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Research Article

Microplastic pollution in two zooplankton groups on the southern coast of the Caspian Sea

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Abstract

The high volume of plastic waste, especially microplastics (MPs), has caused a global concern in the last decades. The present study aims to investigate the presence of MPs in two zooplankton groups in the southwest of the Caspian Sea due to the importance of zooplankton populations in the bottom levels of the marine food pyramid and the probability of their transfer to the human food chain. The samples were collected from four stations over one year (2020-2021) using 100 µm plankton nets. After separation and assessment of MP particles, fragments and fibers were the predominant forms of MPs. Analysis of 5123 Acartia tonsa and 1528 Pleopis polyphemoides, resulted in 38 (22 fragments and 16 fibers) and 28 (15 fragments and 11 fibers) MPs, respectively, and 42 MPs (24 fragments and 17 fibers) in the seawater. The average size of the ingested pieces in the zooplankton communities was in the range of 35-46.5 µm and 56.25 µm in the seawater in four stations. The most commonly observed colors in A. tonsa were orange and white, and black in P. polyphemoides and seawater. The samples were also inspected using FTIR-ATR method and confirmed the presence of polymeric compounds and the probable types of polyester, polyethylene, and polyethylene terephthalate in the zooplankton and seawater samples at all stations. Since seafood is an important source of nutrients in coastal areas, the polymers present in the zooplankton's bodies can transfer to higher trophic levels, including humans.

Keywords: Caspian Sea, Microplastics, Zooplankton, FTIR, Polymer

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Introduction

Plastic pollution in marine ecosystems is a recognized environmental threat on a global scale (Galgani *et al*., 2015; Rochman, 2018; Choi *et al*., 2020). The effects of microplastics (MPs) (1 µm–5 mm) on ecosystems are more severe than large plastic waste due to their potential to enter food webs through lower trophic levels. MPs have a global distribution, and a plastic bottle thrown away on land may end up in the ocean as MPs (De Troyer, 2015). MPs are ingested by a wide range of organisms, including commercially important fish, crustaceans, and molluscs, and enter their food chain, which can have negative effects on organisms, especially humans, who consume those contaminated seafood or water (Wright *et al*., 2013). Phytoplankton is considered the primary producer of any ecosystem and is sensitive to environmental changes (Norian *et al*., 2022). Furthermore, the absorption of MPs by organisms from primary food sources such as phytoplankton and zooplankton can be a pathway for their transfer into the food chain.

Zooplankton is one of the essential and valuable groups in the food chain of aquatic ecosystems and plays a fundamental role as an intermediate links between phytoplankton and macrozooplankton in the food chain, affecting organisms dependent on other trophic levels as well (Souissi *et al*., 2001). Zooplankton rapidly ingests microscopic plastics (<1mm) and then excrete them in their waste pellets. These waste pellets are a source of food for other marine animals such as fish, crabs, shrimps, and other groups of aquatic animals. The consumption of these aquatic animals by humans can lead to the transfer of MPs into humans and cause health problems (Cole, 2016). Recent studies indicate that a wide range of zooplankton communities, including copepods, can also ingest MPs (Cole *et al*., 2013). Exposure to MPs can have a significant impact on the health and biomass of copepods (Cole, 2014).

The Caspian Sea is the world’s largest lake with an area of 390000 square kilometers (Kosarev, 2005). Like most parts of the world, plastic pollution, particularly MPs, can now be found along the coastline of the Caspian Sea (Mataji *et al*., 2020). The coasts of the Caspian Sea have a very high volume of plastic waste due to fishing and tourism activities. In addition, surface waters, after passing through agricultural and residential areas and industrial zones, flows into the sea, and a significant volume of wastewater and agricultural runoff ultimately enters the Caspian Sea (Krdvany, 1995). Therefore, the present study aims to investigate the presence of MPs in the zooplankton communities on the coastal waters of Guilan Province in the southern coast of the Caspian Sea.

Materials and methods

Study area

This study was carried out along the southern coast of the Caspian Sea (Guilan province-Iran) in 2020-2021 at four sampling stations in Chaboksar, Kiashahr, Anzali and Lisar (Fig. 1) on zooplankton and Seawater.

Sampling

Zooplankton samples were collected with vertical closing Juday net, diameter - 36 cm, mesh size 100 µm (Vinogradov *et al.*, 1989). Tows were performed from the surface down to the bottom for all stations. Then the net was washed down into the cod end, removed, and the samples were washed into glass containers. Samples were preserved in formalin (4% formaldehyde) buffered solution and transferred to the lab (APHA, 2005). Also Seawater was collected from each sampling point and passed through a 100 µm mesh, transferred to the lab and utilized for MPs analysis.

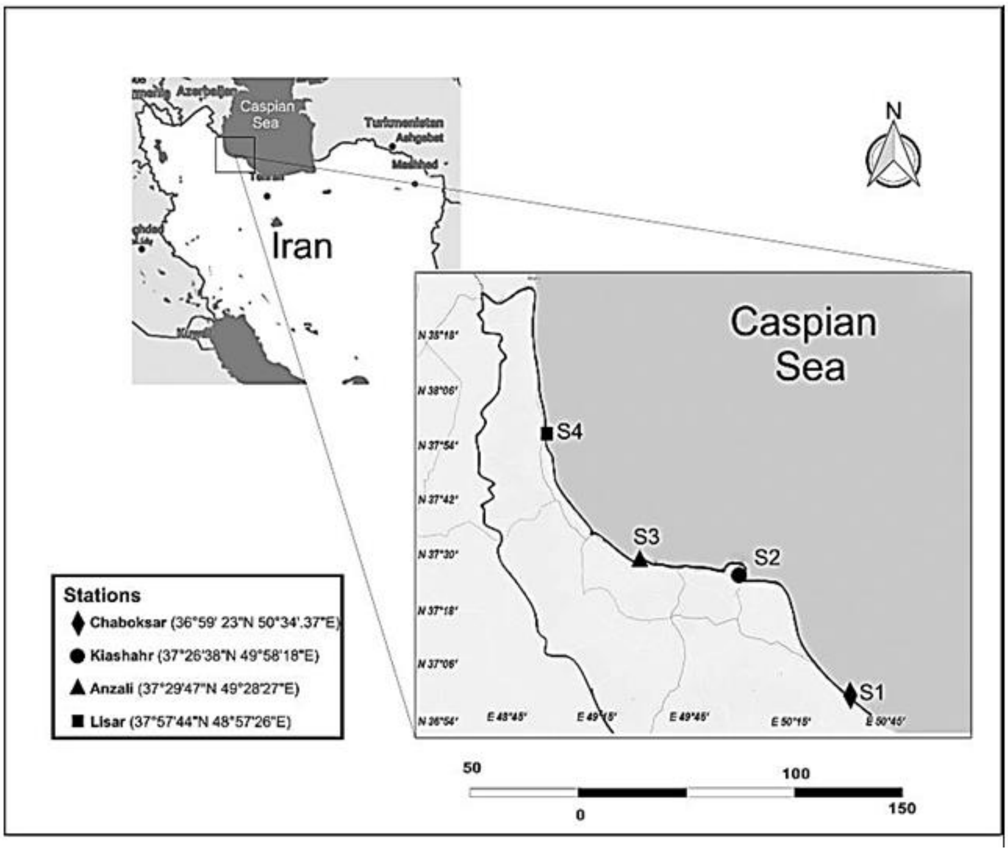

Figure 1: Sampling stations along the coastline of the Caspian Sea (Guilan Province).

Zooplankton analysis

Samples were split in to two parts and the zooplankton communities were identified using a stereo microscope (Nikon-SMZ1). All samples were transferred to Bogorov chambers (Hydro-Bios KIEL) with a 5 mL glass pipette, after homogenization. After one hour of sedimentation, identification and counting were performed using an inverted microscope (Leitz- LABOVERT FS).

Zooplankton total abundance was calculated based on filtered water volume (m^3) by multiplying the area of net opening (36cm) with sampling depth (m) (Harris *et al.*, 2000). Samples were initially identified, using identification keys such as Birshtain *et al.*, (1968), Ruttner-Kolisko (1974), Kasimov (2000), and Postel *et al.*, (2000) and observed and photographed, then were counted using the zooplankton counting chambers.

Microplastics analysis

Each sample was checked under an inverted microscope (Leitz- LABOVERT FS). All copepods and cladocerans were picked out from each sample and rinsed with ultrapure water to make sure that no MPs were found on the exterior parts of their bodies. Each group was placed in a scintillation vial and then, 30% Hydrogen peroxide (H_2O_2) solution was added (Masura *et al.*, 2015; Aytan *et al.*, 2022) to destroy their body tissue. The mixture was placed in a temperature-controlled oven at 45°C for 12 h. If the biological material was not completely digested, more H_2O_2 solution was added (Aytan *et al.*, 2022). After digestion, to density separation, NaCl was used to isolate the plastic debris through flotation (Mathalon and Hill, 2014). Then the floating solids were rinsed with ultrapure water and separated from the denser undigested components. The mixture was filtered on to a WHATMAN Filter Paper 2.5 μm/pore-size, air-dried, removed and then checked under a Stereo microscope for the presence of MPs. Microplastics were identified according to morphological characteristics and physical response features (Desforges *et al.*, 2014). Then MPs were visually classified according to shape and color and photographed using a Zeiss Stemi 508 Stereo microscope (zoom range 8:1, Total Magnification: 50), armed with a digital camera and AxioVision (Zeiss Microscopy) software. The size of MPs was measured manually using the software in the images taken with the digital camera. Sizes of the largest cross-section was measured (total length in the case of fibers).

Water samples were treated with hydrogen peroxide (H_2O_2) 30% solution to digest organic matter. To density separation, NaCl was used to float the plastic debris. Then they were rinsed with ultrapure water and separated from the denser undigested components. The mixture was filtered through Filter Paper, air-dried, removed and then observed under a stereo microscope for the presence of MPs.

The MP particles were analyzed by using the Fourier transform infrared spectroscopy (FTIR-ATR) to confirm their composition chemically. The spectrum range was 4000–460 cm^{-1} with 16 scans for each measurement (JASCO 4700). The mixture from each station was carried out using the FTIR technique to confirm the synthetic polymer origin of the particles and the polymer types were identified by comparing the sample spectra with FTIR spectral libraries.

Quality assurance & quality control

To minimize sample contamination, cotton laboratory coats and nitrile gloves were worn. Analyses were done in a laminar flow hood. All equipment and dissection tools were washed with ultrapure water and used in non-plastic containers to prevent cross-contamination between samples. Procedural blanks without any zooplankton and only H_2O_2 were run for each batch of samples.

Data analysis

Based on the Kruskal-Wallis test for non-parametric data there were significant differences in the abundance of zooplankton across the four seasons among sampling stations ($p<0.05$). Before statistical analyses, data was tested for normal distribution (Shapiro-Wilk test). Pearson correlation analyses were applied to assess the relationships between MPs in seawater and zooplankton and for statistical analysis, one-way ANOVA was performed. Data were analyzed using SPSS 27 software and the figures were created with Microsoft Excel 2013.

Results

Zooplankton abundance and species composition

In the present study, a total of 22 zooplankton species were found.1 species from ciliata, 1 species from Foraminifera, 2 larval stages from cirripedia, 1 larval stage from polychaete, 1 larval stage from bivalvia and 1 larval stage from fishes were identified. From holoplankton, 4 species from cladocera, 5 species from copepoda, 1 species from ostracod, and 5 species from rotifera were identified (Table 1).

Table 1: Zooplankton groups identified in the southwest of the Caspian Sea (the coasts of Guilan province) 2020-21.

No	Group	Taxa	Average	Std
1	Ciliata	*Tintinnopsis tubulosa* Levander, 1900	4795.104	1869.212
2	Foraminifera	*Ammonia* sp. Brunnich, 1772	0.5625	0.521
3	Cirripedia	*Balanus improvisus* (Cypris) Costa, 1778	8.458	2.992153
4	Cirripedia	*Balanus improvisus* (Nauplius) Costa, 1778	1981.208	380.204
5	Cladocera	*Evadne anonyx Sars, 1897*	0.666	2.309
6	Cladocera	*Podonevaden camptonyx* Sars, 1897	19.130	131.149
7	Cladocera	*Podonevaden trigona* Sars, 1897	0.791	5.484
8	Cladocera	*Pleopis polyphemoides* Leuckart, 1859	267.444	1852.909
9	Copepoda	*Acartia tonsa* (Nauplius) Dana, 1849	27981.937	4729.191
10	Copepoda	*Acartia tonsa* (Adult) Dana, 1849	19497.25	2583.707
11	Copepoda	*Calanipeda aquaedulcis* (Adult) Kritchagin, 1873	0.562	0.413
12	Copepoda	*Halicyclops sarsi* Akatova, 1935	0.2291	0.161
13	Copepoda	*Halicyclops sarsi* (Nauplius) Akatova, 1935	0.333	o.333
14	Ostracoda	*Ostracoda* Latreille, 1802	0.104	0.104
15	Bivalvia	Bivalvia (larvae) Linnaeus, 1758	4486.708	1733.476
16	Fish	Pisces (larvae)	0.854	0.496
17	Polychaeta	*Hediste diversicolor* (larvae) Muller, 1776	4487	10549
18	Porifera	*Dosilia radiospiculata* Mills, 1888	1.458	1.458
19	Rotifera	*Brachionus calyciflorus* Pallas, 1766	1.437	1.044
20	Rotifera	*Keratella cochlearis macracantha* Lauterborn, 1898	0.312	0.312
21	Rotifera	*Trichocerca capica* Tschugunoff, 1921	0.041	0.041
22	Rotifera	*Synchaeta* sp. Ehrenberg, 1832	11080.312	5972.024

Zooplankton abundance variations in this study were related to 6 main zooplankton groups: *Acartia tonsa*, *Pleopis polyphemoides, Balanus improvises* (larvae) , Synchaeta sp., Bivalvia larvae, Polychaete larvae. Two groups: *Acartia tonsa* (Dana, 1849, Copepod) (high abundance) and *Pleopis polyphemoides* (Leuckart, 1859, Cladocera) (low abundance) were chosen and analyzed to investigate the presence of MPs (Fig. 2).

There were seasonal changes in abundance of *A. tonsa* (Copepoda). The highest average number observed was 87703 ind/m^3 in autumn 2021. The annual average was 47480. Kruskal-Wallis test showed significant differences in different seasons ($p< 0.05$). Also based on duncan's multiple range test when there are no significant differences between two bars they got the same letter, otherwise showed with different letters. Also there were seasonal changes in abundance of *P. polyphemoides* (Cladocera). The highest average number observed was 1680 ind/m^3 in autumn 2021 (Fig. 3). Based on the Kruskal-Wallis test, significant differences were found in different seasons ($p<0.05$). Also, zooplankton and seawater samples were selected from autumn for assessment of MP particles and the polymer type identification.

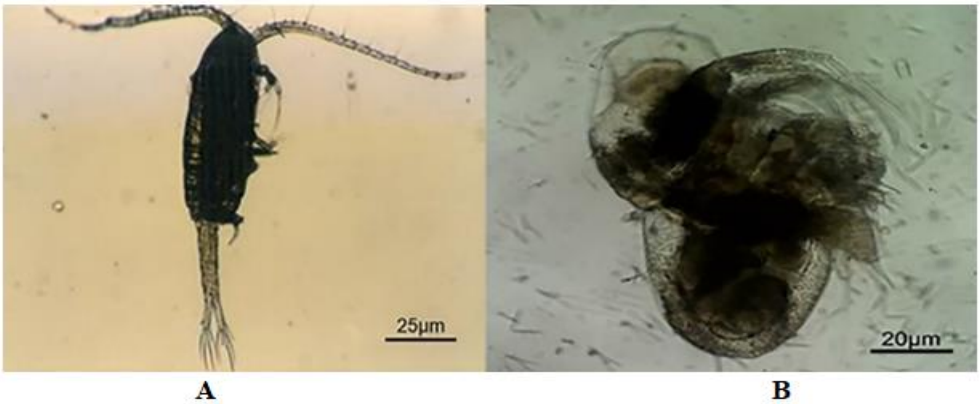

Figure 2: Zooplankton in the study area, *Acartia tonsa* (Copepoda) (A) and *Pleopis polyphemoides* (Cladocera) (B).

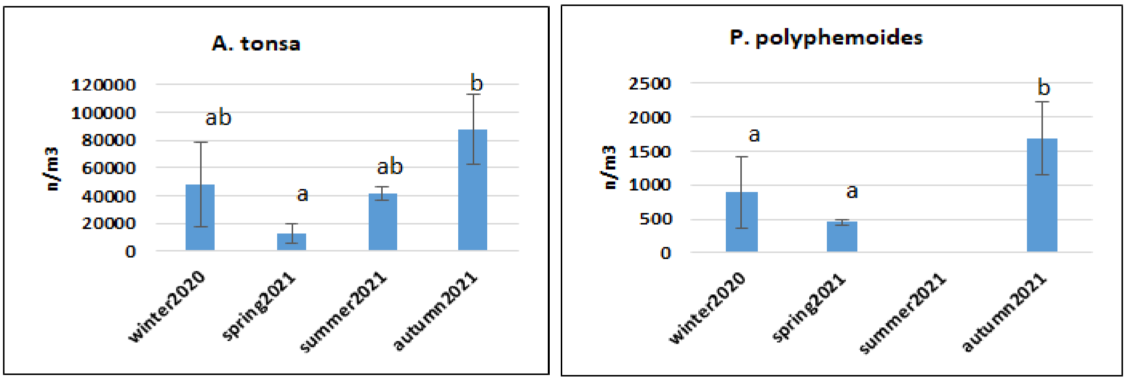

Figure 3: The average number of *Acartia tonsa* (Copepoda) and *Pleopis polyphemoides* (Cladocera) in 4 seasons in the south of the Caspian Sea (the coasts of Guilan Province) 2020-21.

Assessment of MP particles and the polymer type identification in A. tonsa (Copepods) and P. polyphemoides (Cladocera) and Seawater

In all stations, microplatics were detected in *A. tonsa* (Copepods) and *P. polyphemoides* (Cladocera). In *A. tonsa* a total of 38 microplatics (22 fragments and 16 fibers) were detected after analysis of 5123 individuals. The color of MPs were black, orange and white. In *P. polyphemoides* a total of 28 MPs (15 fragments and 11 fibers) were detected after analysis of 1528 individuals . The color of MPs were black, orange and blue. In Seawater a total of 42 MPs (24 fragments and 17 fibers) were detected (Table 2). The color of MPs were black, pink, orange, white, and blue. Figure 4 shows some examples of detected MPs in *A. tonsa* and *P. polyphemoides* and Seawater in the southwest of the Caspian Sea.

Table 2: Characteristics of MP particles ingested by *Acartia tonsa* and *Pleopis polyphemoides* and Seawater in 4 stations in the southwest of the Caspian Sea 2020-21.

Species	Station	Number of zooplankton	Number of MP	MP Mean size (μm)	Color of MP	Shape of MP
Acartia tonsa (Copepoda)	Chaboksar	1064	3	45 ± 2.01	Orange	Fibers
	Kiashahr	1117	9	47 ± 6.18	Orange, White	Fragments/ Fibers
	Anzali	1919	22	48 ± 6.70	Orange, White	Fragments/ Fibers
	Lisar	1023	4	46 ± 5.16	Black, White	Fragments
	Total	5123	38			
	Average		9.5	46.5		
Pleopis polyphemoides (Cladocera)	Chaboksar	297	4	32 ± 4.11	Black	Fibers
	Kiashahr	474	6	37 ± 4.07	Orange, Blue	Fragments
	Anzali	603	15	35 ± 6.25	Black, Blue	Fragments / Fibers
	Lisar	154	3	36 ± 3.60	Black	Fragments
	Total	1528	28			
	Average		7	35		
Seawater	Chaboksar		6	46 ± 4.93	Black, Blue	Fibers
	Kiashahr		8	69 ±12.71	Orange, White, Pink	Fragments
	Anzali		24	71 ± 10.63	Black, Orange, Pink	Fragments / Fibers
	Lisar		4	39 ± 2.82	Black, White	Fragments
	Total		42			
	Average		10.5	56.25		

Based on Table 2, a total of 38 MPs were found, fragments were the most common MPs found in *A. tonsa* followed by fibers. In *P. polyphemoides*, a total of 28 particles, fragments and fibers were also the most common MPs found. In the Seawater, a total of 42 particles, fragments, and fibers were the most common MPs detected. In *A. tonsa*, orange and white, and in *P. polyphemoides* black was the most common color were found among sampling stations. In Seawater the most common color in different stations was black. There were no significant differences in the number of MPs among sampling stations and the Seawater based on one-way ANOVA ($p \geq 0.05$). The compositions of the MPs in *A. tonsa* and *P. polyphemoides* and Seawater in terms of shape, in four stations, are shown in Figure 5. Also color composition of MPs in *A. tonsa* and *P. polyphemoides* and Seawater, in four stations in the southwest of the Caspian Sea, are shown in Figure 6.

For the FTIR analyses, the polymer types were identified by comparing each obtained sample spectra with FTIR spectral libraries. Analysis of particles from all four stations revealed their composition based on Functional groups and spectral peaks in *A. tonsa* and *P. polyphemoides* and Seawater, which were similar to Functional groups and spectral peaks in three known polymers: polyethylene (PE), polyester (PS), and polyethylene terephthalate (PET) (Figs. 7 and 8).

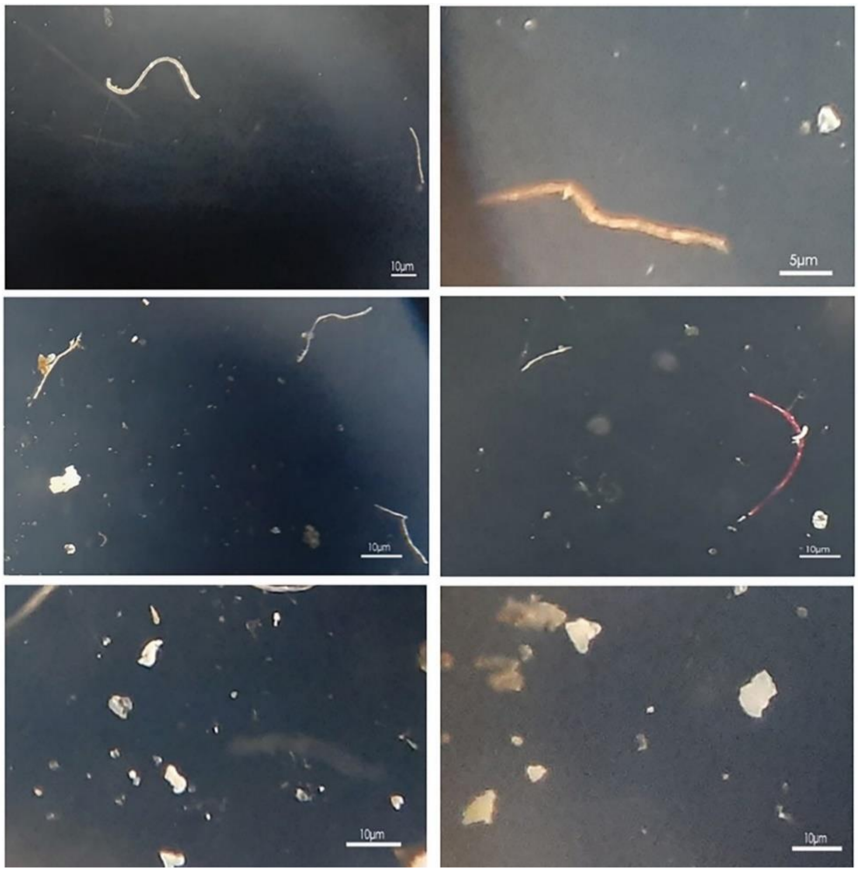

Figure 4: Examples of detected MP particles in *Acartia tonsa* and *Pleopis polyphemoides* and Seawater in the southwest of the Caspian Sea detected by Zeiss Stemi 508 Stereo microscope.

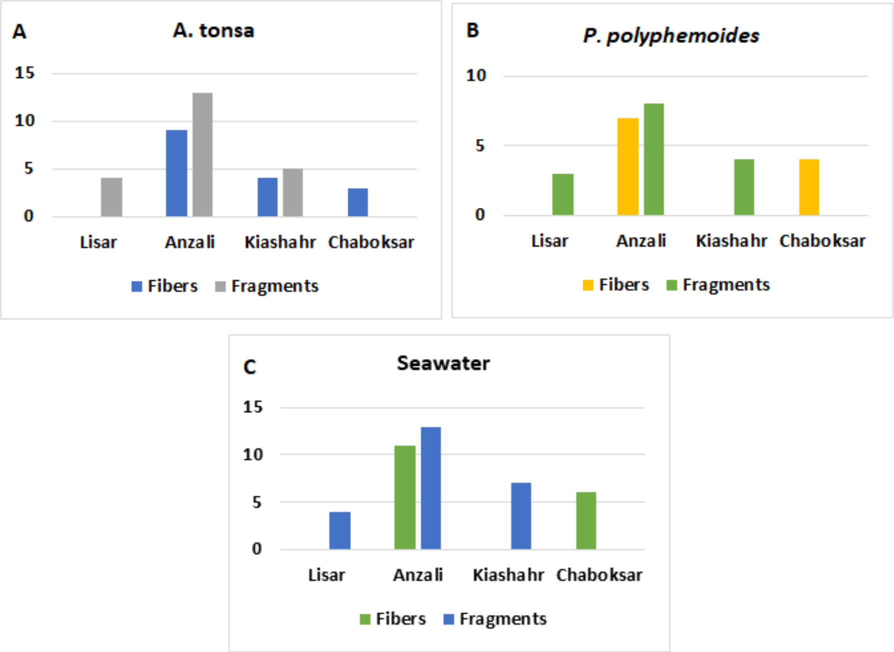

Figure 5: The most common type of MPs found in *Acartia tonsa* (A), *Pleopis polyphemoides* (B), and Seawater (C) in 4 stations in the southwest of the Caspian Sea 2020-21.

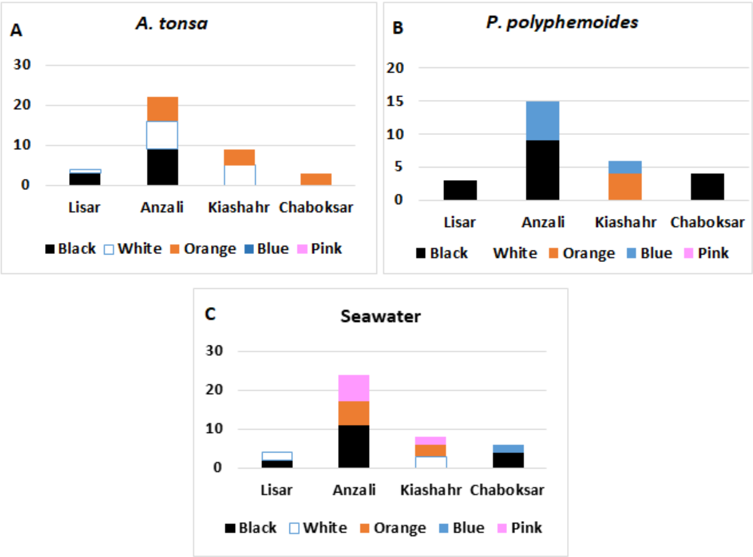

Figure 6: Color composition of MPs found in *Acartia tonsa* (A), *Pleopis polyphemoides* (B) and Seawater (C) in 4 stations in the southwest of the Caspian Sea 2020-21.

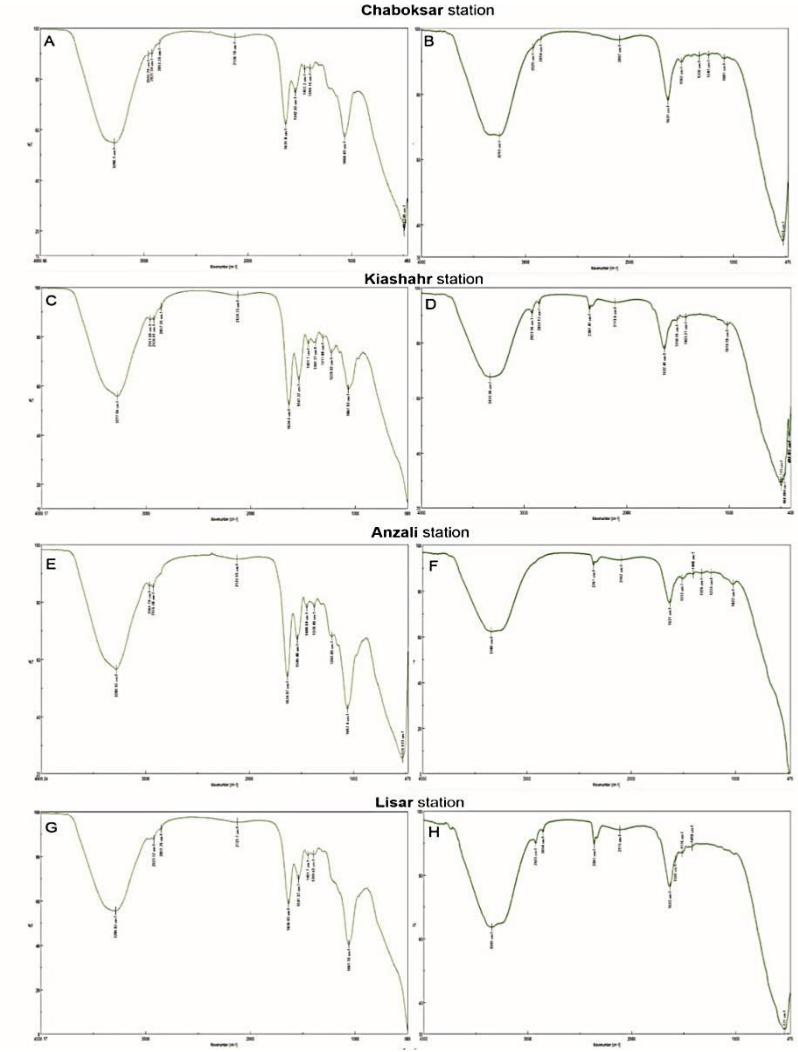

Figure 7: FTIR spectra of MP particles in *A. tonsa* (A, C, E, and G), *P. polyphemoides* (B, D, F, and H) in 4 stations in the southwest of the Caspian Sea 2020-21.

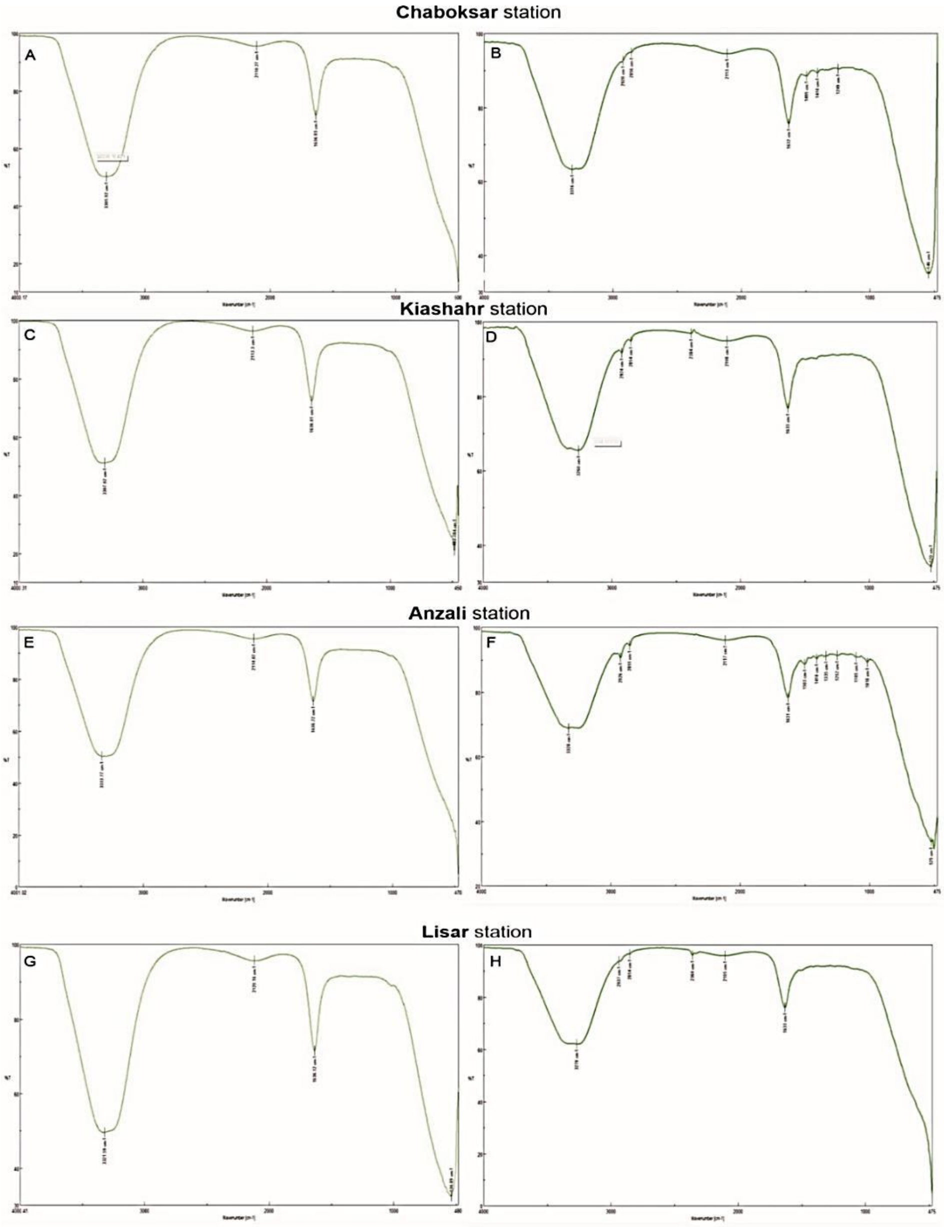

Figure 8: FTIR spectra of MP particles in blank (H_2O_2) (A, C, E, and G) and Seawater (B, D, F, and H) in 4 stations in the southwest of the Caspian Sea 2020-21.

Discussion

In the present study, the presence of MPs and some of their characteristics, including size, shape, color, and potential polymer type, were investigated. The observed shapes of the particles in the zooplankton samples were identified as fragments and fibers in *A. tonsa* and *P. polyphemoides* at four different stations along the southern coast of the Caspian Sea and also in the seawater (Table 2). At the Anzali station, both types of fragments and fibers were detected in both zooplankton species and in the seawater (Fig. 5). These results could be due to the abundance of these shapes in the sampled locations or because these shapes have had the highest bioavailability for these zooplankton samples from MPs. So far, no systematic study has been conducted on the presence of MPs in zooplankton communities in the southern waters of the Caspian Sea. However, according to the studies on MP pollution in this region (southern coast of the Caspian Sea), the results indicated that the highest amount of ingested MPs was attributed to fibers, followed by fragments and films (Zakeri *et al.*, 2020; Manbohi *et al.*, 2021; Nematollahi *et al.*, 2021; Rasta *et al.*, 2021). In general, multiple studies on the ingestion of MPs in the natural environment have shown that the majority of ingested MPs are in the form of fibers (Desforges *et al.*, 2015; Salvador *et al.*, 2017; Steer *et al.*, 2017; Sun *et al.*, 2017). In addition, fibers are the predominant forms of plastic in the bodies of various invertebrate and vertebrate species (Mizraji *et al.*, 2017). In the studies by Sun *et al.* (2018a, 2018b) and Steer *et al.* (2017) on amphipods, copepods, and several other groups, the observed types of particles in the samples were reported to be fibers and fragments, which is consistent with the results of this study.

The number of ingested MPs in the zooplankton samples and the seawater did not show significant differences. According to the one-way analysis of variance (ANOVA) test. The correlation between MP pollution in zooplankton and seawater was also investigated. Based on the normality of the data, the correlation results between the number of MPs in zooplankton and seawater, as well as the Pearson correlation coefficient, showed that this correlation was not statistically significant. In their 2022 study on the ingestion and excretion of MPs by copepods in the Black Sea, Aytan and colleagues also reported that there was no significant correlation between the ingestion of MPs by copepods and the concentration of MPs in the water column.

In the present study, the possible composition of visible particles in the zooplankton and water samples was determined by comparing the results of FTIR spectroscopy analysis of MPs in the samples with the spectra of three standard types of common polymers, including PE, PS and PET (Figs. 7, 8). PS is mostly used in textile, shipbuilding, boats, and transportation equipment industries (Scheirs and Long, 2003). It seems that fishing activities, as well as the use of recreational and fishing boats and ships in this area, maybe the reason for the abundance of this polymer. Nowadays, PE has the most usage in the packaging of food and agricultural industries, and it can be observed in aquatic environments (Zhao *et al.*, 2015). The studied area likely has some amounts of this polymer in the samples due to its proximity to fishing activities, tourism, human activities, and the presence of fishing gear that may have been discarded or left in the environment. PET is used in the production of various types of bottles, straps, and injection-molded parts. PET bottles are used for packaging a wide range of products, including mineral water, carbonated drinks, pharmaceuticals, hygiene and cosmetic products (Wegelin *et al.*, 2001; Mishra, 2016). Therefore, it appears that the direct release of these bottles and parts into the sea or through rivers that flow into the sea is one of the reasons for pollution and the presence of this polymer in the samples of the studied area. Also, there are several plastic manufacturing companies such as plastic bag supplier, plastic fabrication company, plastic granules supplier and manufacture along the southern coast of the Caspian Sea which could be the possible Pollution Sources in these areas. According to limited studies on the presence of MPs in this area, including research by Manbohi *et al*. (2021), the polymers found were PE, PS and PET. Rasta and colleagues identified four types of polymers with polypropylene being the dominant one. Also, according to previous studies by other researchers including Browne *et al*. (2011), Claessense *et al*. (2011), and Sun *et al*. (2017), the major constituents of MPs in marine environments are PS compounds, PE, and PET. The results of all these studies are consistent with the possible polymers found in the samples from the Caspian Sea in this study.

Few studies have been conducted on the effect of color on MP ingestion by zooplankton. The color and shape of MP pieces may be effective in the availability and selection of ingestion by marine organisms due to their similarity to prey (Wright *et al*., 2013). The colors observed in the particles in the zooplankton and seawater samples were similar to those reported in studies by other researchers, such as Desforges *et al*. (2015) and Steer *et al*. (2017). In general, the ingestion of MPs by marine organisms occurs accidentally and depends mainly on the abundance and size of the particles (Rodríguez-Seijo and Pereira, 2017).

The average size of the ingested pieces observed in the zooplankton samples was in the range of 35-46.5 µm. This value was recorded 56.25 µm in seawater after passing water samples from the four stations through a mesh with a pore size of 100 µm, (Table 2). In general, the size of MP pieces ingested by zooplankton in the Caspian Sea was much smaller due to the smaller size of the samples (<100 µm) compared to reported samples from other sources. In some studies, it has been reported that different zooplankton groups ingest a range of 0.5 to 816 µm (Cole *et al*., 2013; Cole and Galloway 2015; Desforges *et al*., 2015). It seems that the diversity in the size of MPs ingested by zooplankton may be related not only to the size and bioavailability of the pieces (Vroom *et al*., 2017) but also to the size of the organisms and the opening range of their mouthparts (Botterell *et al*., 2019). Comparison with some other studies reporting MPs in zooplankton (copepods) is provided in Table 3.

In this study, we investigated the presence of MPs in the zooplankton's bodies and Seawater in the southwest of Caspian Sea in Iran for the first time. The results indicate the contamination and presence of MP particles in the bodies of these two groups of zooplankton, which play a key role in the food chain. Considering the importance of fishing in this area, the presence of polymers in the zooplankton's bodies from the southern coasts of the Caspian Sea can be transferred to higher trophic levels, including humans, through the consumption of planktivorous fish such as Kilka (Clupeidae) and whitefish (*Caspian kutum*), which feed on these zooplankton (the food of white fish larvae in the early stages). Therefore, considering the importance of the issue for human health, management of plastic waste in coastal areas should be given a high priority.

Table 3: Comparison with other studies reporting MPs in copepods.

Location	MP size (µm)	MP composition	MP color	References
Coastal British Columbia	555.5 ± 148.7	-	Black, red, blue	Desforges *et al*. 2015
The northern slope of the South China Sea	125-140	Unidentified fibers, particles, & irregular shapes-Polyester	-	Sun *et al*. 2017
The Yellow Sea	154.62 ± 152.90	Organic oxidation polymers, polypropylene, polyethylene, low-density plastics	-	Sun *et al*. 2018a
The East China Sea	295.2 ± 348.6 (fiber), 20.3 ± 11.0 (pellet), 82.4 ± 80.5 (fragment)	Polymerized oxidized organic material, polyester	--	Sun *et al*. 2018b
Jiaozhou Bay, the Yellow Sea	526 ± 395	Polyester, cellophane, polyethylene	--	Zheng *et al*. 2021
The southern shore of the Caspian Sea, Iran	46.5	Polyethylene, Polyester, polyethylene terephthalate	Black, orange, blue, white	This study

In addition, comprehensive and extensive studies are needed to determine the level and type of contamination in the Caspian Sea ecosystem and its fish to control pollution and improve the situation.

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