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Investigation of Microplastic Contamination in *Diadema Setosum* Obtained from a Fishing Barn

Balıkçı Barınağından Elde Edilen Diadema *Setosum*'da Mikroplastik Kontamisyonunun Araştırılması

Türk Denizcilik ve Deniz Bilimleri Dergisi

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ABSTRACT

This study is undertaken to evaluate microplastic contamination levels in *Diadema setosum specimens* obtained from a fishing barn. Microplastic (MP) pollution levels and their potential impacts on marine biota are still unknown compared to coastal and offshore environments. For this purpose, 19 individuals of *D. setosum* were collected and microplastic abundance in their gastrointestinal tract (GIT) and gonad were investigated. Mean microplastic abundance in GITs was 3.0 MPs±3.1 MPs per individual and 0.9±1.0 MPs per g wet weight. Mean microplastic abundance in the gonads was 0.3±0.6 MPs per individual and 0.08±0.2 MPs per g wet weight. Among all MPs, 45% of extracted MPs were fibers, followed by fragments (44%) and pellets (11%). Regarding size, the majority of the MPs extracted from GITs and all of the MPs extracted from gonads were small size MPs (less than 1 mm in size). FTIR analysis validated the plastic nature of suspected particles. Polyethylene (PE) (50%) and polypropylene (PP) (50%) were the most common type of polymers. These are the main polymers used in production of fishing nets; therefore, this result seems to validate the anthropogenic influence in the study area. This study contributes to the knowledge of the transfer of microplastics to the marine food web and highlights the need for protective measures.

Keywords: İskenderun Bay, Microplastics, Microplastic ingestion, Pollution, Türkiye

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ÖZET

Bu çalışma, bir balıkçı barınağından toplanan *Diadema setosum*'u örneklerindeki mikroplastik kontaminasyonunun varlığını araştırmak amacıyla yapılmıştır. Mikroplastik (MP) kirlilik seviyeleri ve bunların deniz biyotası üzerindeki potansiyel etkileri, kıyı ve açık deniz ortamlarıyla karşılaştırıldığında hala bilinmemektedir. Bu amaçla 19 *D. setosum* bireyi toplanmış ve gastrointestinal sistem (GİS) ve gonaddaki mikroplastik bolluğu araştırılmıştır. GİS'teki ortalama mikroplastik bolluğu kişi başına 3,0 MPs±3,1 MP ve ıslak ağırlığın gramı başına 0,9±1,0 MP olarak bulundu. Gonaddaki ortalama mikroplastik miktarı birey başına 0,3±0,6 MP ve yaş ağırlık başına 0,08±0,2 MP olarak bulundu. Tüm MP'ler arasında, ekstrakte edilen MP'lerin %45'inin fiber, %44'ünün fragment ve %11'inin pellet olduğu bulunmuştur. Boyutla ilgili olarak, GIT'ten çıkarılan MP'lerin çoğunluğu ve gonaddan çıkarılan MP'lerin tümü küçük boyutlu MP'lerdi (boyutu 1 mm'den küçük). FTIR analizi, şüpheli parçacıkların plastik yapısını doğruladı ve yaygın polimer türü olarak polietilen (PE) (%50) ve polipropilen (PP) (%50) bulundu. Bu polimerler balık ağlarının üretiminde kullanılan başlıca polimerlerdir, dolayısıyla bu sonuç, çalışma alanındaki antropojenik etkiyi doğrulamaktadır. Bu çalışma, mikroplastiklerin deniz ürünleri ağına aktarımı konusundaki bilgi birikimine katkıda bulunmakta ve koruyucu ölçümlerin gerekliliğini vurgulamaktadır.

Anahtar sözcükler: İskenderun Körfezi, Mikroplastik, Mikroplastik alımı, Kirlilik, Türkiye

1. INTRODUCTION

The plastic industry continues its significant growth due to the common usage of plastics in almost every area of our daily lives. Yet, low recycling rates and the absence of clear efficient management systems (Jambeck *et al*., 2015) cause the entrance of plastic waste into marine environments. Once these persistent polluters enter marine environments, they break down into smaller particles (Manzoor *et al*., 2022).

Microplastics are defined as plastic particles which are less than 5 mm in size. Even though their first appearance in the aquatic environment was back in the 1970s (Carpenter *et al*., 1972), their significant threat to marine biota was recently understood. Recent studies showed that microplastic pollution is a paramount concern even in the deepest part of the ocean (e.g., Mariana Trench (Peng *et al*., 2018).

The Mediterranean Sea is known as one of the most important hotspots of microplastic contamination since it has a semi-enclosed water circulation system with limited water flow (Everaert *et al*., 2020). The contamination level in the coastal regions is more alarming due to river discharges, anthropogenic activities such as industrial and domestic wastewater discharges, shipping, tourism, fishing industry, etc. (Suaria *et al*., 2016). Thus, marine organisms living in coastal regions are at more risk than those living in offshore waters (Compa *et al*., 2019; Lebreton and Andrady, 2019).

Sea urchins live in the rock and reef habitats of coastal environments (Nyawira and McClanahan , 2020). They are important benthic grazers and they have a crucial function in "material circulation and energy flow" in benthic environments (Dethiera *et al*., 2019; Feng *et al*., 2020). These species are commonly omnivorous grazers and detritus feeders, feeding on the algae or seagrass by scraping from hard substratum (Nyawira and McClanahan, 2020). Since seaweeds are potentially sink of microplastic particles (Gutow *et al*., 2016), these small home range species are more susceptible to microplastic contamination in their natural habitat (Murano *et al*., 2020). Laboratory studies showed that microplastic ingestion causes oxidative stress (Richardson *et al*., 2021), decreases fertilization rate (Lyons *et al*., 2021), deforms morphology (Bertucci and Bellas, 2021), and impacts the immune system (Murano *et al*., 2023). Besides, microplastic particles act like a gate for toxic compounds (Rendell-Bhatti *et al*., 2021; Di Natale *et al*., 2022) and increase the toxicity risk of hazardous chemicals like heavy metals (Rial *et al*., 2023). It needs to be emphasized that these species are potential candidates for protection according to the

International Union for Conservation of Nature (IUCN) (Manzo and Schiavo, 2022). Therefore, microplastic contamination have become more alarming and threatening issue for sea urchins.

For more than three decades, sea urchins have been used as model organisms for ecotoxicological studies (Manzo and Schiavo, 2022). These species are used as an effective bioindicators for the monitoring of several pollutants including microplastics (Lawrence, 2001, 2013; Bayed *et a*l., 2005; Soualili *et al*., 2008; Hennicke *et al*., 2021; Huseini *et al*., 2021), since they have low mobility and good response to benthic biota (Huseini *et al*., 2021). Previous studies have demonstrated that microplastic concentration in the sea urchins' bodies is strongly correlated with the sediment (Hennicke *et al*., 2021; Huseini *et al*., 2021; Rahmawati *et al*., 2023), indicating that these organisms are more likely to reflect the pollution status of the surrounding environment into their body (Pinheiro *et al*., 2020).

In addition to their ecological role, sea urchins are used for human consumption due to their delicious gonad. Stefánsson *et al*. (2017) reported that 50000 tons of sea urchins were harvested and consumed in Japan, Chile, New Zealand, and the Philippines, globally. They also reported that their consumption become popular in European countries such as Italy, France, and Spain, and 3000-3500 tons of sea urchins are harvested from the Mediterranean Sea annually (Stefánsson *et al*., 2017).

Current literature mainly focuses on microplastic occurrence in vertible species i.e. fish. Yet, information related to microplastic occurrence in invertebrate species is scant (de Sa *et al*., 2018). Among invertebrate species, most of the studies have focused on mollusks especially the bivalvae class (Kögel *et al*., 2020), and the presence of MPs in other taxons seems to be ignored. On the other hand, information regarding microplastic presence in sea urchins is highly valuable considering their ecological role, market value, nutrition value, and endangered potential.

This study was conducted to examine the microplastic abundance in the gastrointestinal tract and gonad of *Diadema setosum* specimens collected from a fishing barn in İskenderun Bay. Sampling location has particular importance because microplastic pollution levels in fishing barns are particularly unknown compared to other coastal and offshore environments. Today, it is a well-known fact that the degradation of fishing nets/ropes in shallow marine environments like fishing barns causes the release of microplastics (Welden and Covie, 2017). Similarly, a recent study has demonstrated that a significant portion of microplastics found in the sediment of fishing barns originated from the abrasion of fishing gears (Xue *et al*., 2020). This study is the first report that evaluates the MPs presence in a sea urchin species from Türkiye.

2. MATERIALS AND METHODS

2.1 Study area and sampling

Morphologically undamaged 19 *D. setosum* specimens were collected from the İskenderun fisheries barn (36°35'34"N 36°10'33"E) in the Hatay province of Türkiye, northeastern Mediterranean Sea (Figure 1).

Field observation revealed that the study area is severely contaminated with plastics. For that reason, it is hypothesized that the study area is severely contaminated with microplastics; since, the fisheries industry is one of the major sources of microplastics (An *et al*., 2020; Xue *et al*., 2020).

Figure 1. The sampled area and samples of *D. setosum* specimens.

2.2 Sampling dissection and digestion

The methodology was adopted from Feng *et al*. (2020). Each specimen was weighed, thawed, and washed with distilled water to remove any sediment or outdoor contamination. The diameter length was measured by a vernier

caliper. Next, each specimen was dissected from the mouth of the sea urchin carefully to avoid harm to internal tissue. Then, the gastrointestinal tract and gonad of each individual were extracted, separately weighted, placed into glass beakers, and covered with aluminum foil immediately.
After the dissection process, 50 mL of H_2O_2 was added to the beakers. H_2O_2 was selected as a digestion agent due to environmental concerns. The beakers were placed on a hot plate and kept at 50 °C for 12 h until the solution was homogenized, and tissues were completely digested. Later, the remaining solution was filtered through 0.47 µm glass filters through a glass filter system at low vacuum. Then, filters were placed into sterile Petri dishes until microscopic examination.

2.3 Microscopic examination

Filters were examined for the existence of microplastic particles by Olympus SZX7 microscope. Plastic nature was pre-validated by the hot needle method (Hanke *et al*., 2013; De Witte *et al*., 2014). Identified particles were classified according to type (fiber, fragment), color (black, red, blue, white, transparent, green, brown, and yellow), and their size. Filters that have MPs larger than 1 mm were placed in glass Petri dishes and set aside for Fourier transform infrared (FTIR) analysis.

2.4 Polymer identification

Fourier transform infrared spectroscopy (FTIR) was employed to validate the plastic nature of identified MPs. At this stage, out of 62 MPs, 6 MPs suitable in size (>1 mm) were taken as subsamples and used in spectroscopic analyses. FTIR analysis was carried out on a SHIMADZU QATR10 FTIR spectrophotometer equipped with single reflection attenuated total reflectance (ATR) accessory. The spectrum range was arranged as 4000–400 cm-1 and a resolution was set to 4.0 cm-1 with 32 scans for each measurement. The polymer type was identified by comparing absorbance spectra to reference libraries of SHIMADZU library.

2.5 Quality assurance and control

Collected specimens were quickly placed into pre-washed (i.e. three times with prefiltered distilled water) glass jars to eliminate airborne contamination.
Extensive precautions were taken in the closed laboratories with restricted access. Windows and air conditioners were always kept closed and turned off to minimize the airflow (Bessa *et al*., 2019). Before each analysis, laboratory surfaces, dissection equipment, and glass beakers were cleaned three times with pre-filtered distilled water (Torre *et al*., 2016). Used chemicals and distilled water were filtered before use at all times. Only authorized personnel were allowed to enter the laboratories and they wore nitrile gloves and cotton aprons at all times. Filters were checked under the microscope for the presence of MPs before use. Three wet blank filters were placed in the laboratory during the dissection and microscopic examination steps. Only one fiber sample was detected in the blank samples which suggests that the results are scientifically acceptable. The dataset was corrected by extracting the contamination data.

2.6 Statistical analysis

The normality of the data was checked by Shapiro-Wilk test. Since normality was not validated, Spearman correlation analysis was used to evaluate the correlations between morphological parameters (test diameter, test height, total weight, weight of GIT and gonad) and MPs abundance in the GIT and gonad.
The Kurskal-Wallis test was employed to examine the variations in microplastic abundance between organs. Calculations were carried out using PAST program version 4.03 (Hammer *et al*., 2001).

3. RESULTS

In this study, 19 individuals of *D. setosum* were employed to investigate the impact of fishing barns on marine biota by examining the microplastic abundance in the gastrointestinal tract and gonad of sea urchin. Mean diameter, mean height and mean weight of sampled individuals were 118.3±12.2 mm, 88.2±4.4 mm,

and 50.3±19.1 g, respectively (Table A1).
Statistical analysis showed that there is no correlation between morphological parameters and microplastic abundance (i.e. MP amount in the GIT & total weight, MP amount in the gonad & total weight, MP amount in the GIT & test diameter, MP amount in the gonad & test diameter, MP amount in the GIT & test height, MP amount in the gonad & test height, MP amount in the GIT & wet weight of GIT, MP amount in the gonad and wet weight of gonad)(Table A9).
In total 62 MPs were extracted from the specimens. Among all, 57 of them were extracted from GIT and 5 of them were extracted from gonad. Mean microplastic abundance was estimated as 3.0 MPs±3.1 MPs per individual (ind) and 0.9±1.0 MPs per g wet weight in GIT. When it comes to gonads, mean microplastic abundance was found as 0.3±0.6 MPs per individual and 0.08±0.2 MPs per g wet weight. Among 19 samples, the GIT of 15 specimens and the gonad of 5 specimens were contaminated with MPs.
The results showed that both microplastic presence rate and the extracted microplastic amount are higher in GIT than in gonad tissue. Kruskal Wallis test confirmed a significant variance in sample medians of MPs extracted from GIT and gonad (H (chi^2):13,11; Hc (tie corrected):15,1; p:0.0001021)
Fiber-shaped MPs were found to be dominant in both organs. Among all MPs, 45% of extracted MPs were fiber and that were followed by fragments (44%) and pellets (11%). Extracted MPs were divided into 5 categories in terms of color black, blue, green, red color, and colorless (white and transparent MPs). Blue-colored MPs were dominant (Figure 2). Regarding size, the majority of the MPs extracted from GIT and all of the MPs extracted from gonad were small-size MPs (less than 1 mm in size) (Figure 2).

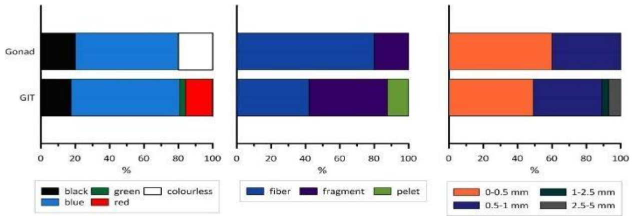

Figure 2. Characteristics of extracted microplastic particles from gastrointestinal tract (GIT) and gonad of *D. setosum*

Six of the MPs extracted from the GIT were validated by FTIR analysis. Analysis showed that all of the suspected particles were plastic in nature and polymer types were determined as 3 polyethylene MPs (50%) and 3 polypropylene MPs (50%) (Appendix Figure A1-A6).

4. DISCUSSIONS

İskenderun Bay is located inside one of the biggest plastic hotspots of the Mediterranean Sea (Papadimitriu and Allinson, 2022) and it is severely contaminated with microplastics (Gündoğdu and Çevik, 2017). Besides, contamination levels become more alarming in the coastal regions due to anthropogenic activities and intense fishing activities. Even though many studies investigate the microplastic pollution levels in the surface water and sediment of the northeastern Mediterranean Sea, there is no information regarding the microplastic pollution levels in fishing barns. Yet, fishing activities have particular importance in the formation and distribution of microplastics. In fact, they are defined as a major source of secondary microplastics (An *et al*., 2020; Xue *et al*., 2020). In this study, microplastic presence in the sea urchin *Diadema setosum* sampled from the fishing barn was investigated to highlight the danger potential of MPs pollution levels in the fishing barns on the marine biota. Besides, this study is the first report regarding microplastic accumulation in *D. setosum* from Türkiye.
In this study, among 19 examined specimens, 15 of them contained microplastic in their GIT (79%) and 5 of them contained MPs in their gonad (22%) (Table A1). The high frequency of occurrence in the GIT points out that these species are highly confronted with microplastics. High frequency of microplastic occurrence in

GIT found in this study is in line with previous reports (Table A2). Feng *et al*. (2020) investigated the microplastic presence (n=210) in the 4 different sea urchin species from the Yellow Sea and reported that 89.5% of examined specimens contained microplastic in their GIT. Other studies reported a 100% microplastic occurrence rate in the *Paracentrotus lividus* sampled from the Eastern Aegean Sea, Greece. (Hennicke *et al*., 2021), *D. setosum* sampled from Barranglompo Island, Indonesia (Savannah *et al*., 2021), *Tetrapygus niger* sampled from Peru (De-la-Torre *et al*., 2020).

Microplastic particles deposited on the sediment cover the top layer of sea gross or rocky bottom and they are eventually consumed by grazer organisms. There seems to be quite a difference in the average amount of microplastics extracted from GIT in the literature. The highest MPs abundance in the GIT of *D. setosum* was reported from Untung Jawa, Jakarta as 2175.55±584.26 MPs per ind (Huseini *et al*., 2021). Followed by a study conducted in Indonesia, which reported the mean MPs abundance as 23.70±2.99 MPs per ind (Sawalan *et al*., 2021). Results obtained in this study (3.0±3.1 MPs per ind) were significantly lower than previous reports which employed *D. setosum* as biomonitoring organisms; however, similar to those employed *P. lividus* (Raguso *et a*l., 2022; Murano *et al*., 2022), *Strongylocentrotus intermedius*, *Temnopleurus hardwickii*, *Temnopleurus reevesii*, *Hemicentrotus pulcherrimus* (Feng *et al*., 2020), *Diadema africanum* (Sevillano-González *et al*., 2022), *Tetrapygus niger* (De-la-Torre *et al*., 2020) from different parts of the ocean (Table A2). Previous reports showed that microplastic concentration in the sediment severely impacts the microplastic abundance in the GIT (Hennicke *et al*., 2021; Huseini *et al*., 2021; Rahmawati *et al*., 2023). Therefore, variations in the reported mean abundance seem to primarily result from microplastic concentration in the ambient environment rather than species type.

Only five specimens contained MPs in gonads with a mean value of 0.9±1.0 MPs per ind, which is coherent with the previous reports (Feng *et al*., 2020; Murano *et al*., 2020). Statistical analysis showed that MPs abundance in the organs was significantly different ($p<0.05$). Previous research speculated that MPs accumulated in the gonads were not transfer from the intestine (Leddy and Johnson, 2000; Feng *et al*., 2020). Gonads receive MPs found in the coelomic fluid while they fill the whole-body cavity which accumulates MPs originate from peristomal gill and tube feed (Leddy and Johnson, 2000; Feng *et al*., 2020). Our results support this idea because of their significantly smaller size and lower amount compared to GIT.

Ingested MPs size is restricted to the animal size (Jâms *et al*., 2020). Coherent to this, almost all ingested MPs extracted from the GIT were smaller than 1 mm. In addition, the size of MPs extracted from gonads were varied between 218 µm to 658 µm. Pyl *et al*. (2022) reported that MPs which are greater than 10 µm could not originate from intestinal wall of *P. lividus*. Therefore, extracted MPs could originate from ceramic fluid (Leddy and Johnson, 2000; Feng *et al*., 2020) or an alternative route that needs to be tested in the future.

Microplastic type (fiber, fragment or pellet) is an important parameter in determining exposure risk and bioavailability of MPs. Fiber-shaped MPs were considered to be the most harmful type for marine invertebrates (Wright *et al*., 2013). Fiber microplastics are commonly attributed to the fragmentation of fishing nets (Koongolla *et al*., 2020) and it is the most commonly ingested type of MPs in the marine biota of the Mediterranean Sea (Koraltan *et al*., 2022; Kılıç and Yücel, 2022; Yücel and Kılıç, 2023a, b). Similarly, both this study and previous studies conducted in sea urchins reported the dominance of fiber microplastic (Hennicke *et al*., 2021; Sawalman *et al*., 2021; Sevillano-González *et al*., 2022; De-la-Torre *et al*., 2020; Raguso *et al*., 2022; Murano *et al*., 2022; Huseini *et al*., 2021; Feng *et al*., 2020). Therefore, sea urchins seem to have a higher ingestion preference for fiber microplastics compared to other types. Apart from this, sea urchins generate secondary microplastics while grazing on plastic surfaces (Porter *et al*., 2019). So, it is possible that urchins fed on newly generated fiber or fragment-type microplastics while grazing on the contaminated seaweed of fishing nets on the sea bottom. This condition is significantly important for fishing barns. Because, in a high microplastic-containing region like fishing barns, they accelerate the biodegradation of macroplastics (Boudouresque and Verlaque, 2007; De-la-Torre *et al*., 2020). While rasping on the surface of the plastic materials by calcionic teeth, they leave scratchers and generate secondary microplastics (Boudouresque and Verlaque, 2007; De-la-Torre *et al*., 2020). That in turn increases the ecotoxicological risk of microplastics for themselves and other marine animals.

Blue was the most dominant color in the extracted MPs, which overlaps with the previous reports in sea urchins (Hennicke *et al*., 2021; Sawalman *et al*., 2021; Sevillano-González *et al*., 2022; De-la-Torre *et al*., 2020; Murano *et al*., 2022; Huseini *et al*., 2021; Feng *et al*., 2020), fish (Güven *et al*., 2017; Giani *et al*., 2019). Previous reports demonstrate that *P. lividus* might select blue-colored particles due to their similarity to the main food source. Besides, the study area is filled with the remaining blue-colored fishing nets, which seems to increase the ingestion rate of MPs.

Microplastic abundance in the fishing area is linked to the fishing effort (Wright *et al*., 2021), and higher MPs abundance is observed in the regions where fisheries activities are dominant (Xue *et al*., 2017). Today, the majority of fishing gears are produced from synthetic polymers such as polyethylene and polypropylene (Nelms *et al*., 2021). In this study, half of the examined MPs were PE, and the remaining were PP. Since the polymer is an important factor that is used to link between contamination source and ecotoxicity of animals, the results presumably imply that the majority of the extracted MPs were derive from fishing activities.

It is a well-known fact that microplastics entering the food chain might cause adverse effects on the upper trophic levels including humans (Hennicke *et al*., 2021). Since sea urchins are a traditional food source for highly populated countries such as Japan, China, and Mediterranean European countries (Stefánsson *et al*., 2017). Therefore, microplastics found in sea urchins might create a pathway for microplastics to the human body. Species that are eaten with gastrointestinal tracts and gills such as small-size fish and bivalves may pose a greater risk to human health (Smith *et al*., 2018; Baechle *et al*., 2020; Hennicke *et al*., 2021). Previous studies in the İskenderun Bay reported 2.9±2.7 MPs per ind in fish *Mullus barbatus*, 3.4±2.7 MPs per ind in fish *Saurida undosquamis* (Kılıç and Yücel, 2022), 0.26±0.5 MPs per ind in patella *Patella caerulea* (Yücel and Kılıç, 2023a), 0.2±0.5 MPs/ind in mussel *Brachidontes pharaonis* (Yücel and Kılıç, 2023b). Compared to other species like fish, the edible parts of this species contained fewer MPs. Thus, it is reasonable to assume that consumption of the gonads of *D. setosum* is safe in terms of microplastic accumulation. In other words, this species poses a considerably low to moderate threat to human health. More detailed studies are required to evaluate the potential hazards of consumption of microplastic-containing aquatic products.

5. CONCLUSIONS

This study is conducted to investigate the microplastic contamination levels in sea urchin *D. setosum* since sea urchin species are a good reflector of microplastic pollution in the ambient environment. Results validate the omnipresence of microplastics since the majority of the specimens contained microplastics in the bodies. Polypropylene and polyethylene were the main types of polymers extracted from the GIT which might reveal the impact of the fishing industry since these are the main types of polymers used in fishing nets. As far as we know, this is the first focusing on the marine animals habituated in a fishing barn in Turkiye; therefore, the results demonstrate the importance of the fisheries industry in the formation and distribution of microplastics. There is still little known in the transfer of microplastics throughout food web, yet studies like this will provide insight to the microplastic contamination in different trophic levels and different environments.

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AUTHORSHIP CONTRIBUTION STATEMENT

Ece KILIÇ: Conceptualization, Methodology, Validation, Formal Analysis, Resources, Writing - Original Draft.
Erkan UĞURLU: Validation, Formal Analysis, Resources, Writing-Review and Editing, Data Curation, Software, Visualization, Writing-Review and Editing.

CONFLICT OF INTERESTS

The author(s) declare that for this article they have no actual, potential or perceived conflict of interests.

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Appendix I

Table A1. Morphological statistics i.e. test diameter, test height, GIT weight, gonad weight (mean ± standard deviation) and microplastic abundance and frequency of occurrence (%) in the examined organs

Test diameter (mm)	Test height (mm)	GIT weight (g ww)	Gonad weight (g ww)	# of MPs	# of fiber MPs	# of fragment MPs	# of pelet MPs	MPs per ind	MPs per g ww	FO (%)
118.3±12.2	88.2±4.4	0.37+0.16	5.25+3.5	GIT						
				57	24	26	7	3±3.1	0.8±1.0	79
				Gonad						
				5	4	1	0	0.3±0.6	0.08±0.2	22

Appendix II

Table A2. Recent literature indicating mean microplastic abundance in organs as well as predominant type of microplastic in similar species

Species	Location	GIT MPs per ind	GIT MPs per g ww	Gonad MPs per ind	Gonad MPs per g ww	Common type	Common color	References
Diadema setosum	İskenderun Bay, Türkiye	3.0 MPs± 3.1	0.9±1.0	0.3±0.6	0.08±0.2	Fiber	Blue	This study
Paracentrotus lividus	Greece	26	1.9			Fiber	Blue	Hennicke et al., 2021
Diadema setosum	Barranglompo Island, Indonosia	23.70±2.99	-			Fiber	Blue	Sawalman et al., 2021
Diadema africanum	Canary Island, Spain	9.2 ± 3.0				Fiber	Blue	Sevillano-González et al., 2022
Diadema africanum	El Porís	10.0 ± 4.5				Fiber	Blue	Sevillano-González et al., 2022
Tetrapygus niger	Peru	3.22 ± 0.49				Fiber	Blue	De-la-Torre et al., 2020
Paracentrotus lividus	Sardinia, Italy	1.0 ± 0.3				Fiber	Grey	Raguso et al., 2022
Diadema setosum	Untung Jawa, Jakarta	2,175.55 ± 584.26				Fiber	-	Huseini et al., 2021
	Tidung, Jakarta	1,786.66 ± 451.17				Fiber	-	
Paracentrotus lividus	Southern Italy,	1.2		0.83		Fiber	Black and blue	Murano et al., 2022
4 different species	Yellow Sea, China	3.04		0.82		Fiber	Blue, gray	Feng et al., 2020

Appendix III

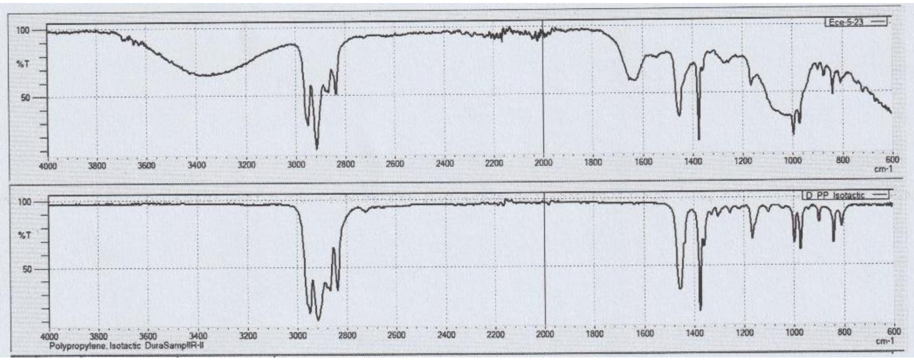

Figure A1. FTIR spectra of microplastic particle 1 (*Polypropylene)*

Appendix IV

Microplastic Particle 2:

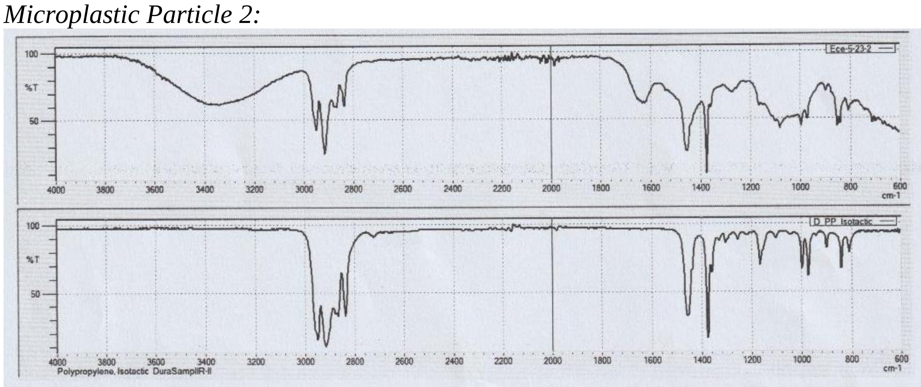

Figure A2. FTIR spectra of microplastic particle 2 (*Polypropylene)*

Appendix V

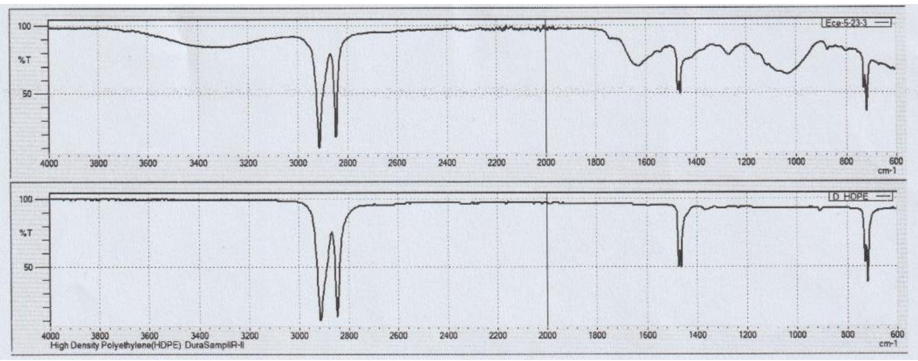

Figure A3. FTIR spectra of microplastic particle 3 (*Polyethylene)*

Appendix VI

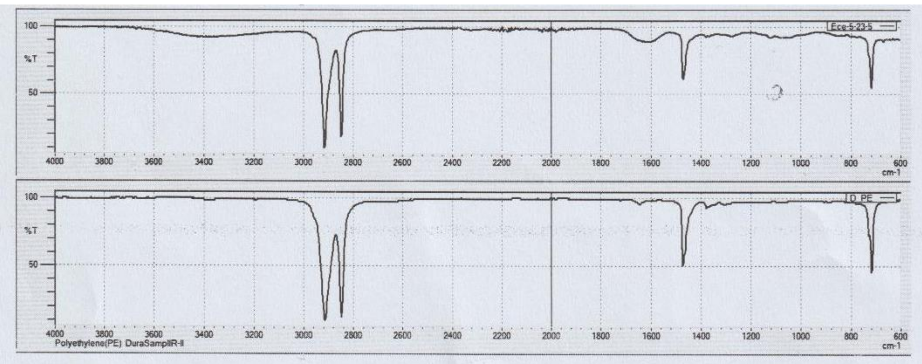

Figure A4. FTIR spectra of microplastic particle 4 (*Polyethylene)*

Appendix VII

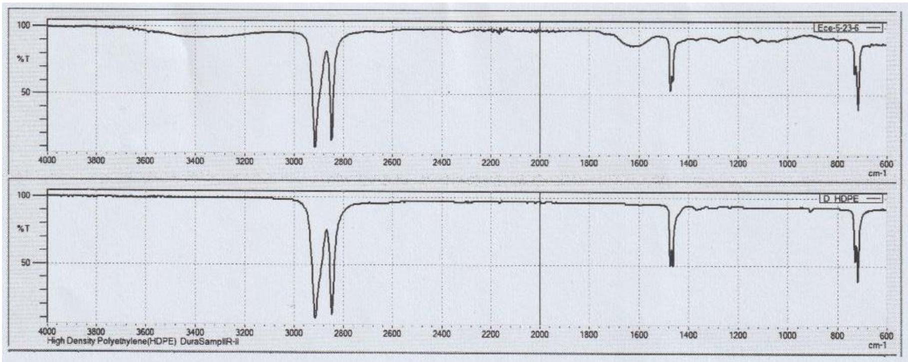

Figure A5. FTIR spectra of microplastic particle 5 (*Polyethylene)*

Appendix VIII

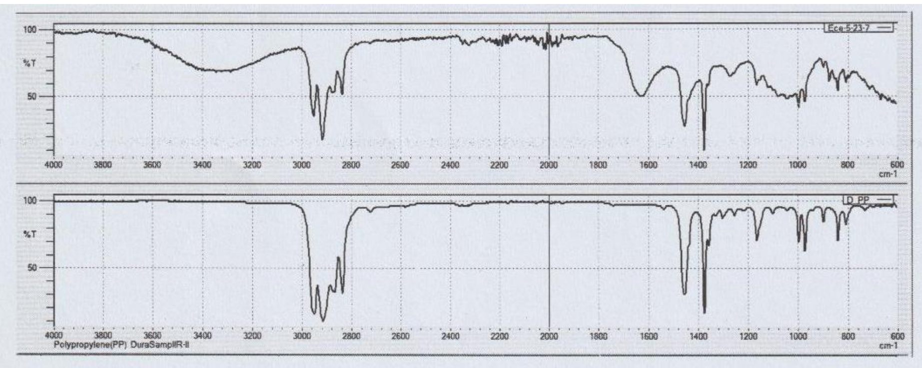

Figure A6. FTIR spectra of microplastic particle 6 (*Polypropylene)*

Appendix IX

Table A9. Results of correlation analysis (Below column shows statistical value, while upper column show p value)

	Test diameter	Test height	Weight	GIT weight	Gonad weight	MP in GIT	MP in Gonad
Test diameter		1,30E-07	6,63E-05	0,054845	0,00018	0,14943	0,9042
Test height	0,90215		0,000104	0,13054	0,000317	0,61684	0,66162
weight	0,78596	0,77315		0,1659	0,000744	0,1793	0,75556
GIT weight	0,44728	0,35956	0,33128		0,097013	0,57951	0,36812
Gonad weight	0,75614	0,73717	0,70526	0,39192		0,15055	0,96001
MP in GIT	-0,34388	-0,12268	-0,32166	-0,13574	-0,34299		0,72934
MP in Gonad	-0,02962	0,10741	0,076512	-0,21881	0,012341	-0,085	