Beadlet Anemone: A Novel Bio-Indicator of Microplastic Pollution in the Marine Environment

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List of abbreviations including units and nomenclature

Please give all abbreviations:

MPs: Microplastics

SEM: Scanning Electron Microscope

FE-SEM: Field Emission Scanning Electron Microscope

Abstract: Extensive usage of plastic in different industries and household usage has degraded to microplastic due to environmental conditions over the last year. While several researchers conducted the determination of microplastic (MP) bioaccumulation from rivers to stormwater, except for some filter-feeding species used as a bioindicator, to achieve a holistic approach to the fate of MPs in the marine system, sea anemone was used as an indicator. Microplastics were extracted from surface seawater, sediment, and sea anemones from the same sampling area and characterized. The extracted MPs were confirmed by confocal micro-Raman spectroscopy, and the morphology of the MPs was investigated by scanning electron microscopy (SEM). The results showed that the accumulation of microplastics and the type of polymer, shape, colour, and size of these persistent pollutants varied in sediment, surface water, and sea anemone. The abundance of MPs was different, and grey-coloured MPs were detected at a relatively higher level in sea anemones, from 8.7 to 13.3%, and more minor MPs (less than 0.5 mm) at 43.4 to 56.8% were detected in sea anemones among the sampling stations. In terms of polymer type, there are relatively more types of polymers classified in sea anemones among the sampling stations (six different types of polymers). These results indicated that the utilization of sea anemones as a bioindicator for MPs could be a critical factor in a better understanding of their pathway in the marine ecosystem. This study proved that analysing sea anemones as an indicator could offer a reliable, fast, and time-saving approach for detecting microplastic accumulation in marine systems.

Keywords: Beadlet anemone, Bioaccumulation, Microplastic Pollution; Bioindicator, Filter-Feeding,

1. Introduction

There is a significant increase in plastic production at a 3% rate annually across the globe, and this rate will reach 70 million tons by 2050 (Vo and Pham, 2021; Mishra et al., 2022). Interestingly, the total mass of plastic worldwide is more than 10 billion metric tons, which is 25 times more than the global population (Shekhar et al., 2022; Bostan et al., 2023). Microplastics (MPs) pollution has been growing attention in environmental concerns globally over the last decades due to their higher bioaccumulation rate as a result of degradation capacity; they are the candidate to be persistent pollutants due to their specific large surfaces; and last but not least, they can be ingested by living organisms (Wang et al., 2018; Hu et al., 2019). The bioaccumulation of these environmental pollutants is inevitable due to their high discharging rate to nature, low recovery rate, and long stability (Shen et al., 2020; Ya et al., 2021). While the detection of microplastics has been reported in all natural ecosystems, from air to terrestrial sources, water bodies are more vulnerable to these pollutants than other environmental media (Jeyavani et al., 2021). Climate change, excessive industrial spills, and rising tourism stress cause an expansion in MP pollution in water bodies (Deme et al., 2022; Yuan et al., 2022).

The bioaccumulation of these persistent pollutants in aquatic sources has been driven by the type and source of polymers and environmental conditions (Yusof et al., 2023) The cumulative effect of MPs bioaccumulation causes several negative impacts on the reproductivity, feeding, and energy metabolism of living species (Wang et al., 2020; Ali et al., 2021). In addition to the direct impacts of microplastic exposure on the aquatic system, these pollutants act as a vector for other marine contaminants (Jeyavani et al., 2021). Monitoring of other aquatic pollutants is accepted as an essential step to understanding the progression and long-term results of pollutants and determining mitigation strategies in water bodies (Kentin and Battaglia, 2022). Biomonitoring is one of the most preferred monitoring approaches owing to its economic, environmental, and user-friendly advantages (López-Pedrouso et al., 2020).Fish are considered the most appropriate organisms for biomonitoring aquatic contamination owing to their wide distribution and ecological functionality across the food and energy systems (Freire et al., 2023) Like other aquatic pollutants, fish have been accepted as one of the essential biomarkers in the microplastic debris in the water system; Jabeen et al. (2017) highlighted that microplastic bioaccumulation has been reported in more than a hundred fish species. Oysters and mussels are also commonly used as indicators in MPs monitoring via their filter-feeding system (Costa et al., 2023) Besides these benefits, several studies have reported that MPs have a higher adhesion capacity on the gills, skin of fish, and even the edible part of mussels (Makhdoumi et al., 2021). These factors tend to lead researchers to find alternative filter-feeding aquatic species that humans do not consume in the design of MP monitoring approaches.

Sea anemones are known predatory animals for many kinds of marine organisms, from fish and crustaceans to zooplankton to molluscs, and they play a key role in understanding the relationship between pelagic and benthic ecosystems. Due to the fact that they are present from the tropical zone to the polar zone and have well-inhabiting properties from the intertidal zone to more than 10,000 m depth, these species are essential in the better understanding of marine ecosystems (Sun et al., 2022; Menezes and Thakur, 2022). The potential bioaccumulation of MPs in the predator animal as a result of continual feeding of a wide range of MPs ingested by zooplankton, phytoplankton, and crustaceans has been indicated (Debnath et al., 2022). Despite the abundance of research related to MP bioaccumulation performed by traditional sampling of sediment and surface water, there is still a pressing need for alternative bioindicators that are outside the food chain monitoring of MPs. Thus, the aim is to evaluate a first attempt at the feasibility of using sea anemones as a monitoring tool for microplastics correlated with sea surface water and sediment in a holistic framework. Within these efforts, the presence of MPs in sea anemones, surface water, and sediment from the same locations was investigated.

2. Materials and Methods

2.1. Sampling location

Coastal surface water, sediment, and sea anemones were collected from three different stations in Mersin, Türkiye, in early May 2023. Mersin is in the Northeast Mediterranean part of Turkey at latitude 36° 48' 8" N and longitude 34° 37' 58" E (Figure 1). The land border is 608 km2, the sea border is 321 km2, and the surface area is 15.953 km2. The sampling station was chosen as a case study area because of its different environmental pollution stresses. Yeşilovacık 1 (36° 10′ 7790″ N; 33° 39′ 5710″ E) was defined as Station 1 and represents a sample under industrial pollution stress due to its closeness to the thermal power plant. Yeşilovacık 2 (36° 11′ 4670″ N; 33° 38′ 29″ E) as Station 2 as the sample under pressure from anthropogenic activities and commercial fishing ports. Taşucu (36° 18′ 9790″ N; 33° 52′ 3750″ E) as station 3 represents a bay where a relatively higher intensity of tourism activities than other areas.

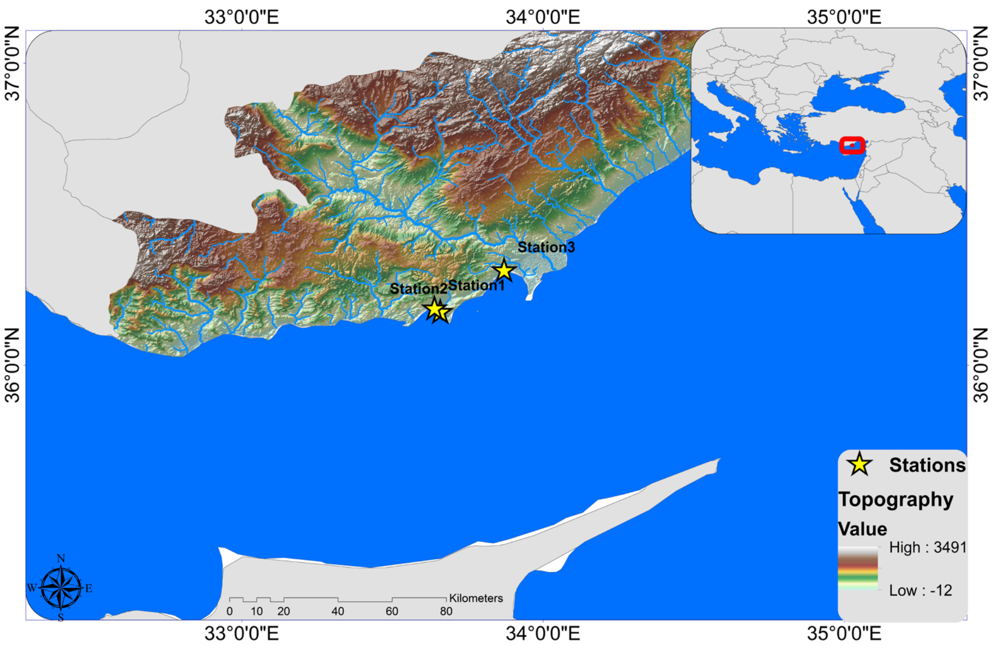

Fig.1.Sampling map

Sampling procedures were based on previous studies where 10 litres of surface water were collected from depths of 0–20 cm with the aim of capturing as many MPs as possible on the water surface at each sampling station. Sea surface water was collected using a stainless-steel Teflon pump and filtered using a mesh sieve with a pore size of 50 μm (Yabanlı et al., 2019; Mak et al., 2020), with three replicates collected separately at each sampling location (Qu et al., 2018). Surface water samples (1 L each) were rinsed into a sterile glass sampling jar, 4% formalin solution was added, covered with aluminium foil, closed, and cold-preserved in a portable refrigerator until reaching the laboratory (Herrera et al., 2018). Sediment samples were collected from the same location as the surface seawater. The sediment samples passed through a sieve with a mesh size of 5 mm, and a 300-gram sample was put into the glass jar using the metal spoon, covered with aluminium foil, and closed. The lips were preserved at 4 ◦C before analysis. Anemone samples were collected manually from the sampling zone on the rocky shore. Thirty individuals of sea anemones (Actinia equine), which were closed between each other with scales in weight and length, were collected for each sampling area. Sea anemones were collected from the same location as surface seawater and sediment to achieve representative samples. A total of 90 sea anemones (30 individuals from each site) were gathered randomly from beach rocks at three sampling stations in the intertidal zone. Sea anemone samples were immediately placed into the glass sampling jar and covered with aluminium foil and lips. They reached the laboratory in a freezer at -20 °C. To avoid possible MP contaminations during the sampling of surface water, sediment, and sea anemones, and within the laboratory approaches, all precautions were applied.

2.2. MP extraction

Each surface water sample was filtered through a 0.45 μm filtration membrane initially, and then the filter paper was pre-treated with hydrogen peroxide (H2O2, 30% v/v) to eliminate organic material (Zhang et al., 2017). Then, 1 L of surface water was filtered through a multi-branch filtration system aided by a vacuum pump (Sartorius, AG, Germany) with a GF/F glass fiber filter (diameter 47 mm, pore size 0.7 mm, Sartorius, Germany). Then the filter paper was placed into glass Petri dishes and dried at 40 ◦C for 24 h in an incubator (Heratherm IVH180-S, Thermo Scientific, Germany). MP extraction from sediment samples was performed using the method described by Lots et al. (2017) and Besley et al. (2017).

The sediment samples were sieved with a stainless-steel sieve with a 5 mm mesh size. 100 g of sediment was placed into the glass and sterile flask and desiccated at 60 °C overnight prior to the MP extraction process. Then 130 mL of hydrogen peroxide (H2O2-Merck) was added to the sediment sample to remove the organic matter, stirring slowly at ambient temperature for 24 h. Then the hydrogen peroxide solution was removed, and the sediment sample was washed through sterile and filtered water and dried at 60 °C over the night. Sodium chloride (NaCl-Merck for analysis EMSURE®) at the 1.2 g/cm3 final density was added to the secondly dried sediment samples, and the mixture was dispersed in an ultrasonic water bath for 15 min. The stirred sediment and sodium chloride mixture were kept at ambient temperature over the night to achieve good sedimentation (Yabanlı et al., 2019). The solution was filtered through GF/F Whatman® filter paper (47 mm diameter, 0.70 μm pore size) by the above-mentioned filtration system, and the filter paper was dried at 40 ◦C into glass Petri dishes over the night. Microplastic extraction from sea anemones was performed based upon Hurley et al.'s (2018) method with minor revisions. Fenton's reagent was prepared by 15 g of ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) crystals (Merck, Germany) in 1 L of 0.2 μm Filtered ultrapure water, and the pH of the reagent was adjusted to 3.0 by 3 mL of sulphuric acid (H_2SO_4; 95%; Fisher Scientific). Then the reagent was mixed with hydrogen peroxide (H_2O_2; 30%; Fishers Scientific) at a 1:1 ratio.

The volume of Fenton's reagent used in the microplastic extraction from sea anemone flowed by Savage et al.'s (2022) methodology was 40 mL for the large anemones (22.5–25 g/each individual), 20 mL for the mid-size anemones (10–15 g/each individual), and 10 mL for the smaller anemones (5–6 g/each individual). Extraction of MPs from anemones was performed in a shaker incubator at 40 °C and 60 rpm at 40–50 °C and stabilized by an ice bath due to the exothermic properties of Fenton's reagent. The extraction was repeated until all the organic material was digested to achieve a clear solution (approximately 48 hours). All the digests were then cooled to ambient temperature, vacuum filtered, and dried as the above-mentioned methods. All the surface water, sediment, and sea anemone samples from each sampling station were analyzed three times.

2.3. Characterization of Microplastic and Visualization

Microplastics are characterized visually depending on their shape and colour by a stereo microscope (Leica M165FC, Wetzlar, Germany) equipped with a camera (Leica DFC450C) according to previous classifications (Xu et al., 2021). The size of MPs was analyzed by Image J software. Following the classification of MPs on the microscope, the type of polymer was verified by laser confocal micro-Raman spectroscopy (NRS-4500 JASCO) with specific wavelengths ranging from 532 to 785 nm (Xi et al., 2019). The size of the laser spot was 0.6 m, the integration time was 10 s, the spectral resolution was 10 cm^{-1}, and the laser intensity was 0.1%. The type of MP's was identified by the system's own special software and reference spectral library. A field emission scanning electron microscope (FE-SEM) (Hitachi Hi-Tech, Ltd., SU5000, Japan) was used to characterize the surface composition of the microplastic particles (Hitachi SU5000, Japan). As suggested by Foo et al. (2022), ten randomly selected microplastic particles that have different morphologies were used for SEM analyses. The MPs were glued to double-adhesive carbon tape and covered by gold for 60 s using a sputter coater (Leica ACE 200, Leica Microsystems GmbH, Wetzlar, Germany) with a thickness of about 6 nm gold. SEM imaging of MPs was performed with a 0.25 nA electron beam accelerated at 10 KeV under a vacuum pressure of 10-3 mbar.

2.4. Statistical analysis

The abundance of MPs in surface water, sediment, and sea anemone was calculated in terms of particles. A one-way analysis of variance (ANOVA) test was performed using SPSS 22.0

software (Chicago, IL, USA) to determine the differences in terms of colour, size, and shape of MPs in all environmental media from all stations. All statistical analyses were conducted in triplicate from each sampling area.

3. Results and Discussion

3.1. Spatial differences of MPs in sea surface water, sediment and indicator Sea anemone (*Actinia equina*)

The average number of microplastics detected in the individuals of Actinia equina was 19.3, with an average of 1.95 microplastic particles per gram of sea anemone regardless of the sampling station. The spatial differences of polymer types of MPs in the sea surface water, sediment, and sea anemone along Mersin Bay are shown in Table 1. The main composition of the polymer was polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyurethane (PU), and polyamide/nylon (PA/NY) at diverse ranges in different environmental media. Polyethylene (PE; St. 1–52.7%; St. 2–39.3%; St. 3–37.1%) was the dominant type of polymer. In the sea surface water, the PE abundance was found to be relatively higher in station 1 than in other sampling stations. Spatial variations were also found in other types of polymers. Polypropylene (PP) abundance also differs among sampling stations (PP; St. 1–52.7%; St. 2 39.3%; St. 3 37.1%). The quantity of polyethylene terephytalate (PET) was relatively higher in station 3. (PET; St. 1–52.7%; St. 2 39.3%; St. 3 37.1%).

The amount of polystyrene (PS) was found to be relatively higher in stations 2 and 3 (PS; St. 1–52.7%; St. 2 39.3%; St. 3 37.1%). Polyurethane (PU) was not detected in sea surface water from Station 1 and Station 2, and this polymer was detected only at Station 3. The abundance of polyamide (nylon) (PA) also differed depending on location. While PA was not detected in the MPs from station 1, PA was detected at the 52.7% rate from St. 2 and 39.3% from St. 3.

Table.1. Microplastic counts from different station categorized by polymer type.

		Type of Polymer						
Location		**Polyethylene (PE)**	**Polypropylene (PP)**	**Polyethylene terephytalate (PET)**	**Polystyrene (PS)**	**Polyurethane (PU)**	**Polyamide (PA)**	**Total**
Station 1	SSW	210	160	58	70	-	-	498
	S	129	112	90	90	4	20	445
	SA	100	85	69	31	12	49	346
Station 2	SSW	340	380	110	150	-	20	1000
	S	320	300	170	220	-	50	1060
	SA	130	110	104	101	45	90	580
Station 3	SSW	410	430	220	310	100	80	1550
	S	340	300	240	360	120	20	1380
	SA	185	145	179	133	69	101	7671

SSW: Sea surface water; S: Sediment; SA: Sea anemone.

Similar static differences were observed in terms of the polymer type of the tested MPs among the sediment samples from different locations. The microscope view and Raman results of microplastic are shown in Figure 2.The total number of MPs of sediment was higher (135 MP kg^{-1}) in Station 3, following Station 2 (109 MP kg^{-1}) and Station 1 (59 MP kg^{-1}). While polyethylene (PE) was dominant at Station 1 (35.6%), the abundance of polypropylene (PP) was higher than other polymer types in Station 2 and Station 3 (34.9% and 31.9%, respectively. The level of polyethylene terephytalate (PET) was found to be different among sediment samples; the highest level of PET was determined in the sediment samples from station 3 (16.3%), and the lowest level of PET was observed in the sediment samples from station 1 (11.9%). While the amount of polyamide (nylon) MPs were found to be relatively lower than other kinds of polymers in all sediment samples, the highest and lowest rates were found in the sediment samples from stations 3 (5.9%) and 1 (3.4%), respectively.

The MP contents of sea anemones from different sites in Mersin Bay are shown in Table 1. In total, 1768 MP particles were counted in the 90 individuals (30 from each station) analyzed. The lowest number of MPs particles was found in Station 1 (346 items), and relatively higher MPs items were observed in Stations 2 and 3, with 699 and 723 items, respectively. Polyethene (PE) was the dominant MP found in all the sea anemones from all sampling beaches. While sharp variations were observed in terms of polymer type in the anemones from Station 1, the abundance of detected polymer types was relatively close to each other in the anemone samples from Station 2 and Station 3. While the PP mount was found to be relatively higher than the PET in anemone samples from stations 1 and 2, the PET was found to have a higher number of items than the PP in station 3. Interestingly, polyurethane (PU) was not detected in surface water from stations 1 and 2, but it was found in anemone samples from relevant stations (12 and 45 items, respectively). Polyamide (nylon) was also found to be relatively higher in anemone samples than in sediment samples from all stations.

The spatial differences in the polymer type of MPs were observed in sea surface water, sediment, and sea anemone samples from different stations, which are under different environmental stresses. In terms of sea surface water, a total of 389 items were found in station 1 (Yeşilovacık 1). 1070 and 1680 items were determined in station 2 (Yeşilovacık 2) and station 3(Taşucu), respectively, and all these variations were found to be statistically significant. The amount of microplastic in station 3 was found to be almost three times higher than that in station 1, which is clear evidence that the accumulation of microplastic differed depending on location. The significant variations in the number of MPs among stations could be related to the distance of the sampling location and anthropogenic impacts such as tourism, fishing, and household activities. The abundance rate of polyethylene (PE) was found to be relatively higher in Station 1 at 52.7 % than in other stations (39.3 and 37.1% for Station 1 and Station 2, respectively). Station 1 was a relatively remote area from human communities compared to other stations, and the higher amount of MPs in Station 2 and Station 3 could explain the effect of fishing and tourism. Cabansag et al. (2021) and Concato et al. (2023) also reported similar differences in the number of MPs driven by the presence of humans, tourism, and fishing activities. Significant differences were also found in the spatial distributions of MPs in the sediment sample among sampling stations. The abundance of MPs differed from highest to lowest as follows: St. 3 (1950) > St. 2 (1090)> St. 1 (534). The highest MP ingestion rate of sea anemone was observed in Station 3, which is in accordance with the spatial differences observed in sea surface water and may partially explain the relative abundance of MPs in the sediment sample, attributed mostly to the presence of human-related activities and fishing (Agharokh et al.,2022).More specifically, Gedik et al. (2022) reported that there were spatial differences in different sampling sites in the Mediterranean Sea and a positive correlation between the density of the human population and the abundance of MPs.

Overall, the abundance of MPs in sea anemones was significantly lower than that in sea surface water and sediment ($P < 0.05$) in all stations from Mersin Bay, which is also reported in different marine organisms (Agharokh et al., 2022). The spatial distribution of microplastics in the sea anemones matched that of surface seawater sediment samples at all stations. The dominant polymer types in sea anemones samples were polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET) in order from highest to lowest, and the type of polymer dominancy differed among the samples from different sampling stations. The spatial variations in sea anemones were consistent with the results of previous research performed by Morais et al. (2020). They researched the MP bioaccumulation on different anemones (Bunodosoma cangicum) across different locations on the Brazilian Amazon coast and highlighted that the ingested polymer type in sea anemones varied among sampling stations. de Orte et al. (2019) also reported that the dominance of the polymer type of ingested MPs from the sea anemone Aiptasia pallida changed by the ingestion mechanism of the organism. More specifically, Janssens and Garcia-Vazquez determined the polymeric differences depending on location in the same sub-species (Actinia equina (Linnaeus, 1758)) across the north coast of Spain. The properties of polymers and environmental pollution stress are the main factors in the bioaccumulation of MPs in sea anemones as filter-feeding animals (Xu et al., 2020).

The dominance of polymer types was found to be different in various media in this research. While polypropylene (PP) and polyethylene (PE) rates were found to be higher in surface sea water than sediment, polystyrene (PS) and polyethylene terephytalate (PET) rates were found to be higher in sediment samples than surface sea water, regardless of the spatial differences. These differences might be explained by the density of polymers and sea water; PP and PE have a relatively lower density than seawater, and they tend to float in the sea water. PET and PS tend to sink to sediment due to their higher density (Patterson et al., 2019). Polyamide (nylon) abundance found higher than that in sea water and sediment could be explained by the higher density of PA than sea water. The density of the detected polymer also had an impact on the digested MPs by sea anemone at different ranges, which is consistent with de Orte et al. (2019), who reported that most of the MPs ingested by sea anemone had a higher density than seawater.

3.2. Shape, Size and Colour Differences of MPs:

The shapes of MPs in different environmental media are classified into four main groups: fragments, fibers, films, and pellets. Figure 3. Fragment and fiber were dominant in the surface seawater samples from all sampling stations. The distribution of fiber and fragments in the surface sea water detected the following: St. 1: fragment: 47.6% >fiber; 25.9%; St. 2: fragment: 29.3%; fiber > 28.0%; and St. 3: fragment: 31.9% > fiber; 37.7%. The abundance of pellets was found to be relatively lower than other polymer shapes in all the surface seawater samples. The most dominant shape of MPs in the sediment sample was fiber. In addition to fiber and fragments, the amount of film was also significantly higher in the sediment samples. St.1: fiber; 37.1%; fragment; 38.8%; film: 29.4; St.2: fiber; 32.6%; fragment; 25.6%; film: 24.4; and St.3: fiber; 29.6%; fragment; 24.6%; film: 24.1. While sharp differences were observed in terms of the shape of MPs in stations 1 and 2, the differences in the shape of MPs were of relatively lower importance. Spatial differences were observed in the percentages of MPs from sea anemones. The fiber was the dominant shape of MPs found in sea anemones from all the sampling stations. The rates of fragment and film were also found to be higher than pellet in all anemone samples: St. 1: fiber; 56.1%; fragment; 30.3%; film: 12.4; pellet: 1.2; St. 2: fiber; 56.9%; fragment; 28.6%; film: 11.9; pellet: 2.6; and St. 3: fiber; 47.0%; fragment; 28.2%; film: 19.0; pellet: 5.8.

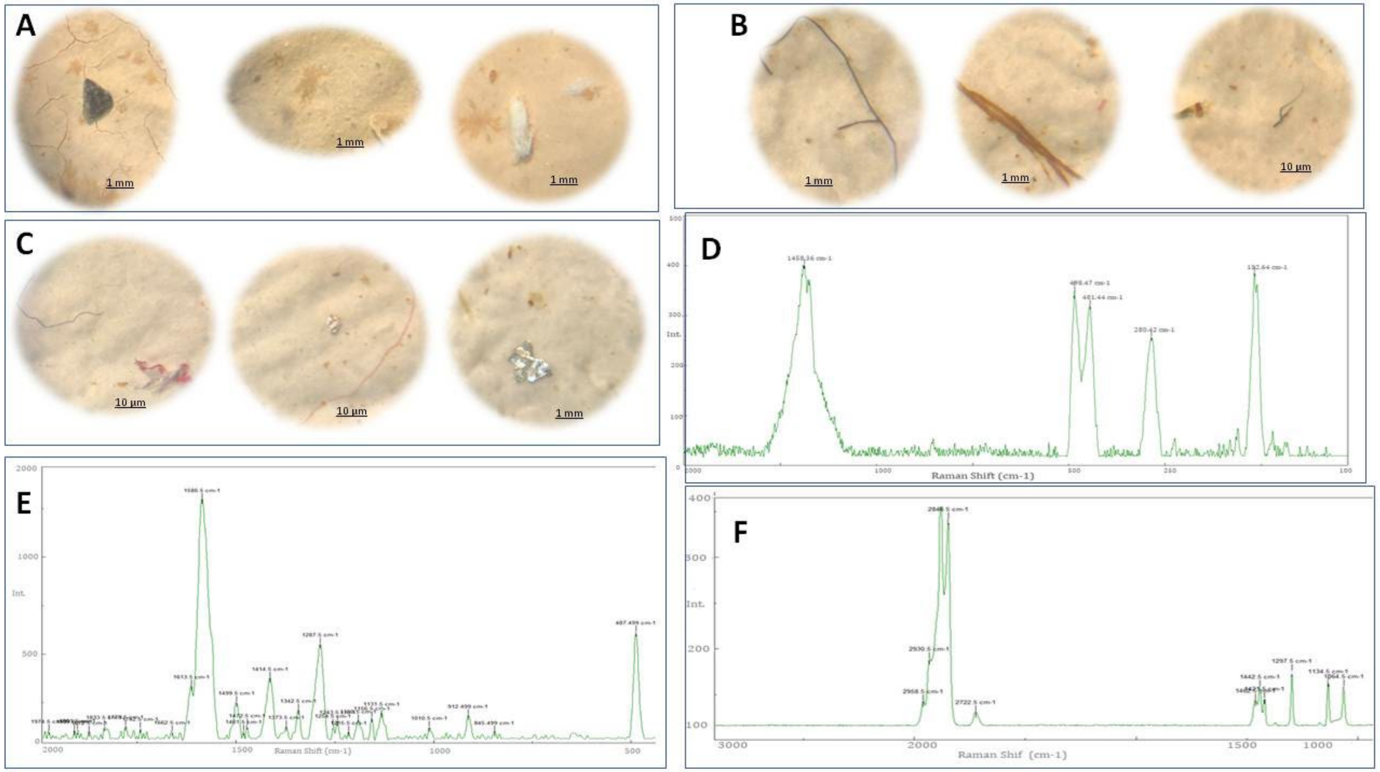

Figure 2. Microscope images and Raman results of randomly selected MPs from A) SSW;B: S ;and C) SA, D-F: Raman microscope view of MPs

Six different colours (transparent, black, blue, red, yellow, and grey) of MPs were found in this study. For all sampling sites, regardless of environmental media, from most to least frequent were: transparent (30.7%), black (21.8%), blue (20.9%), red (13.5%), yellow (8.8%), and grey (4.4%) (Fig. 3-A). Particularly, while transparent was the dominant colour in all samples, the second dominant colour of MPs differed in the surface seawater, sediment, and sea anemone groups; blue was found to be the second dominant colour of MPs in surface seawater. The colour of MPs in the SSW from highest to lowest order as SSW1: transparent: 33.1%; blue: 25.7%; black: 16.5%; red: 13.1%; yellow: 7.2%; grey: 4.4% In SSW2, transparent: 28.4%; black: 23.1%; blue: 20.1%; red: 14.5%; yellow: 11.9%; grey: 2.0%. In SSW3, transparent: 25.4%; black: 22.5%; blue: 19.7%; red: 17.4%; yellow: 12.3%; grey: 2.6%. Spatial differences were also determined in the sediment samples as follows: transparent: 32.4%; black: 28.5%; blue: 22.77%; red: 8.5%; yellow: 6.5%; grey: 1.3%. In S2, transparent: 28.0%; black: 23.5%; blue: 16.7%; red: 15.6%; yellow: 11.7%; grey: 4.5%. In S3, transparent: 30.4%; black: 26.4%; blue: 22.0%; red: 10.9%; yellow: 9.2%; grey: 1.1%. The Colour of sea anemones was found in a different order from station 2, as: SA1: transparent: 36.1%; blue: 24.3%; black: 16.2%; red: 14.2%; grey: 8.7%; yellow: 0.6% MPs Colour was found in the different order from station 2, SA2: transparent: 42.4%; blue: 16.9%; black: 13.4 %; grey: 13.3%; red: 10.5%; yellow: 3.4%. In the same order within different ranges as SA3: transparent: 34.1%; blue: 25.1%; black: 17.2%; grey: 11.2%; red: 9.2%; yellow: 3.1%. (Fig. 3-B). The size range for all MPs varied from 0.023 mm to 4,83 mm, regardless of the total 7671 MPs items, generally distributed from highest to lowest as 1-3 mm (29.6%), 0.5-1 mm (28.5%), 3-5 mm (23.6%), and <0.5 mm (18.4%) (Fig. 3-C). In addition to statistically important differences among SSW, S, and SA samples, the dominance of MP size was also influenced by spatial variation. The most abundant particle size of MPs was 3-5 mm in the surface seawater at a rate of 37.4–57.4%. The dominant size of MPs from sediment was 1-3 mm, ranging from 37.5% to 46.5%. (Fig. 3-D).

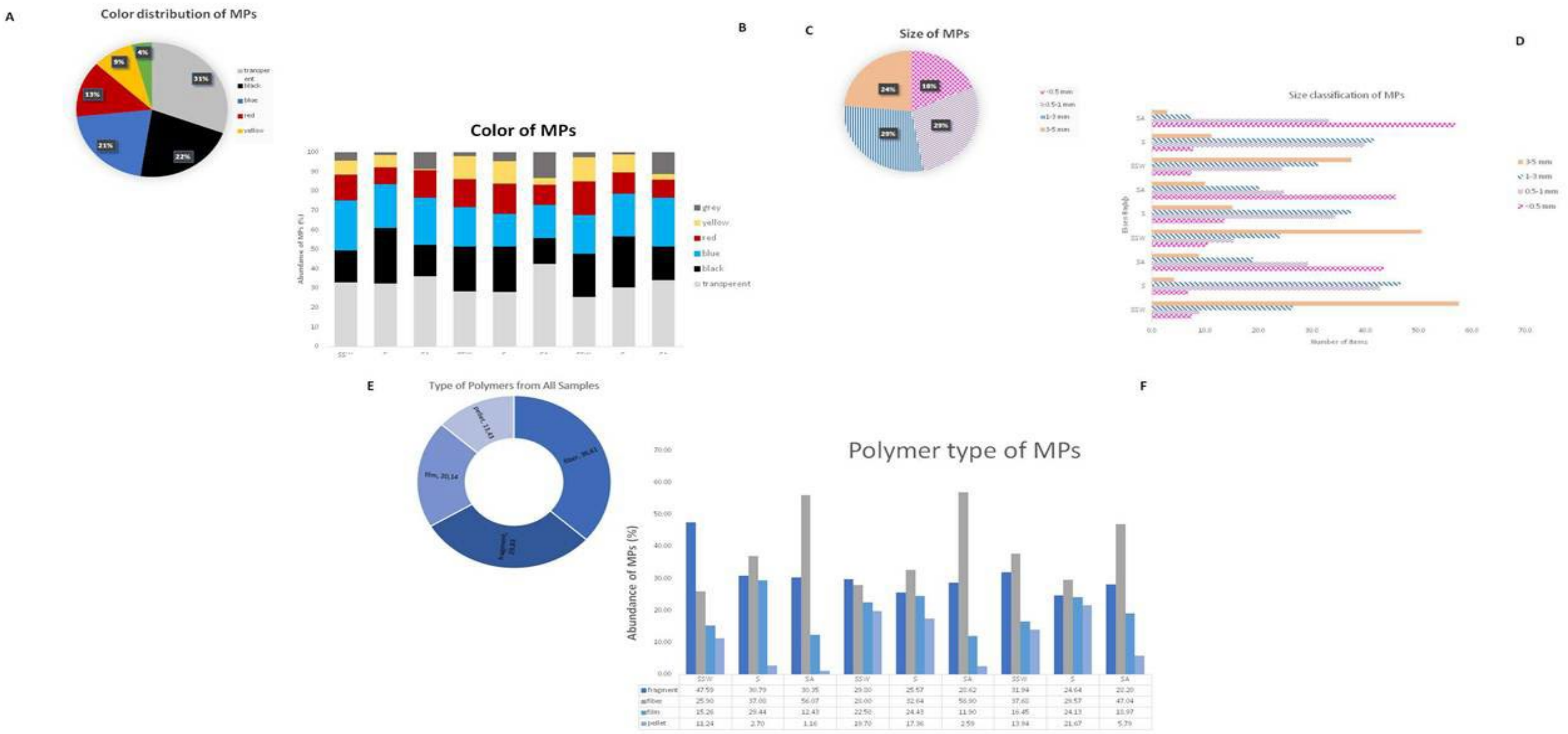

Figure.3. Type of polymer, size, and Colour distribution of MPs. A: Colour distribution of all microplastic, B: Colour distribution of microplastic in SSW, S,SA from each station, C: Size of all microplastic; D: Size of all microplastic in SSW,S,SA from each station; E:Morphology of all microplastic; F :Morphology of all microplastic in SSW,S,SA from each station

The abundance of fragments and fibers was dominant in the surface sea water from all sampling stations. This dominance and the lowest rate of pellet shape of MPs could be a result of fragmentation or degradation of primary MPs (pellets) into secondary MPs (fiber, film, fragments) (Ranjani et al., 2022). The highest rate of fragment was observed in the surface sea sample from station 1, which is very close to a thermal power plant, and the degradation of MPS was caused by temperature differences in both water and air temperatures. Microplastic fibers dominated the sediment sample at all sampling stations, accounting for 37.1% for St. 1, 32.6% for St. 2, and 29.6% for St. 3 of the total plastics for each location. This dominance of fiber was also highlighted by Gündoğdu et al. (2018) and Gedik et al. (2022) in the Mediterranean. Relatively more clear dominancy was found in the sea anemone sample than in the surface sea water and sediment samples in terms of the shape of MPs. As observed in sediment samples, the fiber concentration is highest in the anemone samples. The fiber rate in sea anemones was 56.1%, 56.9%, and 47.0% in ST1, ST2, and ST3. A high amount of fragments was also observed in the sea anemone sample, both film and pellet, which overlapped with the findings of Ruairuen et al. (2022), who reported that fiber abundance in the filter fed marine organisms. There were statistically significant differences observed among the sampling stations.

Fragments are known secondary microplastics that are formed by the oxidative, thermal, or photodegradation of larger-sized plastics used in the thermal power industry and construction sectors. Due to the fact that this polymer can be carried by the wind, the transformation of MPs from vehicles in the traffic and industrial gas streams into marine ecosystems is accepted as a serious concern (Neto and da Silva, 2023). A possible explanation for the higher abundance of fragments in surface seawater and sediment samples from Station 1 is influenced by proximity to the thermal plant. Since the usage of fibers has increased extraordinarily in the textile, fishing, aquaculture, and industries, and it cannot be removed by wastewater treatments.

Table.2. Examples of different aquatic organisms used for the monitoring of MPs.

Aquatic Species/organism	Sampling Area	Analytical approach	Main conclusions	References
Beadlet anemone, *Actina equina*	Mersin bay, Türkiye	FE-SEM, micro Raman stereoscopy	More Colourful and smaller MPs ingested by beadlet anemone.	This study
Acorn barnacles, *A.s amphitrite*, *F. albicostatus, T.*and goose barnacle, C. mitella	Hong Kong	Micro-FTIR; Attenuated total reflection (ATR)	Eight types of polymers were identified in barnacles and positive correlation between sediment	Xu et al., (2020)
Gastrointestinal tract and gill of (*M, barbatus,M.surmuletus, M.cephalus,S. undosquamis*	Northeastern Mediterranean, Türkiye	FTIR; Attenuated total reflection (ATR)	Fiber type-, black Coloured microplastics were dominant in the Gastrointestinal tract of fish	Kılıç and Yücel (2022)
Mangrove snails and mangrove crab	Pramuka Island, Jakarta Bay, Indonesia	Visualization	Positive correlation between abundance of microplastic and the body weight of snail and crab	Patria et al., (2020)
Green mussel,*Perna viridis*	Sungai Balang, Johor.	Fourier Transform Infrared (FT-IR)	Polystyrene and polyethylene were dominated and the age of mussel impact on the bioaccumulation of MPs	Buyong and Zulbakia, (2023)
Red swamp crayfish (*Procambarus clarkii*)	Northwestern Italy	µFT-IR technique	Gender and size of crayfish impact on bioaccumulation of MPs and crayfish can be used as indicator.	Pastorino et al., (2023)
Mnemiopsis leidyi	Southeast coast of Brazil	Raman spectroscopy	Blue Coloured fibers and fragments were dominant more kinds of polymers offer that *Mnemiopsis leidyi* can be used as a bioindicator for MPs	Otegui et al., (2023)

Discharging of this nonbiodegradable polymer by synthetic ropes used for fishing and clothes and other household textiles into the water bodies is accepted as one of the most common ways of MP contamination (Ranjani et al., 2022; Kentin and Battaglia, 2022). The higher concentration of fiber-type MPs in stations 2 and 3 than in station 1 might be related to these two stations being under tourism household and fishing stress.

The colour of MPs in the marine ecosystem can be altered by degradation under thermal or light stress, and besides the type of polymer, this physical parameter provides information about the primary source of MP (Hartmann et al., 2019). Regarding **Colour,** transparent MPs occupy 30.6% of total extracted MPs, followed by black (21.8%), blue (11%), red (13.5%), yellow (8.8%), and grey (4.4%). Transparent MPs were dominant in all environmental mediums at all sampling stations. The dominance of transparent MPs from sediment, surface sea water, and marine organisms is also stated by previous research (Xu et al., 2021). The predominance of transparent or white MPs in the marine ecosystem could be related to the extensive usage of light-Coloured plastic products in food packaging, household, and transportation (Ferreira and Lôbo-Hajdu, 2023). Besides the origin of plastic, photolysis and aging of MP driven by environmental factors also increase the proportion of transparent and white MPs in marine debris. Zhang, X., et al. (2021). Black was the second dominant Colour of MPs in the sediment samples from all the sampling stations, whereas blue was found to be the second dominant Colour in the sea anemones regardless of the sampling station. The detected relative abundance of black and blue MPs in the surface sea water, sediment, and marine biota in this study is consistent with previous findings by Kazour et al. (2019) and Gurjar et al. (022). The relatively higher density of black MPs may be related to the shorter time span of MPs in the relevant environmental media and short-term exposure to sunlight (Rasta et al., 2021). The higher abundance of blue MPs is most sourced from fishing gear and synthetic textile items (Simantiris et al., 2022). While gray MPs were found to be the least abundant in surface sea water and sediment groups for all sampling stations, this Colour was higher in abundance than yellow MPs in the sea anemone sample. These findings are in accordance with Janssens and Garcia-Vazquez (2021), who stated that the most prominent Colours were transparent, blue, and black in the beadlet anemone. Transparent MPs predominance can be explained by filter feeding of most commonly transparent or white-Coloured algae and plankton by anemones accidentally (Wang et al., 2020). In sea anemone samples, the Colour composition of MPs was different from that of surface seawater and sediment. The grey-Coloured MPs rate was relatively higher in sea anemone than in surface seawater and sediment from all sampling areas, most probably associated with the ingestion of MPs by the filter feeding mechanism of sea anemone. This result is in line with Abbasi et al. (2018), who reported the high abundance of grey MPs in brown shrimp. The Colour differences of MPs in the present study could be a result of consumer goods and fishing activities.

The size of MPs in aquatic systems is another crucial factor in better understanding the pathway of these persisting pollutants from the main polymer source to water bodies or even to humans. The extracted MPs ranged in size from 0.023 mm to 4.83 mm. MPs in the 1–3 mm size range were the most dominant, accounting for 29.6% of the total, followed by the 0.5–1 mm size class (28.5%) and the 3-5 mm size class (23.6%). The remaining MPs were larger than 0.5 mm, which constitutes 18.4% of the total MPs from three environmental samples. The predominant size of MPs differed among surface seawater, sediment, and sea anemone groups. The size of the MPs determined in surface seawater was found to be bigger than MPs from sediment and sea anemone groups. These environmental media-influenced size abundance variations were also represented by several studies in the Mediterranean coastal area by Güven et al. (2017) and Gedik et al. (2022). Relatively smaller MPs were found in sea anemone samples than in sediment and surface sea water from all sampling sites. These results clearly show that as MPs decrease in size, their ingestion rate by marine organisms increases (Wright et al., 2013). More particularly, Güven et al. (2017) pointed out that most of the MPs ingested by marine organisms were smaller than 5 mm in Mersin Bay.

3.3. Morphology of MPs

The morphology of micro- and nanoparticles characterized by SEM images is most found in different areas (Yousefi et al., 2019; Yousefi et al., 2021). SEM images of randomly selected MPs from surface sea water, sediment, and sea anemone are given in Figure 4. The high-magnification SEM images show surface grooves, fiber reinforcement cracks, irregular surfaces, fractures, pits, and vermiculate textures. Different types of diatoms were detected on the sea surface and sea anemone samples. The size and abundance of externally adhered microparticles on the MPs were found to be different depending on the MPs extracted from environmental media. Photooxidation of MPs also changes their hydrophobic structure and, consequently, their distribution rate in the marine environment (Ranjani et al., 2022). A representative SEM micrograph of the MPs from different environmental media clearly showed a high abundance of residues of biofilm, ruptures, fringe scale, and pits. These morphological differences are mainly caused by the origin of polymers, the life span of MPs in marine environments, and the weathering conditions. Additionally, adhering particles such as diatoms and vermiculate textures were also detected on the MPs, which is consistent with previous research that reported various types of contaminants and adherent items on the MPs extracted from sediment, surface water, and shellfish (Truchet et al.,2021).Due to the attachment of external materials to the surface of MPs, which alters the density of relevant MPs, it directly impacts the MP's life span in the environmental medium and ingestion rate, namely its fate (Aghilinasrollahabadi et al., 2021). This research represents one period of results: monitoring of microplastic pollution by sea anemones during the wet and dry seasons would improve the knowledge of microplastics and sea anemones.

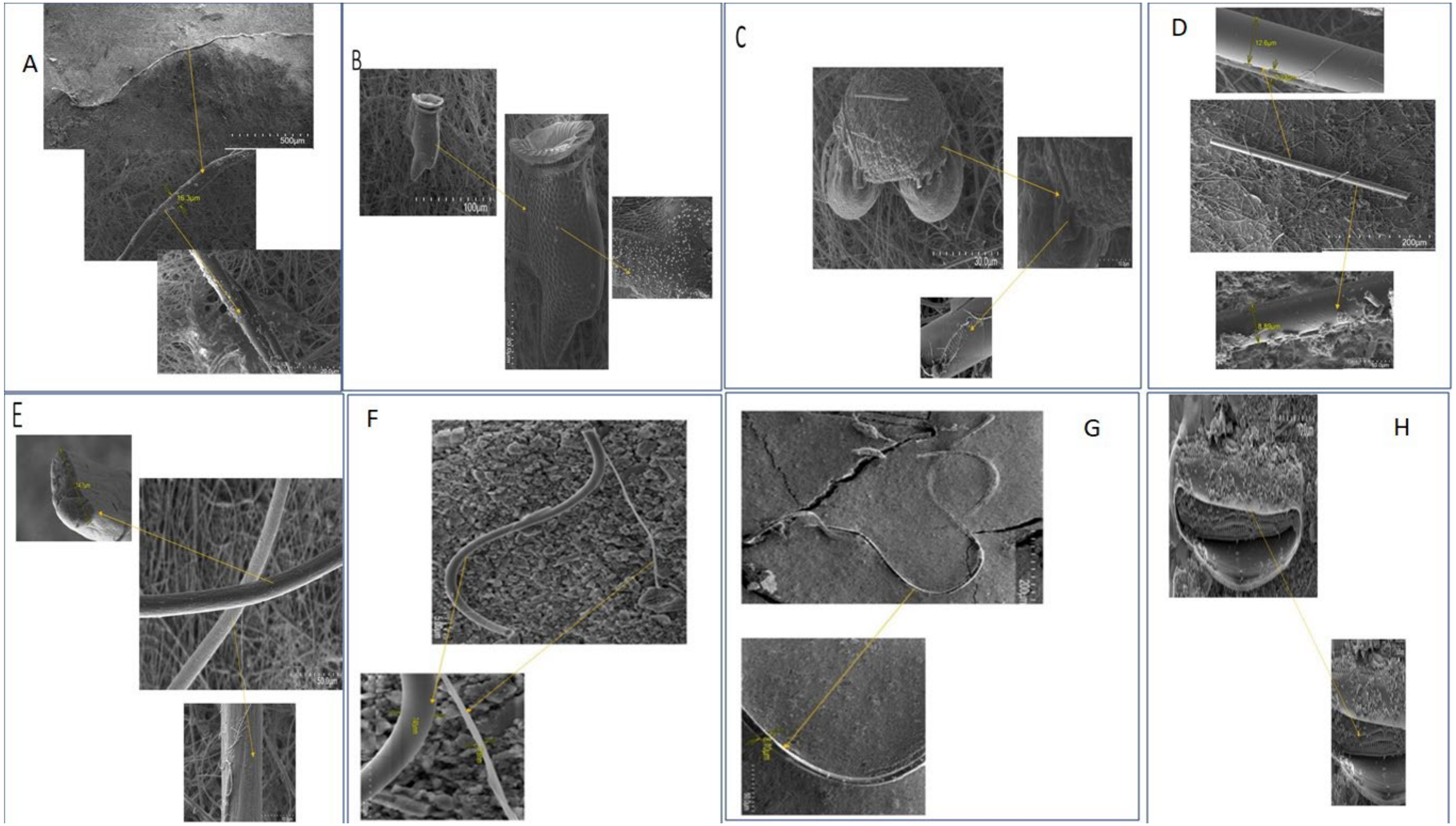

Figure.4. SEM micrograph of MPs and gastropods obtained at different magnifications A)Fiber, B-C: Gastropod, D-G)Fragments, H:Films

4. Limitations of This Study

There are some limitations which we have faced for this research as follows:

- ❖ Represents single term sampling of surface sea water, sediment, and sea anemones as aquatic medias.
- ❖ The sampling sites represent a small area of northeast Mediterranean part of Turkey.
- ❖ An enlarged multi-regional and multiple sampling times of the field study would improve the better understanding of results.
- ❖ The extraction of MPs from surface sea water, sediment and sea anemone by different methods that include several types of chemicals and process, that is the other limitation of this study.
- ❖ The standardized extraction process of MPs from several aquatic medias could be more user friendly and economic.

5. Conclusion

This study investigated the usage of sea anemone as a bioindicator for microplastic in marine systems within surface seawater and sediment from different sampling areas within Mersin Bay, which is in the northeast Mediterranean part of Turkey. The type of polymer, Colour, size, and shape of MPs were clearly different, and it depends on spatial variations. The effects of industry, fishing activity, and other factors were deeply considered, and the results demonstrated that the pathway of MPs is complicated and impacted by several internal and external factors. The results of micro-Raman spectroscopy and SEM images confirmed that sea anemones could be used as bioindicators in marine systems. Ingestion of more Colourful and relatively smaller MPs in the beadlet anemone could offer a better understanding of the fate of MPs in marine ecosystems. The findings of this research provide a holistic approach to surface seawater and sediment samples. Monitoring of MPs by beadlet anemone offers more sensitive and informative data about the bioaccumulation mechanism of these pollutants in marine ecosystems than surface seawater and sediment. Utilization of beadlet anemone as a practical bioindicator for monitoring MPs, reducing the sampling and characterization processes of these pollutants in sea surface water and sediment, could be a robust model for further research.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Graphical abstract