MICROPLASTICS IN THE MARINE ENVIRONMENT, PRESENCE IN WATER AND INTERACTION WITH MARINE ORGANISMS

Nelly Hobeika* and Afaf Younes
Lebanese University, Lebanon
Corresponding author*: Nelly Hobeika

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ABSTRACT

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This study is done in the framework of CLAIM project (Cleaning Litter by developing & Applying Innovative Methods in European seas). The objective is to advance the knowledge on the current status of marine plastic pollution in the Gulf of Gabes area of the Mediterranean Sea, by quantifying and qualifying the microplastics in water and biota samples. The results obtained show a high abundance of microplastics in all marine compartments studied with an average abundance of 1.16 items/m^3 ± 0.83 SD in the water sample. This concentration is relatively high compared to those reported in other Mediterranean regions. Dominance in number of fragments over other shapes of microplastics was reported in all sites. Polyethylene was the main plastic polymer for water samples (73% of the items analyzed are Polyethylene). These data underscore that the Gulf of Gabes region is a hotspot for plastic pollution, and this calls urgently for precautionary measures. Concerning the ingestion of microplastics by marine organisms, one blue plastic particle of 0.13 mm was found in 20 tested fishes. In addition, ecotoxicological tests were run in order to verify whether 1-4 and 20-25 µm polyethylene beads are likely to trigger lethal and sub-lethal responses in marine planktonic crustaceans and the results show that microplastics were accumulated in crustaceans, and may affect mortality.

Keywords: microplastics, Gulf of Gabes, water pollution, crustaceans.

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INTRODUCTION

Plastic pollution is an emerging and growing threat across world oceans and it has been pointed out as one of the most visible problems in Earth's ecosystems. Global plastics production has consistently increased over recent years and it reached 381 million tons per year (MTPY) in 2015 (Geyer et al., 2017). Thanks to its durability, low cost, and widespread application, plastic is a material of vast benefits to society. Therefore, plastics production is likely to increase even further.

Once in the environment, plastics tend to break down into smaller debris called microplastics (MPs), and they can be defined as ubiquitous plastic particles smaller than five millimeters in size, of various shapes, color and polymer composition (Frias & Nash, 2019). The biggest part of these MPs is found in the seas and oceans. In 2010, the amount of plastic waste reaching the oceans was estimated between 4 to 12 MTPY, and without proper management measures, the predictions indicate an increase by an order of magnitude by 2025 (Jambeck et al., 2015)**.**

With a size spectrum and buoyancy comparable to most planktonic organisms, MPs are likely to be ingested by higher trophic level organisms (fish, bivalves) and could accumulate along the food chain (Cole et al., 2011; Lusher et al., 2015).

In the Mediteranean Sea, the MPs analyses increased during the last years but a significant data gap can be identified especially for what concerns Southern Mediterranean countries. To fill this gap, and as part of CLAIM project, this study focuses on sampling and characterisation of MPs in the Gulf of Gabes area in Tunisia which is a partner in CLAIM project and where data are still missing.

The main goals of the present study are:

1) Monitoring and assessment of MPs abundance in two environmental compartments (sea surface water and marine biota) by determining MPs abundance, types, sizes, and polymer composition.

2) Studying the effects of MPs on marine crustaceans represented by the brine shrimps *Artemia.*

MATERIALS AND METHODS

Zone of study and sampling sites

In this project, the study focuses on assessing the MPs abundance in the Gulf of Gabes area. The gulf is 100 km long and 100 km wide and is bounded by the Kerkennah Islands on the northeast and by Jerba Island on the southeast. The Gulf of Gabes is the only part of the Mediterranean with a substantial tidal range causing the uncovering of extensive sandbanks at low water. It is also considered highly productive, contributing approximately to 40% of the national fish production in Tunisia (Béjaoui et al., 2019).

Both Gabes and Sfax are major ports on the gulf, supporting sponge and tuna fisheries, with Gabes being the economic and administrative center.

To asses MPs in this area, 6 stations are selected for sea water sampling (Table 1, Figure 1). The stations are located near ports and rivers which are considered as main sources point for the entry of MPs to the sea.

Table 1. Station number, position (latitude and longitude) and sampling date of water samples.

Sample ID	Station 4	Station 5	Station 7	Station 8	Station 16	Station 17
Start time	13h55	14h37	11h24	12h06	09h25	09h59
Start latitude	N34°00'386"	N 34°00'494"	N33°55'507''	N33°56'691''	N 33°46'789"	N 33°47'769"
Start longitude	E010°03'449''	E010°05'959"	E010°07'044"	E010°08'33"	E 010°15'701"	E 010°16'824"
Stop time	14h10	14h55	11h39	12h21	09h40	10h14
Stop latitude	N34°00'588"	N 34°00'397"	N33°56'248''	N33°57'185''	N 33°47'354"	N 33°48'284"
Stop longitude	E010°04'353''	E010°06'858"	E010°07'863"	E010°09'148"	E 010°16'384"	E 010°17'442"
Volume filtered	240	231	301	254	246	215

Figure 1. Map showing the locations of the sampling stations.

Samples preparation

Sea water samples

Microplastics sampling by manta net is a widely used method for the sampling of microplastics on the sea surface, but to date there has been no unified methodology. In this study, we report the procedure for manta net sampling.

In April 2019, water samples were collected using a 330 µm mesh size towed manta net having the dimension of the opening of 25x65 cm following the below sampling procedure (Gago et al., 2019):

1. Deploy the net out of the wake zone (approximately 3 to 4 m distance from the boat) in order to prevent collecting water affected by turbulence inside the wake zone (figure 2A)
2. Write down the initial GPS coordinates and initial time in the data sheet
3. Start to move in one straight direction for approximately 15 minutes and begin the time measurement.
4. After 15 minutes, stop the boat and write down final GPS coordinates, the length of the route (the most correct way is to calculate the length from the GPS coordinates) and the average boat speed into the data sheet provided and lift the manta net out of the water.
5. Rinse the manta net thoroughly from the outside of the net with seawater using water from the boat water reservoir. Rinse in the direction from the manta mouth to the cod end in order to concentrate all particles adhered to the net into the cod end (figure 2B).

6. Safely remove the cod end and the materials retained is carefully transferred into new plastic bottles. Rinse the cod end thoroughly from the outside and pour the rest of the sample through the sieve. Repeat this step until there are no longer any particles inside the cod end (Figure 2C).

7. Close the bottle, label the lid and outside of the jar with the sample name and date with waterproof marker.

8. Unless required, remove large pieces (algae, wood, organisms such as jellyfish) from the sample. Rinse them thoroughly with water from a wash bottle to ensure that all plastic is retained.

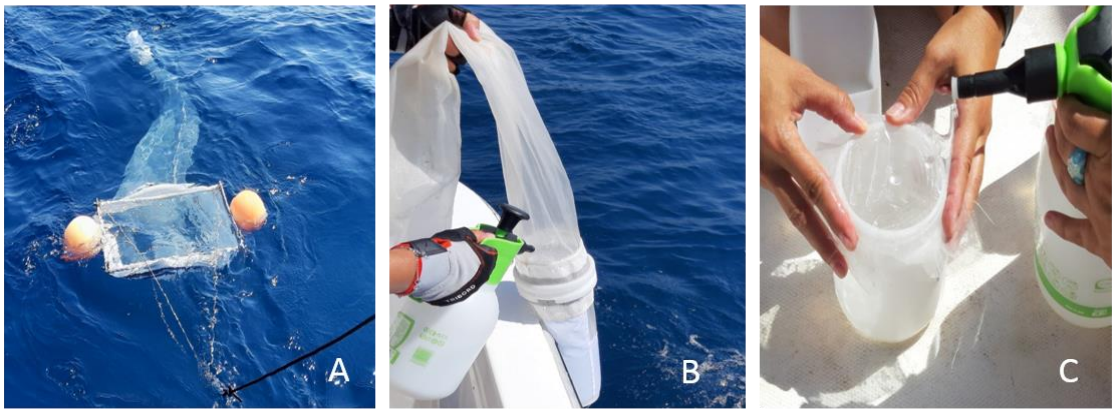


Figure 2. Sea water sampling steps: (A) manta net is deployed in water, (B) the net is recovered, held vertical and washed into the cod end, (C) the cod end is removed and rinsed using filtered sea water.

Biota samples

The fish samples studied were not sampled directly from the sea, they were purchased on May 2019 from the market of Gabes. The samples consist of 20 commercially available fishes: 10 *Pagellus erythrinus (PE)* and 10 *Scomber scombrus* (*SS*) specimens. Table 2 summurizes the details of both fish samples. The total and standard length (the standard length is defined as the total length without the caudal fin) are measured in centimeters (cm). The total weight and the weight without the guts are recorded in grams (g).

Table 2. Dimensions and weight of the biota samples.

PE sample ID	**PE1**	**PE2**	**PE3**	**PE4**	**PE5**	**PE6**	**PE7**	**PE8**	**PE9**	**PE10**
Total length (cm)	19.2	19.9	18.9	19.9	18.6	18.9	19.7	19.8	19.1	19.4
Standard length (cm)	14.9	15.6	14.6	15.8	15.7	15.2	15.8	15.9	14.9	15.3
Total weight(g)	79.3	104.88	81.16	92.55	78.72	78.92	95.67	97.99	84.73	86.18
Weight without	75.72	93.39	78.83	86.91	74.7	77.92	91.47	93.35	79.41	82.45

guts(g)										
Total length (cm)	18.3	18.3	18.9	18.4	18.8	18.9	19.7	18.9	19.4	18.6
Standard length (cm)	16.9	17.9	17.8	16.2	16.9	16.4	17.2	16.6	16.7	16.4
Total weight (g)	66.23	66.3	44.75	50.38	59.4	56.21	60.86	57.3	55.91	48.12
Weight without guts (g)	60.44	59.16	40.78	46.47	54.63	51.84	55.88	51.36	51.4	44.01

Samples analysis and characterization

Analysis of water samples is achieved by the separation of all potential MP items, classification according to physical properties and chemical properties.

In order to isolate the MP particles from sea water samples, the below procedure is followed:

1) A small amount of the sample (subsample) is poured into a glass Petri dish.

2) All subsamples are then visually examined and sorted under a stereomicroscope Olympus SZX7, 8x-56x) with attached digital camera (Nikon, DSL3).

3) When finding each MP item, it is manually separated using tweezers and then categorized according to physical properties (size, shape, color).

4) Separated MP items are dried and stored on a rectangular glass plate for later identification of polymer composition.

On the other hand, in order to detect the presence of MPs in fish samples, the fishes (Figure 3A) are dissected and the gastrointestinal tract, liver or other tissues are removed.

Each sample tissue is then placed on a glass Petri dish and dried overnight in oven at 50˚C. The dried sample is then triturated using a mortar (Figure 3B), until a powder is obtained, the powder is then put in a graduated cylinder and 100 ml and hyper saline solution is added to the sample (Figure 3C). The solution is stirred and decanted for 10 min. After this, a first aliquot of supernatant is collected (Figure 3D). The materials used for the collection of the supernatant are also washed in order to collect particles potentially attached to the wall of glass tube and cylinder. This resuspension step is carried out twice for a better extraction performance. The supernatant of each resuspension is then collected in a glass beaker and vacuum filtered (Figure 3E). The membranes with retained materials are transferred in a Petri dish with 15% hydrogen peroxide (H_2O_2) solution and put in the oven at 50˚C overnight for the partial digestion of organic matter (Figure 3F).

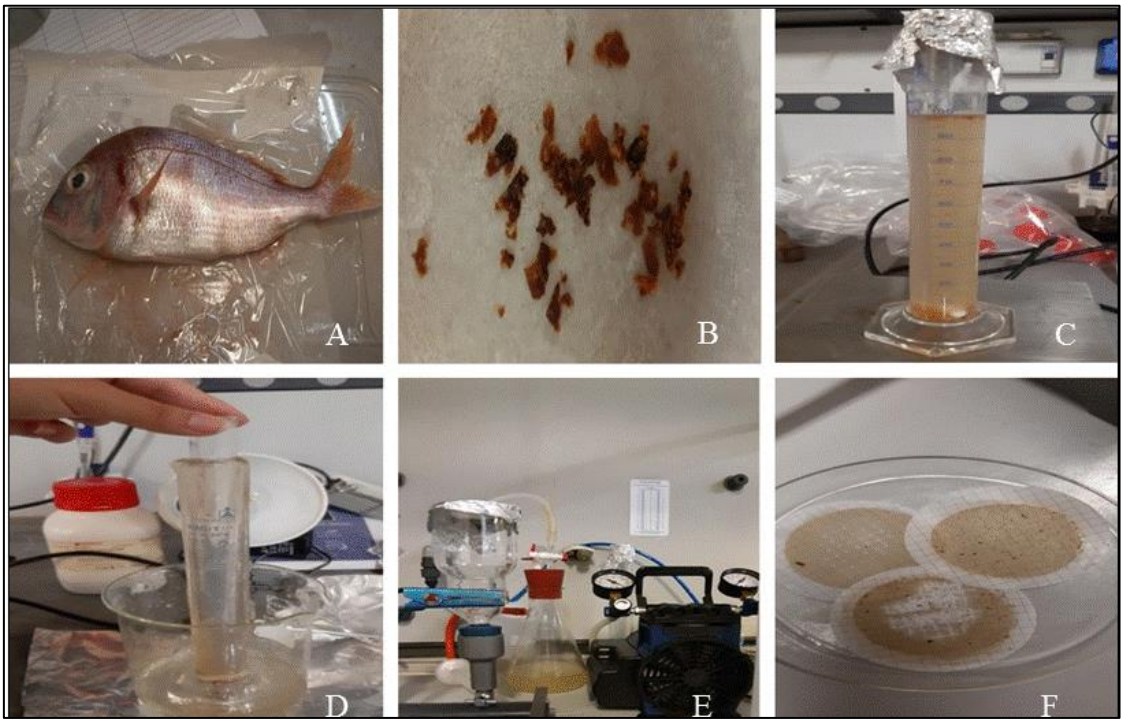


Figure 3. Pictures showing the recovery of MPs from fish sample.

After isolating MP items from water and fish samples, they are characterized using a PerkinElmer Spectrum Two FTIR spectrometer equipped with ATR accessory with a 9- bounce diamond top-plate was used. A computer was connected to the spectrometer to display and print the spectrograms. In this project, 909 MP items were characterized using the FTIR and the MP polymer type was determined by matching the characteristic peaks of recorded spectra with the reference spectra of known plastics.

Ecotoxicological effects of MPs on the marine environment

The effects caused by micro-sized plastics on marine crustaceans are studied. Therefore, we assessed ingestion effects of commercially available PE beads in the planktonic stages of brine shrimp, *Artemia sp*. Brine Shrimps are small shrimps that live in extremely salty water, will hatch within 24 hours, and become mature in about one week. They have short life cycle, high adaptability to wide salinity range of 5–250 g/L as well as to temperatures from 6 to 35°C. Three steps are performed to conduct the test:

1. Hatching of *Artemia* cysts

Dehydrated spherical cysts of *Artemia sp* (averaged 250-350 µm) were purchased and used for the experiments. The nauplii of the *Artemia sp.* were obtained by incubating the cysts for 24 h at 28 °C under light source and continuous aeration of the cyst suspension in seawater (37% salinity). The hatched organisms were transferred with a pipette into a beaker containing 0.22 µm filtred sea water (FSW).

2. Microplastics suspension preparation

Two sizes of MPs were used in the tests: 1-4 µm fluorescent PE and 20-25 µm PE MPs.

MPs were sonicated for 1 min using a bath sonicator and then suspended in 0.22 µm filtered natural seawater up to 100 mg/L concentration. This stock concentration was used to bring MPs to the various concentrations used in the tests (0.001–0.01–0.1–1–10 mg/L). The tests were performed immediately after MPs suspension preparation.

3. Contact between the nauplii of the *Artemia and the MPs solution*

To perform the test, organisms were transferred from the beaker into each well of a multi-well plates containing 1 mL of different MP concentrations. They were incubated in the dark, for 24 and 48 h, at 25 °C. Controls using only seawater and the brine shrimps were run simultaneously.

After exposure, mortality analysis was performed under a stereomicroscope: completely motionless larvae were counted as dead organisms, and the percentage of mortality was compared to the controls. Organisms that do not change their own barycentre position and do not move their appendages in 5 seconds are referred to as 'motionless' or 'not moving'. Mortality was evaluated in order to see whether such MPs rates may have toxic effects on the selected crustacean species.

RESULTS AND DISCUSSION

Quality control

During sampling and sample handling, it is important to identify potential sources of plastic contamination. And for that purpose, a blank was checked continously in the lab during the sample handling. As seen in the figure 4 below, fibers of different colors, mainly black and blue, were identified in the quality control and consequently, these items were excluded from the total count of detected MPs.

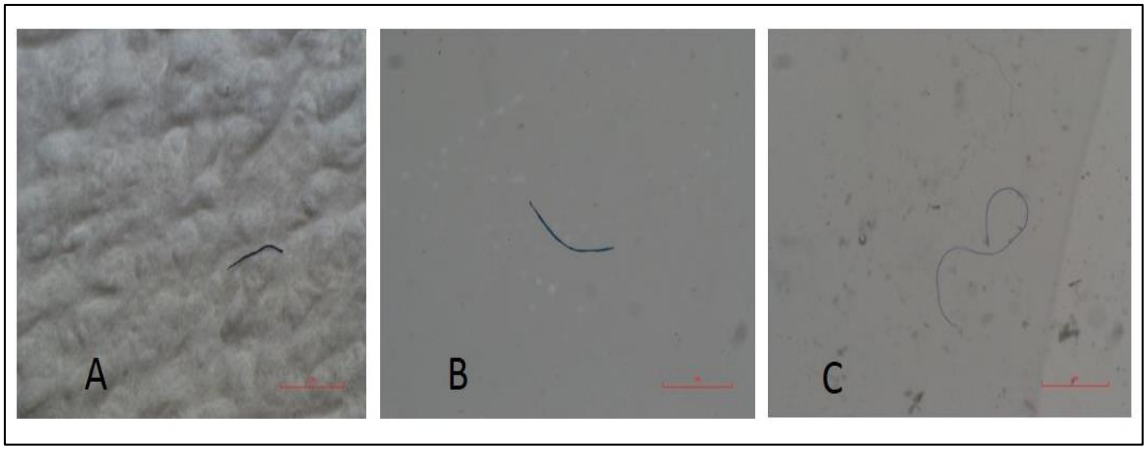


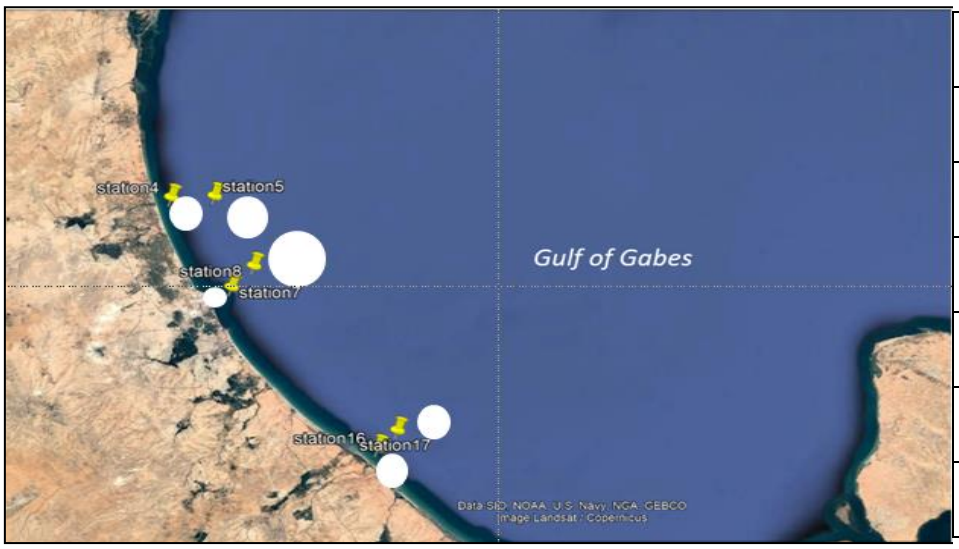
Figure 4. Examples of fibers found on the procedural blank during the analysis of water and fish samples. (A) black fiber, (B) and (C) blue fibers.

The main origin of these fibers is associated with airborne contamination, such as synthetic fibres stemming from clothing. For mitigating these contaminating risks, the sources of contamination should be eliminated and/or substantially reduced by wearing polymer-free clothing or cotton coveralls and gloves for example.

Microplastic abundance in water samples

For ease of comparability with other studies, the plastic concentrations in this study are expressed as plastic abundance per unit volume (Items/m^3). Attention should be given to the method used to measure the filtered and the analyzed volume. The filtered volume is the total volume from which we collected the MPs and it is calculated by multiplying the area of the mouth of the net by the distance covered during the tow. The later is obtained from the GPS data. This volume should be used to calculate the MPs abundance. In the other hand, the analyzed volume is the water volume that we added in the laboratory to the Petri dish for MPs observation under the microscope.

The abundance of MPs at 6 stations in the Gulf of Gabes is illustrated in figure 5.



Station	Abundance (items/m^3)
Station 4	0.81
Station 5	1.78
Station 7	2.50
Station 8	0.23
Station 16	0.89
Station 17	0.73

Figure 5. Abundance of plastic items at each sampling station.

MPs are present in all stations, suggesting the pervasiveness of MPs pollution in the area. A total of 1162 counts of MPs were detected at the 6 stations.

The concentration of MPs differ among the stations. It ranged between 0.23 and 2.50 items/m^3 with a mean value of 1.16 items/m^3 (± 0.83SD).

The relatively high MP abundance at station **7** (2.5 item/m^3) may be attributed to its geographic proximity to Gabes port. This result confirms that the shipping and fishing industries are potent sources for MPs pollution at sea and that the discharge of ship-generated waste has a substantial environmental impact.

Taking into account the content of ships' garbage (Plastics for example), this type of waste might be a relevant source for reusing or recycling supporting the transition towards a circular economy.

A comparison between each 2 adjacent stations shows that:

1) The MPs abundance in station 5 is higher than station 4($\Delta = 0.97$).
2) The MPs abundance in station 7 is higher than station 8($\Delta = 2.2$).
3) The MPs abundance in station 16 is almost equal to that in station 17($\Delta = 0.16$).

A trend of increasing abundance with distance from the shore is evident (the MPs abundance is lower in stations close to the shore and higher in the stations that are far from the shore). This result can be explained by the environmental factors and mainly the tidal movements in this area.

The tides observed in Gulf of Gabes are among the highest in the Mediterranean Sea (Up to 2 m in height) (Othmani et al., 2017) and they can help with the transport of MP items from the shore to the sea which result in higher MP concentrations in farther stations.

Comparison with other Mediterranean regions

Various units for MPs abundance (e.g., items/m^2, items/m^3, g/m^2, items/kg, etc.) have been used. Inconsistency in the sampling approach and reporting units make it difficult to compare MPs measurements results. In table 3, we compare our results with other studies with similar sampling methods and reporting units.

Table 3. Comparison of floating MP concentrations obtained in previous studies performed in the Mediterranean Sea.

Site	Mesh size (µm)	Abundance (Items/m^3)
Tuscany coastal water	330	0.26 (Baini et al., 2018)
Turkey (South East of Turkey)	330	0.27 (Güven et al., 2017)
Mediterranean surface (Italy, Spain, France, and Cyprus)	200	0.83 (Ruiz-orej, 2018)
Gulf of Gabes	**300**	**1.16 (present study)**
Palestine	333	7.68 (van der Hal et al., 2017)

The mean MPs abundance reported for the Gulf of Gabes region fall in the range of values reported elsewhere and it is relatively high compared to those reported in other Mediterranean regions and particularly the Western part where MPs concentrations vary between 0.15 and 0.62 items/m^3. This relatively high concentration of MPs compared to other Western Mediterranean regions is explained by the tidal effects present in the Gulf

of Gabes. In the Eastern Mediterranean, the MPs concentration fluctuates importantly across regions, ranging from 0.7 items/m^3 in Northeast coast of Turkey to 7.68 items/ m^3 in the Israeli surface water (Güven et al., 2017; Van der Hal et al., 2017). Although a small mesh size is used to collect MPs in Lebanon, a relatively high concentration is obtained which highlight the potential contribution of coastal landfills to this pollution (Kazour et al., 2019).

Our result is relatively low compared to the Eastern Mediterranean regions. This result can be explained by many factors. First, the Eastern Mediterranean has been reported for its high contamination of floating plastic debris in comparison to the Western part. The difference between the two parts of the Mediterranean Sea is due to the different circulation patterns: water currents in the Western part promote circulation; whereas currents in the Eastern part are somewhat attractors leading to the accumulation of floating debris (Baini et al., 2018).

Abundance of MPs according to their shape

The MPs in the Gulf of Gabes has a broad range of shapes, including fragments, fibers, foams, pellets and films. Fragments (Figure 6A) were the most abundant particle shape found within the study area accounting for 96% of the total microplastics found. Films (Figure 6B) were the second most abundant particle shape and contributed to 3% of the total plastic count. The abundance of fibers (Figure 6C) never exceed more than 1% of the total composition. Sometimes we saw few foam shaped and pellets shaped like the ones in figure 6.

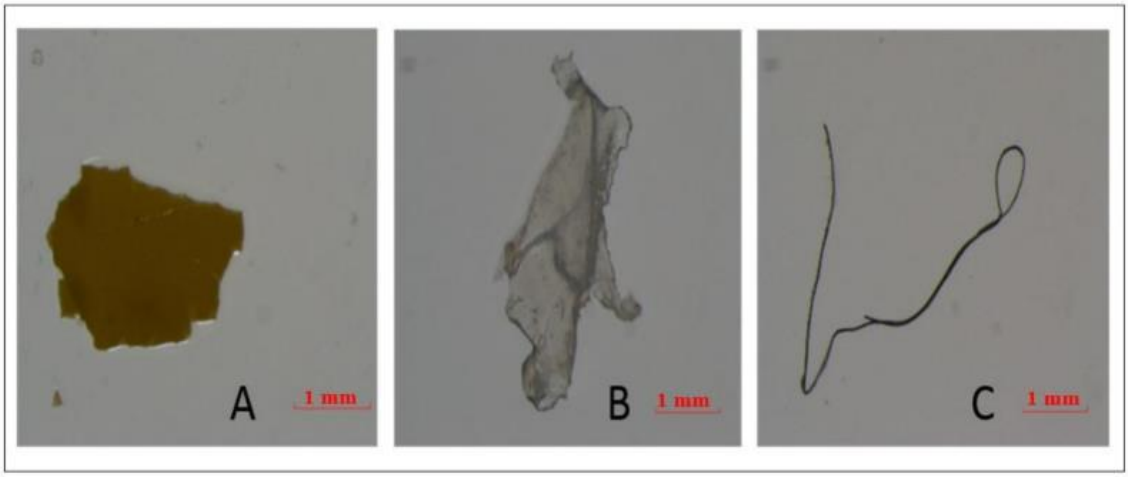
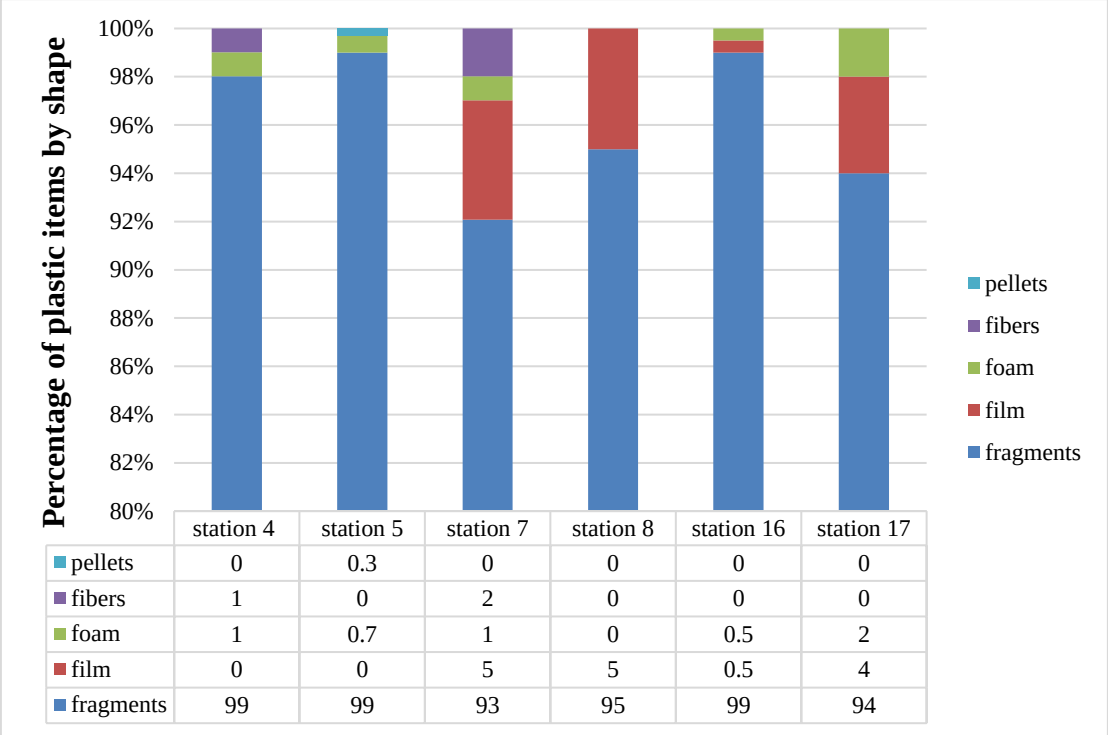


Figure 6. Pictures showing the shapes of microplastic debris detected: (A) yellow fragment, (B) transparent film, (C) black fiber.

Figure 7 illustrates the distribution of MP items by shape in each sampling station. Overall, there is evidence of a predominance of secondary MPs (Fragments) making up 96% of the total particles number. In total, 1162 MP items have been recorded within this study and 1131 of them were fragments and this suggests that the

main driver of MPs pollution in the study zone is from the degradation of larger plastics from terrestrial sources.



	station 4	station 5	station 7	station 8	station 16	station 17
pellets	0	0.3	0	0	0	0
fibers	1	0	2	0	0	0
foam	1	0.7	1	0	0.5	2
film	0	0	5	5	0.5	4
fragments	99	99	93	95	99	94

Figure 7. Distribution of plastic items by shape at each sampling station
Size classes of MPs.

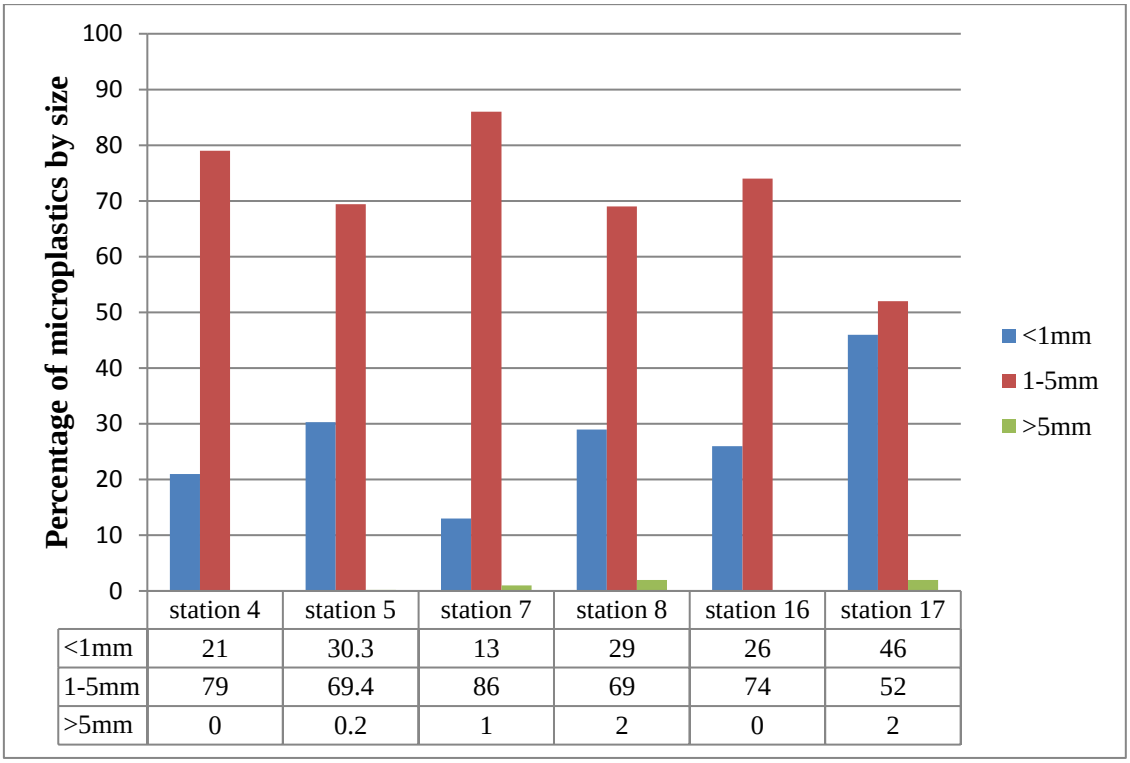
The high proportion of MP fragments over other shapes of MPs is consistent with those in previous studies of plastic debris in surface seawater (Güven et al., 2017). The fragments have various surface features, such as sharp edges with cracks or rounded shapes with smooth surfaces. Although we couldn't identify the sources of the fragments, their appearance may relate to their origin or history of degradation in the environment (sharp edges might indicate either recent introduction into the sea or the recent break-up of larger pieces, while smooth edges are often associated with older fragments that have been continuously polished by other particles or sediment (Ruiz-orej, 2018).

Different sizes of MPs were detected and in order to better quantify the amount of MPs, which are the center of this study, the items were separeted in 3 size classes:

- Macroplastics: > 5 mm.
- Large microplastics: 1- 5 mm.
- Small microplastics: < 1 mm.

The diagram below (Figure 8) shows the distribution of each size class in the stations. Our results show that the size of 72% of the detected MPs spans from 1 to 5 mm. 27% of MPs are small size (<1mm), while 0.86% of MPs are macro sized. Meso

sized plastics were the least dominant in all stations and they never exceed 2% of the total count.



	station 4	station 5	station 7	station 8	station 16	station 17
<1mm	21	30.3	13	29	26	46
1-5mm	79	69.4	86	69	74	52
>5mm	0	0.2	1	2	0	2

Figure 8. Distribution of plastic items according to their size.

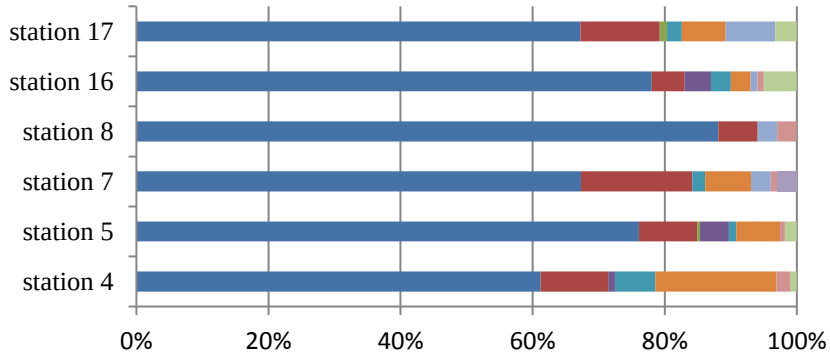
In addition, the MPs (Large and small) were the most dominant class size in all the stations thus confirming that MPs as the most abundant type of marine debris in sea water (Van der Hal et al., 2017). Since the smallest particles are most abundant here, it is probable that these MPs are in their early stage of fragmentation.

This result also confirms that the fragmentation of larger plastic items into smaller ones is the main driver of MP pollution. We can also note that the percentage of the small MPs (<1 mm) increase with distance from shore which is explained by the fact that smaller particles can be transported for longer distances compared to bigger particles (1 to 5 mm).

The mesh size of the net can influence the size distribution as well as the speed of the tow, as smaller particles avoiding the net can be forced aside from the net opening or large particles can squeeze out through the mesh. This study used a 330 µm mesh sized net.

Polymer characterization of MPs

PE was found to be the most abundant (73%) plastic type among the identified MP items in the research area. Other identified polymers include PP (10%) and Ethylene-vinyl acetate EVA (7%). The proportion of the polymers found in our study roughly corresponds to the global production stocks of plastic materials, with polyolefins (PE and PP) accounting for 62% of the global plastic demand and for 83% of our sampled items. (Figure 9)



	station 4	station 5	station 7	station 8	station 16	station 17
PE	60	74.7	68	89	78	66
PP	10	8.7	17	6	5	11.8
PVC	0	0.4	0	0	0	1.1
Pest	1	4.3	0	0	4	0
EPDM	6	1.1	2	0	3	2.2
EVA	18	6.5	7	0	3	6.5
ELASTOMER	0		3	3	1	7.4
PVA	2	0.7	1	3	1	0
Pacrylic	1	1.8	0	0	5	3.2
PA	0	0	3	0	0	0

Percentage of microplastics by polymer composition

Figure 9. Distribution of MPs according to their chemical composition.

PE and PP are the two predominant plastic polymers in our MPs samples, consistent with earlier findings (Ghosal et al., 2018). Being widely used in the disposable packaging industry and having lower densities than seawater, it is not surprising that these polymers consistently account for the majority of the plastic particles floating in surface waters worldwide. PE is the most common plastic on a global scale and consequently the most dominant plastic debris in the Mediterranean Sea and worldwide, deriving mainly from plastic bags and bottles.

Further studies should be done in order to know and to locate the types of industries in the area, the types of effluent and the types of landfills. Those information are strongly needed for a better interpretation of the results. A total number of 909 items were examined with ATR-FTIR spectroscopy. 850 items of them were assessed to be plastic polymers, 57 particles were assessed to be "non plastic" which means that 6.27% of the analyzed items did not consist of plastic but were rather made of viscose, wax, cellulose and other non-synthetic materials (Table 4). This reveal a relatively low misidentification rate during visual sorting. The distribution of the non-plastic items is shown in the table below.

Table 4. Non plastic items found in all the analyzed samples.

Type	viscose	inorganic	cellulose	resin	wax
Number of items	31	21	3	1	1

MPs in fish samples

Plastic items were detected in 5% of the examined fish samples. 10 PE species were examined and we found that they had not ingested any plastic items. One the other hand, plastic was found in one out of 10 SS species (Figure 10). The MP item found is a blue fragment having a size of 0.13 mm. The chemical composition of this item is not confirmed because it is too small and thus we need to characterize it using Micro-Raman spectroscopy which is not available in the laboratory. In our study, most MPs isolated in fishes were fibers, which we exclude to derive from secondary pollution (Fibers from clothes, contamination from the air, etc.). When working on MPs, fiber contamination of samples is a potential problem that needs to be addressed by specific protocols.

Figure 10. The plastic item found in the digestive tract of one specimen of Scomber scombrus (red bar equals 1 mm).

Preliminary ecotoxicological results

The effects of 1-4 µm fluorescent PE MPs on *Artemia sp.* Nauplii

Microscopy observations showed that fluorescent PE MPs (1-4 µm) were ingested within 24h and 48h, and were accumulated in the gut of the crustaceans. Toxicity tests results with nauplii of the Artemia sp. exposed to different MPs concentrations are reported in table 5 and figure 11.

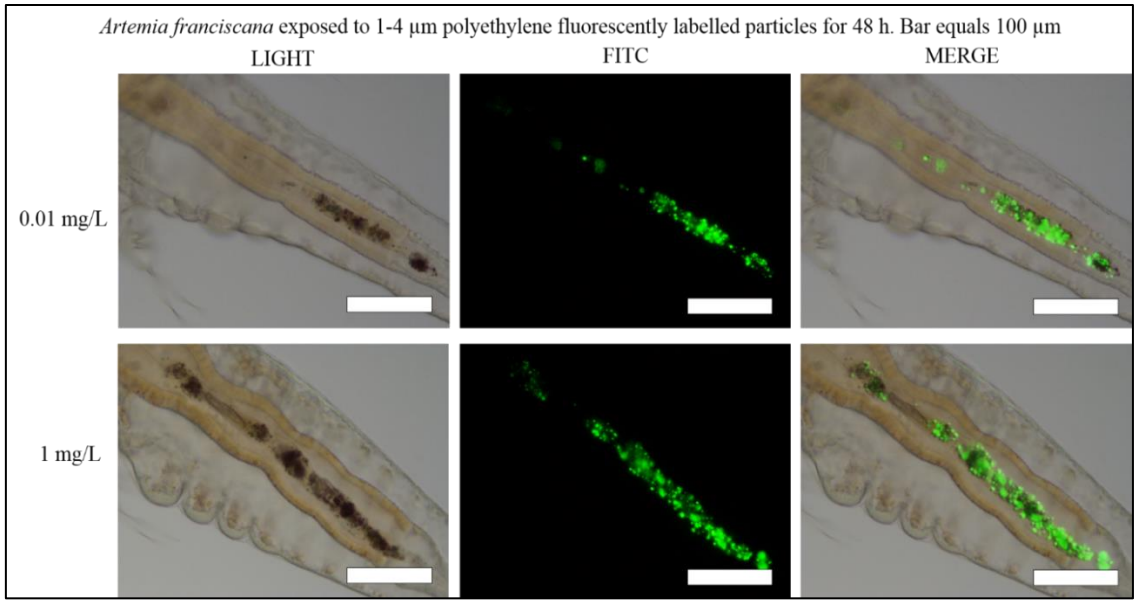


Figure 11. Representative microscopy images of Artemia sp nauplii revealing MPs inside the invertebrates after 48 h of the control and with exposure to 0.01and 1 mg/L.

Table 5. Percentage of mortality and immobilization observed in nauplii exposed to 1-4 µm fluorescent PE MPs.

Concentration (mg/L)	**0 (controls)**	**0.001**	**0.01**	**0.1**	**1**	**10**
After t= 24 hours						
%mortality	1.85	1.39	4.44	0	4.98	2.78
%immobilisation	1.85	1.39	6.67	1.96	4.98	2.78
After t= 48 hours						
%mortality	4.44	0	4.44	3.06	2.90	0
%immobilisation	4.44	1.39	6.67	4.72	4.57	0

Mortality and immobilization were affected by PE MP in Artemia sp. nauplii, and after 24h and 48h of exposure, they were both impaired mainly by 0.01 and 1 mg/L

concentration of MP, where a difference was observed between exposed nauplii and controls. No difference was found at the other concentrations . The highest mortality was observed at a concentration of 1 mg/L and it equals 4.98% which is considered as tolerable effect (<10%). This result may be due to the fact that the organisms will take out the MPs after ingestion. A continuous exposure to MPs is needed in order to accumulate MPs inside the guts of the organisms and thus to see higher effects.

As shown in figure 12, the organisms did not ingest MP in a dose-dependent manner and despite high MP concentrations used in this study (> 1 mg/L), limited mortality was observed in brine shrimps after 2 days. The concentration of 10 mg/L didn't result in the highest effects. These findings suggest that at higher concentrations, MPs tend to aggregate which means that it will be impossible for the organisms to eat it because of its large size.

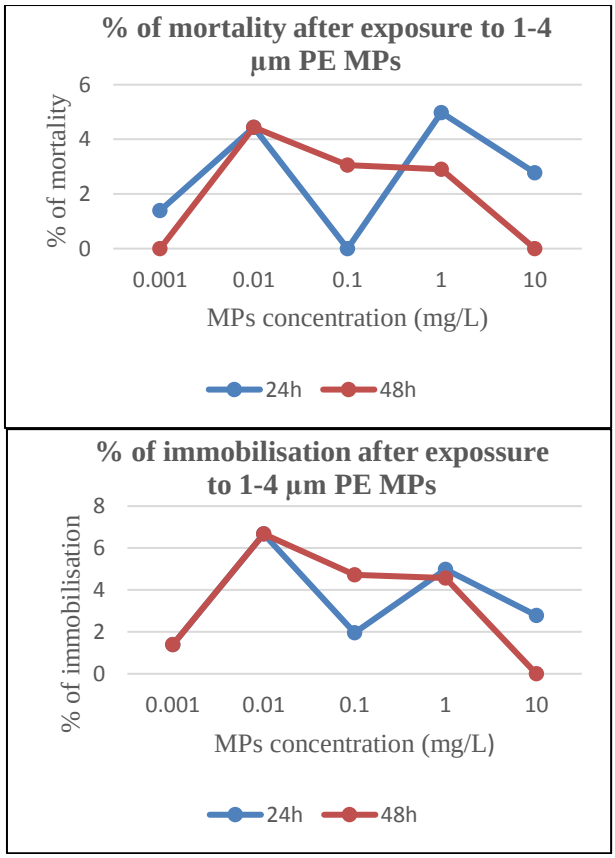


Figure 12. Percentage of mortality and immobilisation of *Artemia* nauplii after 24 and 48 h of exposure to increasing concentrations of 1-4 µm fluorescent PE MPs.

In addition, the effects observed after 24h are higher than those observed after 48h of the test. An exception is seen at a concentration of 0.1 mg/L where the mortality and immobilization highly rise after 48h. This indicates that higher exposure time didn't necessarily result in higher effects and this may be due to the aggregation of MPs after

longer time. The experimental setup and exposure time are two parameters that should be further taken into account for MP toxicity assessment.

The effects of 20-25 µm polyethylene MPs on *Artemia sp.* Nauplii

In the second test where larger MPs particles (20-25 µm) where used, we could not see any effects after 24 hours (Table 6, figure 13) which demonstrates that bigger MP particles aggregate faster and after 24h of the test, the MPs were already aggregated so they didn't cause any effects to the organisms. Another explanation is that bigger MPs particles need more time to be ingested by the organisms and thus longer time to affect the mortality and immobilization.

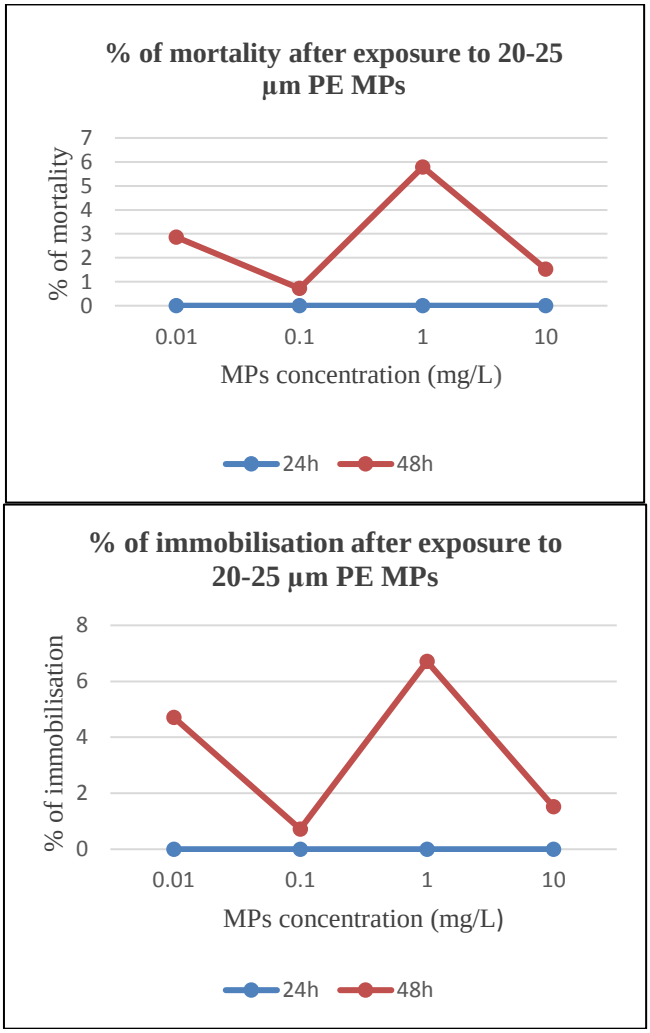


Figure 13. Percentage of mortality and immobilization of *Artemia sp.* nauplii after 24 and 48 h of exposure to increasing concentrations of 20-25 µm PE MPs.

Table 6. Percentage of mortality and immobilisation of Artemia sp. nauplii exposed to 20-25 µm PE MPs.

Concentration (mg/L)	**0**	**0.01**	**0.1**	**1**	**10**
After t=24 hours					
%mortality	0	0	0	0	0
%immobilisation	0	0	0	0	0
After t=48 hours					
%mortality	2.86	2.86	0.72	5.79	1.52
%immobilisation	9.11	4.71	0.72	6.71	1.52

After 48h of the test, we can see that there were little effects with all the concentrations used but these effects are still considerable tolerable (<10%). The highest mortality and immobilization were observed at a concentration of 1 mg/L consistent with the previous test. Overall, we can say that a concentration of 1 mg/L will cause relatively higher effects compared to other concentrations. Repetition of the tests with longer exposure time and continous MPs exposure are needed to obtain powerful results.

Comparaison between the effects caused by 1-4 and 20-25 µm MPs.

The charts in figure 14 compare the effects of the MPs size on the mortality and immobilization. After 48h of exposure, and at lower concentrations, the smaller MP items result in higher effects on both the mortality and the immobilization of the organisms. However, with higher concentrations (>1 mg/L), the bigger MP items caused more effects. This suggest that, for the moment, no correlation exists between the size of the MP items and the effects caused by its ingestion. The results obtained with ecotoxicological tests are preliminary results and in order to confirm them, the tests must be repeated for at least 3 times. We also suggest the use of bigger MPs (1 to 5 mm) which are the most abundant MP size in water samples that we analyzed.

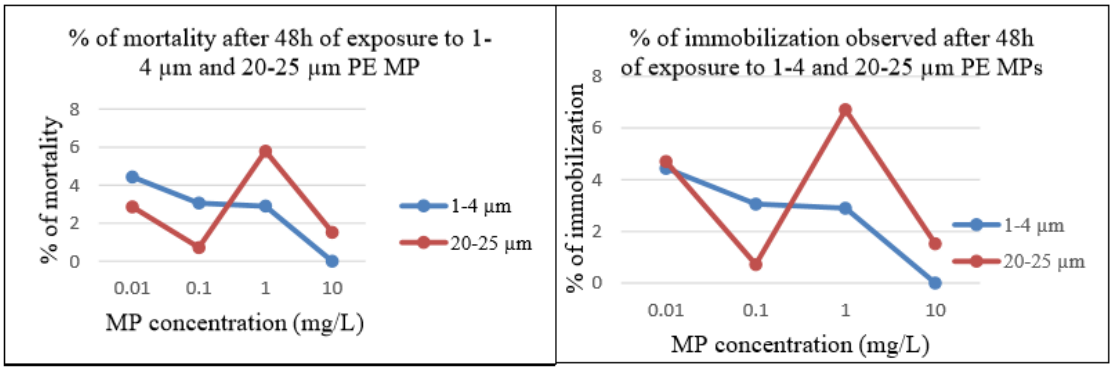


Figure 14. Percentage of mortality and immobilization of Artemia sp. nauplii after 48 h of exposure to 1-4 µm and 20-25 µm PE MPs.

CONCLUSION

The ubiquity of MPs in the marine environment has been previously reported. This is the first study in the Gulf of Gabes presenting an integrated picture of MPs pollution. Our results show that the study area is contaminated by MPs in all studied compartments. MPs have been found in 100% of the seawater, and in 5% of the biota samples examined which underscores that plastic pollution is a real issue for the Gulf of Gabes region. Plastic fibers have been observed in both water and fish samples. Plastic fiber presence could be due to air-borne contamination during sampling procedures. Therefore, when working on MPs, a careful data interpretation as well as standardized methods during sampling and lab activities are mandatory; standardization of methodologies for identification and quantification of MPs in the marine environment and formulation of standard operating procedures is strongly needed.

In addition to water and fish samples, we suggest that sediments samples from beaches should also be analysed.

We demonstrated that polyethylene microbeads which accumulate in the nauplii of the brine shrimp, only affect sub-lethal responses at environmental and high MP concentrations. Ecotoxicological tests could also be repeated in order to get significant results.

Irrespective of different polymers sources and typologies, the problem of plastic pollution is a social and behavioral issue, whose causes require to be mostly sought upstream in the consumption chain. There is no miracle solution! Genuine solutions will arise from collective action by civil society, policymakers and buisness. If just one of these groups doesn't fulfill its role in the tri-party scenario, the solutions will fail.

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