (11.76% at S1, 18.75% at S2), polyethylene terephthalate (PET) (17.64% at S1, 6.25% at S2), and polystyrene (PS) (5.88% at S1, 6.25% at S2) (Figs. 3-4).

Microplastic characteristics in winter (Stations S1–S6): In winter, six stations (S1 to S6) were sampled. Across all stations, fibers consistently dominated, ranging from 65% to 75% of total microplastics.

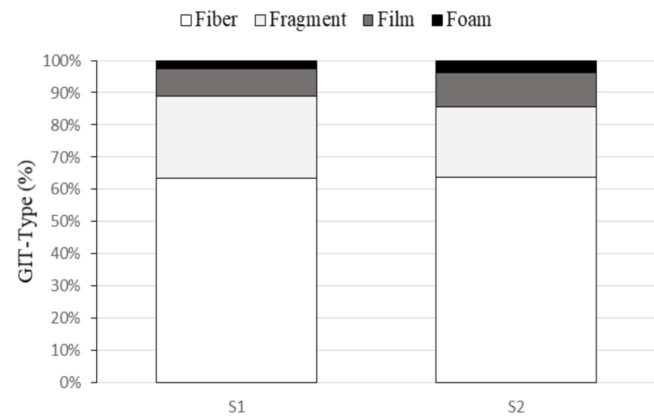

Fig.1. Frequency of microplastic types in the digestive tract of *Clupeonella cultriventris* by percentage at different stations (S1-S2) in the summer season.

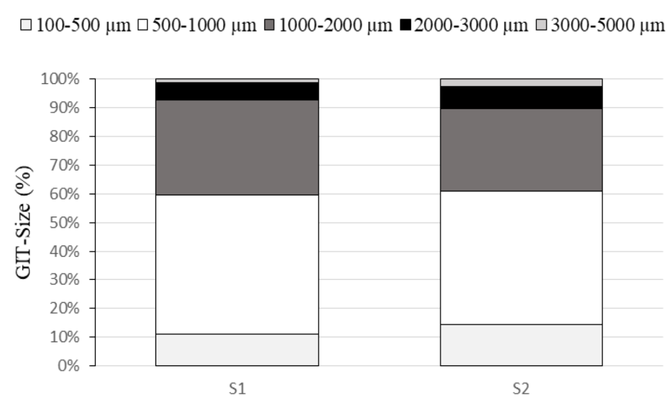

Fig.2. Microplastic size in the digestive tract of *Clupeonella cultriventris* by percentage at different stations (S1-S2) in the summer season.

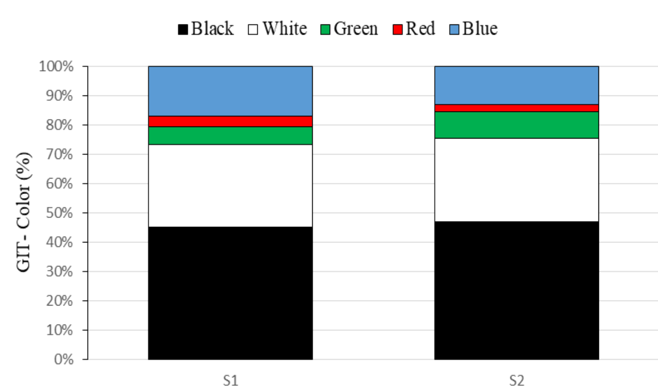

Fig.3. Microplastic color in the digestive tract of *Clupeonella cultriventris* by percentage at different stations (S1-S2) in the summer season.

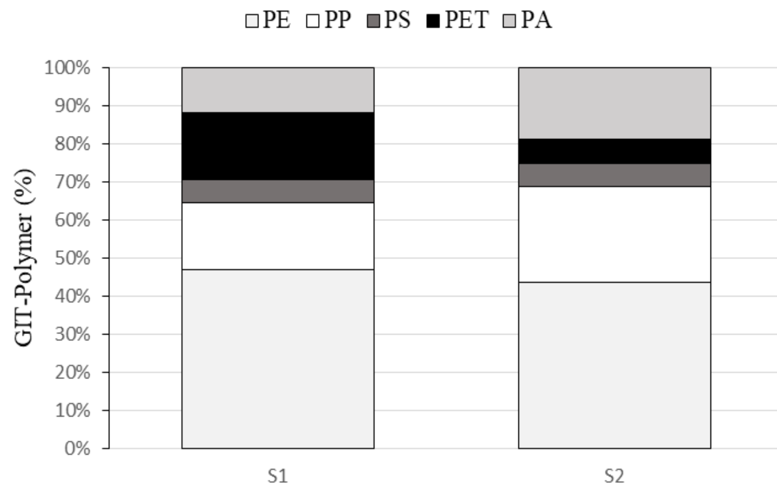

Fig.4. Microplastic polymers in the digestive tract of *Clupeonella cultriventris* by percentage at different stations (S1-S2) in the summer season.

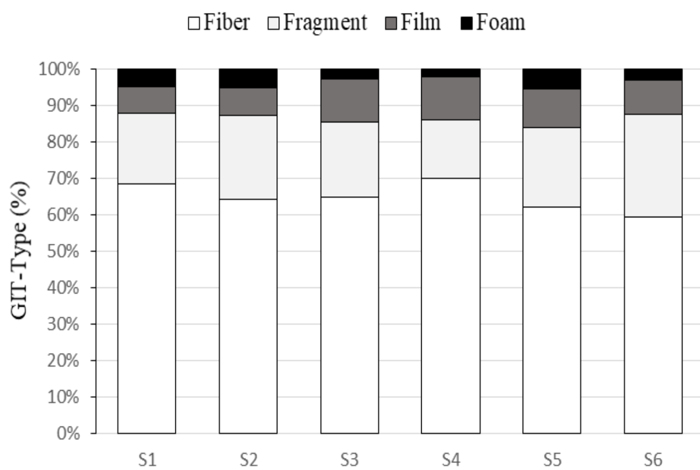

Fig.5. Frequency of microplastic types in the digestive tract of *Clupeonella cultriventris* by percentage at different stations (S1-S6) in winter.

Fragments (approximately 15–20%) and films (5–10%) were secondary, while foams accounted for less than 3% (Fig. 5).

The 500–1000 µm size fraction was again the most prevalent, comprising 40–50% of particles across stations. The 1000–2000 µm class accounted for 25–35%, and particles exceeding 3000 µm were scarce (<5%), with some stations showing none (Fig. 6).

Black microplastics were consistently dominant in winter (45–50%), followed by white (20–25%), blue (10–15%), green (5–10%), and red (3–5%) (Fig. 7).

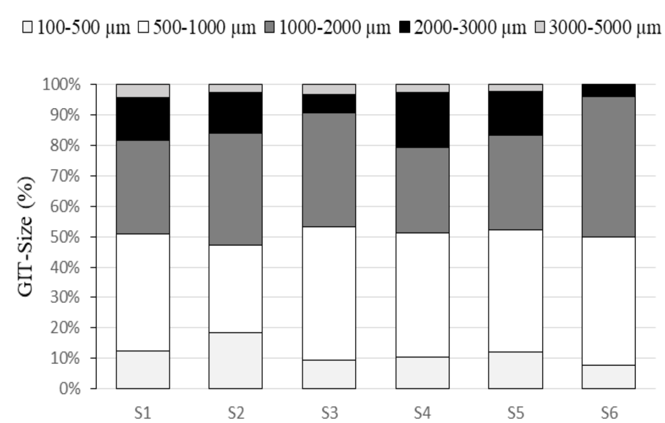

Fig.6. Microplastic size in the digestive tract of *Clupeonella cultriventris* by percentage at different stations (S1-S6) in winter.

Polymer composition in winter mirrored that of summer: PE was the most abundant (40–47% across stations), followed by PP (20–25%). PA ranked third (10–15%), while PET (<10%) and PS (<5%) occurred

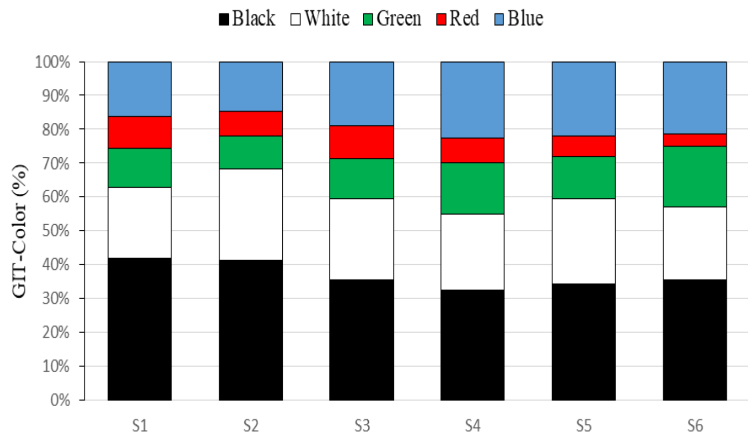

Fig.7. Microplastic color in the digestive tract of *Clupeonella cultriventris* by percentage at different stations (S1-S6) in winter.

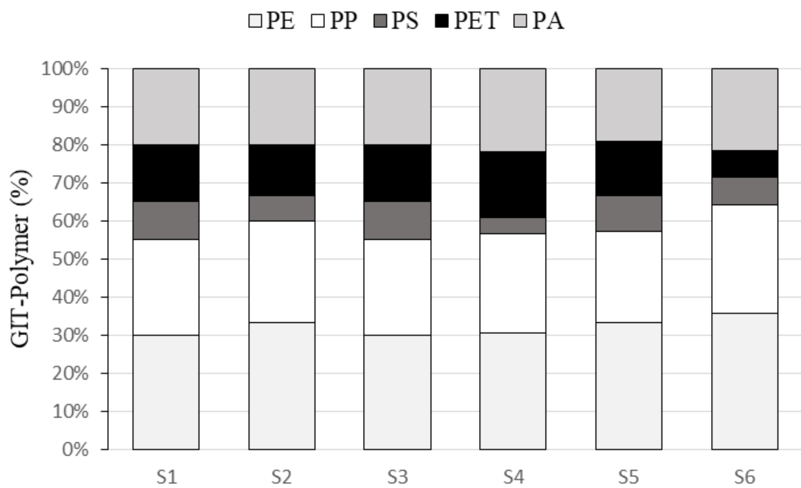

Fig.8. Microplastic polymers in the digestive tract of *Clupeonella cultriventris* by percentage at different stations (S1-S6) in winter.

at lower frequencies (Fig. 8). The results revealed no significant differences among stations for any of the microplastic characteristics (shape, size, color, or polymer type) in either season.

DISCUSSION

The present study provides a comprehensive characterization of microplastic contamination in the gastrointestinal tract of *C. cultriventris* from the Kiashahr coast of the southeastern Caspian Sea. The results demonstrate a uniform spatial and seasonal pattern, with fibers (65–75% in winter, ~63% in summer) being the dominant shape across all stations. This finding is consistent with previous reports from other marine systems. Constant et al. (2022) found that fibers constituted up to 91% of ingested microplastics in fish from the northwestern Mediterranean Sea, while Irnidayanti et al. (2023) reported fibers as the predominant morphotype in *Rastrelliger kanagurta* from Jakarta Bay. The prevalence of fibers is largely attributable to two main sources: (1) laundry discharge from synthetic textiles, which releases hundreds to thousands of fibers per wash into wastewater, and (2) the abrasion and degradation of fishing gear, including nets and ropes. The active fishing pier at Kiashahr and the discharge of untreated municipal sewage into the sea provide plausible local sources for both pathways (Foomani et al. 2020; Maleki et al. 2020).

The dominance of the 500–1000 μm size fraction (40–50% in winter, 46–49% in summer) can be explained by the feeding ecology of kilka. As a planktivorous fish, *C. cultriventris* naturally preys upon copepods and small mysids, which typically fall within this size range. Consequently, microplastic particles of similar dimensions are likely mistaken for prey. This phenomenon, often termed “prey size mimicry,” has been documented in other planktivorous species. Pitt et al. (2024) reported a similar size preference for microplastics (500–1000μm) in *Fundulus heteroclitus* from the Gulf of Maine. The sharp decline in particle frequency above 2000μm suggests either an active rejection mechanism or a physical gape limitation in kilka.

Black was the most frequently observed color (45–50% in winter, 45–47% in summer), a result that aligns with Irnidayanti et al. (2023) in Jakarta Bay. Two non-mutually exclusive hypotheses may explain this observation. First, black particles might be particularly abundant in the ambient environment of the study area. Potential sources include tire-wear debris (which is predominantly black), transported via road runoff and urban drainage into the sea. Second, kilka may exhibit color-selective feeding due to resemblance to natural prey. Many planktonic copepods and insect larvae are dark-pigmented (e.g., dark brown or black), leading fish to ingest black microplastics by mistake.

The predominance of polyethylene (PE) and polypropylene (PP) over other polymers was expected. Both polymers have densities lower than seawater (<1g cm^{-3}), making them positively buoyant and thus highly available to surface-feeding fish such as kilka. Major sources include single-use packaging (plastic bags, bottle caps, food containers) and fishing gear (ropes, nets). Constant et al. (2022) similarly identified PP as a dominant polymer in Mediterranean fish. The relatively low proportion of PET (polyethylene terephthalate), a polymer commonly used in rigid beverage bottles, suggests that soft packaging materials, rather than hard plastics, are the primary source of pollution in the Kiashahr region.

One of the most noteworthy findings is the remarkable uniformity of microplastic characteristics across different stations and between seasons. Despite the lower number of sampling stations in summer (two vs. six in winter), the observed percentages for shape, size, color, and polymer type were highly similar. The absence of significant spatial variation ($P > 0.05$) and the lack of a distinct seasonal signal indicate that microplastic contamination in the Kiashahr coastal area is a persistent, widespread phenomenon rather than a transient or point-source event. This uniformity implies continuous input of similarly sourced plastics throughout the year, likely derived from sustained municipal wastewater discharge and ongoing fishing-related activities.

Four main limitations should be acknowledged. First, the summer sampling campaign was restricted to two stations, whereas the winter campaign included six stations. Second, microplastic concentration (particles per gram of tissue) was not quantified, limiting comparisons with studies reporting abundance metrics. Third, the edible fillet tissue was not analyzed, which is critical for assessing human exposure risk via seafood consumption. Fourth, polymer identification was performed on only 20% of the extracted particles; although this subset was randomly selected, it may not fully represent the entire particle population.

CONCLUSION

In conclusion, this study demonstrates that all examined *C. cultriventris* from the Kiashahr coast were contaminated with microplastics, with black polyethylene fibers of 500–1000 μm being the most prevalent type. The spatial and seasonal uniformity of contamination patterns points to a stable, diffuse source of pollution. Given the key ecological role of kilka as a forage fish in the Caspian Sea, there is a potential for trophic transfer of microplastics to larger predatory fish and ultimately to humans. These findings underscore the urgent need for improved municipal wastewater treatment (e.g., installation of microfiber retention filters) and better management of derelict fishing gear at the Kiashahr pier. Future research should examine microplastic contamination in kilka fillets and in higher-trophic-level species (e.g., *Liza aurata* or *Rutilus kutum*) to fully assess the risks of trophic transfer.

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مقاله کامل

آلودگی میکروپلاستیک در دستگاه گوارش ماهی کیلکای معمولی (*Clupeonella cultriventris*) صید شده در سواحل کیاشهر (جنوب شرقی دریای کاسپین)

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چکیده: میکروپلاستیک‌ها به‌عنوان آلاینده‌های نوظهور در اکوسیستم‌های آبی، قابلیت انتقال در زنجیره غذایی را دارند. هدف این مطالعه، تعیین فراوانی نسبی شکل، اندازه، رنگ و نوع پلیمر میکروپلاستیک‌های موجود در دستگاه گوارش ماهی کیلکای معمولی در سواحل کیاشهر (جنوب شرقی دریای کاسپین) در دو فصل زمستان و تابستان بود. نمونه‌برداری در زمستان ۶ ایستگاه (S1 تا S6) و تابستان ۲ ایستگاه (S1 و S2) انجام گرفت. پس از تشریح، دستگاه گوارش ۸۰ قطعه ماهی با H_2O_2 ۳۰٪ هضم و با شناورسازی در NaCl جداسازی شد. میکروپلاستیک‌ها براساس شکل (الیاف، قطعه، فیلم و فوم)، اندازه (پنج کلاس)، رنگ (پنج گروه) و نوع پلیمر (با استفاده از طیف‌سنجی FTIR) دسته‌بندی شدند. در تمام ایستگاه‌ها و هر دو فصل، الیاف شکل غالب بودند (زمستان ۶۵-۷۵٪، تابستان حدود ٪۶۳). اندازه ۵۰۰-۱۰۰۰ میکرومتر بیش‌ترین فراوانی را داشت (زمستان ۴۰-۵۰٪، تابستان ۴۶-۴۹٪). رنگ سیاه در همه موارد بالاترین درصد را نشان داد (زمستان ۴۵-۵۰٪، تابستان ۴۵-۴۷٪). پلی‌اتیلن (PE) پلیمر غالب بود (زمستان >٪۴۰، تابستان ۴۳-۴۷٪) و پس از آن پلی‌پروپیلن (PP) قرار داشت. تفاوت معنی‌داری بین ایستگاه‌ها از نظر توزیع ویژگی‌های میکروپلاستیک مشاهده نشد (P>۰/۰۵). آلودگی میکروپلاستیک در کیلکای معمولی کیاشهر دارای الگوی فصلی و مکانی یکنواخت است که نشان‌دهنده منبع آلودگی گسترده و پایدار می‌باشد. الیاف سیاه پلی‌اتیلنی با اندازه ۵۰۰-۱۰۰۰ میکرومتر شایع‌ترین نوع آلاینده شناسایی شد. کنترل ورود فاضلاب‌های شهری و پسماندهای صیادی به دریا ضروری به‌نظر می‌رسد.

کلمات‌کلیدی: میکروپلاستیک، ماهی کیلکا، دریای کاسپین، کیاشهر، پلی‌اتیلن، الیاف