

The Effect of Acrylonitrile Butadiene Styrene (ABS) and Polypropylene (PP) Microplastics on *Ulva lactuca* L. and *Ceramium diaphanum* R. Algal Growth

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Abstract

The goal of this study was to determine the effects of acrylonitrile butadiene styrene and polypropylene microplastics as two typical classes for microplastic pollution on algal growth, lipids, carbohydrates, chlorophyll-a and total carotenoid content of *Ulva lactuca* L. and *Ceramium diaphanum* R. collected from the Gulf coast of Izmir province. The results demonstrate that ABS-MPs had no statistical effect ($P>0.05$) on the various physiological parameters of *U. lactuca* macroalgae after 5 days of exposure, except for the total carbohydrate and lipid concentration. The chl-*a* value of *C. diaphanum* was considerably increased under the low concentration of ABS (25 mg L^{-1}), increasing by 81.56% compared with the control while the amount of chl-*a* decreases (32.05%) in parallel with the increasing concentration of PP-MPs. Laboratory incubation experiments showed that MPs affect relative growth rate, pigments efficiency or carbohydrate content of *C. diaphanum* until reaching an extremely high concentration (100 mg L^{-1}), indicating a high tolerance to MPs. The results showed that two macroalgae species, especially *U. lactuca*, were not highly affected at low MPs concentrations under laboratory conditions which were much higher than the levels of environmentally relevant concentrations

Introduction

Nowadays, it is possible to see plastic waste in all marine habitats, and this has caused plastic pollution to become a global problem as it poses a threat to marine life, human and environmental health (Eriksen et al. 2013; Dahl et al. 2021; Sridharan et al. 2021). While plastics are constantly breaking down into smaller pieces in the seas, the threats they pose are also increasing. Due to the difficulties of collecting plastic waste from the seas and the permanent structure of plastic, it is necessary to remove the plastic after it enters the sea. Moreover, these wastes continue to be broken down into smaller pieces due to environmental conditions in the seas. Macroplastics become microplastics, microplastics become nanoplastics, and

their collection becomes impossible (MacLeod et al. 2021). Research studies have created great waves especially in the field of microplastics (MPs), any plastic item with dimensions smaller than 5 mm (Sedlak 2017; Frias & Nash 2019; Hartmann et al. 2019; Kutralam-Muniasamy et al. 2023).

MPs are persistent pollutants that are mostly produced by human activities and accumulate in aquatic ecosystems. MPs can diffuse into the oceans more quickly than large plastics due to their smaller particle size, resulting in a broader impact on the ecosystem, biotic and abiotic (Hermabessiere et al. 2017; Haward 2018). MPs are not only a problem at the macro level, but also break down for different reasons and cause invisible dangers (Kanlı & Kurt 2019; Brander et al. 2020; Kayan et al. 2020). Research shows that MPs pollution,

which increases in parallel with the improvement of civilizations, is a considerably danger, especially for marine ecosystems because of long periods of time, their ability to travel long and intercontinental distances, and their accumulation in many marine habitats (Van Sebille et al. 1995; C'ozar et al. 2014; Law 2017; Van Sebille et al. 2020; Huang et al. 2021; Kutralam-Muniasamy et al. 2023). 1.21×10^5 particles per cubic meter, significant a threshold at which ecological risks occur recommended level. This threshold has been exceeded in areas considered pollution hotspots, including sea ice in the Mediterranean, East China Sea, Yellow Sea and Arctic Ocean. It is expected that the ecological risks caused by microplastic pollution on the sea surface may increase 50 times by the end of the 21st century (Peeken et al. 2018; Everaert et al. 2020). Lenz et al. (2016) state that realistic micro-nano plastic concentrations fall in the range of $0.001-1 \mu\text{g L}^{-1}$. Scientists have used much higher concentrations of microplastics in laboratory ecotoxicology studies than those found especially in the aquatic environment. In studies conducted with different algae groups, it was determined that the growth and photosynthesis of organisms exposed to low microplastic (5 mg L^{-1}) increased, while there was a decrease in high microplastic concentration (50 mg L^{-1}) (Ansari et al. 2021; Nassar et al. 2022; Wang et al. 2022; Zhang et al. 2022). The reason why microplastic removal studies conducted by scientists have been carried out at much higher concentrations than those typically found in natural environments is to determine the response of organismal biochemistry to high exposure to increasing microplastic pollution.

Aquatic plants and macroalgae play an important ecological role such as sediment stabilization in coastal areas, providing habitat for benthos (epifauna and infauna) and animal or human feed. Plastic particles may physically affect organisms via ingestion. In other words, it affects organisms by acting as a carrier for pollutants and a substrate for organisms. As a result, it makes inevitable the transfer of microplastics in or on macroalgae to animals and humans through the food web (Ziani et al. 2023). It reveals that macroalgae, one of the primary producers that play an important role in protecting ecosystems, can adsorb or internalize plastic particles as a result of their interaction with MPs (Reed et al. 2016; Gao et al. 2018; Senturk 2024).

At present, studies have shown that microplastics can affect the growth of microalgae (such as *Chlorella*, *Scenedesmus obliquus* and *Phaeodactylum tricorutum*) (Song et al. 2020; Luo et al. 2020), shellfish or fish (Anastasopoulou et al. 2013; Choy & Drazen 2013; Neves et al. 2015; Li et al. 2016; Nadal et al. 2016; Ory et al. 2017; Ding et al. 2018). Very few studies focus on the accumulation of microplastics in macroalgae in the field (Gao et al. 2020; Li et al. 2020). Therefore, two macroalgae species, *Ulva lactuca* (non-filamentous) and *Ceramium diaphanum* (filamentous), were selected in the study.

Ceramium and *Ulva* is a very common macroalgae worldwide. They are distributed in oceans and seas in the littoral zone. Although not toxic to humans, macroalgal blooms can harm coastal activities due to their sheer physical mass (Arroyo & Bonsdorff 2016). *U. lactuca* macroalgae selected in this study are usually found on rocky shores and can be attached to stones, rocks, etc. It has slightly yellowish-greenish irregular, round-curved edge-shaped, translucent soft leaves, and the leaves are attached to rocks with the holdfast (Yu-Qing et al. 2016). Significant seasonal changes can be seen in its morphology. Young plants are dark green in color and soft to the touch, whereas older thalli become light green and their surfaces become slippery (Baweja et al. 2016). *Ulva* spp. contain a significant amount of 54.0% fiber, 19.6% mineral, 8.5% protein, 20.6% hemicellulose, 9% cellulose, 1.7% lignin, 7.9% lipid and (Yaich et al. 2011; Dominguez & Loret 2019). *Ceramium* genus is erect filamentous, 5-15 cm high, flat and rounded, branching is dichotomous or subpinnate, the tops of the branches are generally curved in the form of pincers. The axis is cylindrical, freely branched. Chloroplasts are numerous and spherical, needle-like, elongate in shape. Cells are mononuclear. *Ceramium* species form brown-red grassland on rocky shores or as epiphytes (Sauvageau 1971; Van Den Hoek et al. 1995).

The current investigation examined the interactions of Acrylonitrile Butadiene Styrene (ABS) and Polypropylene (PP) microplastics (varied concentrations and sizes) with *Ulva lactuca* and *Ceramium diaphanum*, a non-filamentous (parenchymatous) and filamentous macroalgae, respectively. The purpose of this study is to evaluate the effects of ABS-MPs and PP-MPs on algal growth, amount of lipids and carbohydrates, chl-*a* and carotenoid content of *U. lactuca* and *C. diaphanum*. The findings contribute to improving the current understanding of the biochemical mechanism risks of two microplastics in laboratory conditions on *Ulva* and *Ceramium* macroalgae. This study will provide information about the biochemical changes of two identified macroalgae species as a result of exposure to microplastic levels, which are much higher than environmentally relevant microplastic concentration levels under laboratory conditions.

Materials and Methods

Study Area and Organisms

The macroalgae species and seawater used in this study were collected from the coastal zone of İzmir Bay, İnciraltı ($38^{\circ}24'39''\text{N}$, $27^{\circ}02'10''\text{E}$) region between Feb 10 and Feb 11, 2024 (Figure1). After sampling, the *U. lactuca* and *C. diaphanum* samples were quickly transferred in a cooler ($+4^{\circ}\text{C}$) to the laboratory. The samples were then transferred to the laboratory as soon as possible, and *U. lactuca* and *C. diaphanum* were identified and separated. Seawater was collected from coastal zone. To remove microplastics, a $0.22\text{-}\mu\text{m}$ filter

was used. Macroalgae growth in a recirculating seawater system with aeration at 28°C, an irradiance of 125 $\mu\text{mol photons}/(\text{m}^2\cdot\text{s})$ and a 12-h light/12-h dark regime for 3 weeks before being used for the experiment (Pérez-Mayorga et al. 2011). The algal volume was 5g fresh weight (fw) per 200 mL seawater for each test tube (Figure 1).

Chemicals

Clean ABS (100-3500 μm) and PP plastics of (10-1500 μm) different sizes were purchased from RC mold design (Manisa, Turkey). The two microplastics were washed with 70% ethanol twice and double-distilled water. Thus, dust and other contaminants adhering to the MP surface were removed before the experiments. Finally, MPs were dried oven at 40°C overnight (Chen et al. 2020). Two microplastics were obtained using inverted microscope (Olympus IX71-DIC attachment) equipped with a digital camera (Olympus DP25) with an objective magnification of 40 \times to check size and shape (Figure 2).

Macroalgae Exposure to ABS and PP Microplastics

U. lactuca and *C. diaphanum* taken from areas of mass growth of algae macroalgae were transferred individually in sterilized glass vessels filled with one of the two test media, ABS and PP-MPs. These media were prepared by adding a volume of ABS and PP stock solution to obtain a final concentration of 25, 50, 75 and 100 mg L^{-1} in the filtered seawater. In the published study by Feng (2020) and Rozman (2022), the upper limit of the toxicity test was determined as 100 mg/L for MPs.

Macroalgae kept in the filtered seawater growth medium without ABS or PP stock solution were used as negative controls. All vessels were agitated on an orbital shaker at 100 rpm for 15 min before adding the plants. All tools and devices were thoroughly cleaned with 75% alcohol. All chemical reagents and deionized water were filtered with 2.7 μm glass microfiber (Whatman Grade GF/D) before use. Samples with a fresh weight of approximately 5 g fw were placed in a clean 200 mL beaker and exposed to 200 mL of solutions containing microplastics at different concentrations for 5 days.

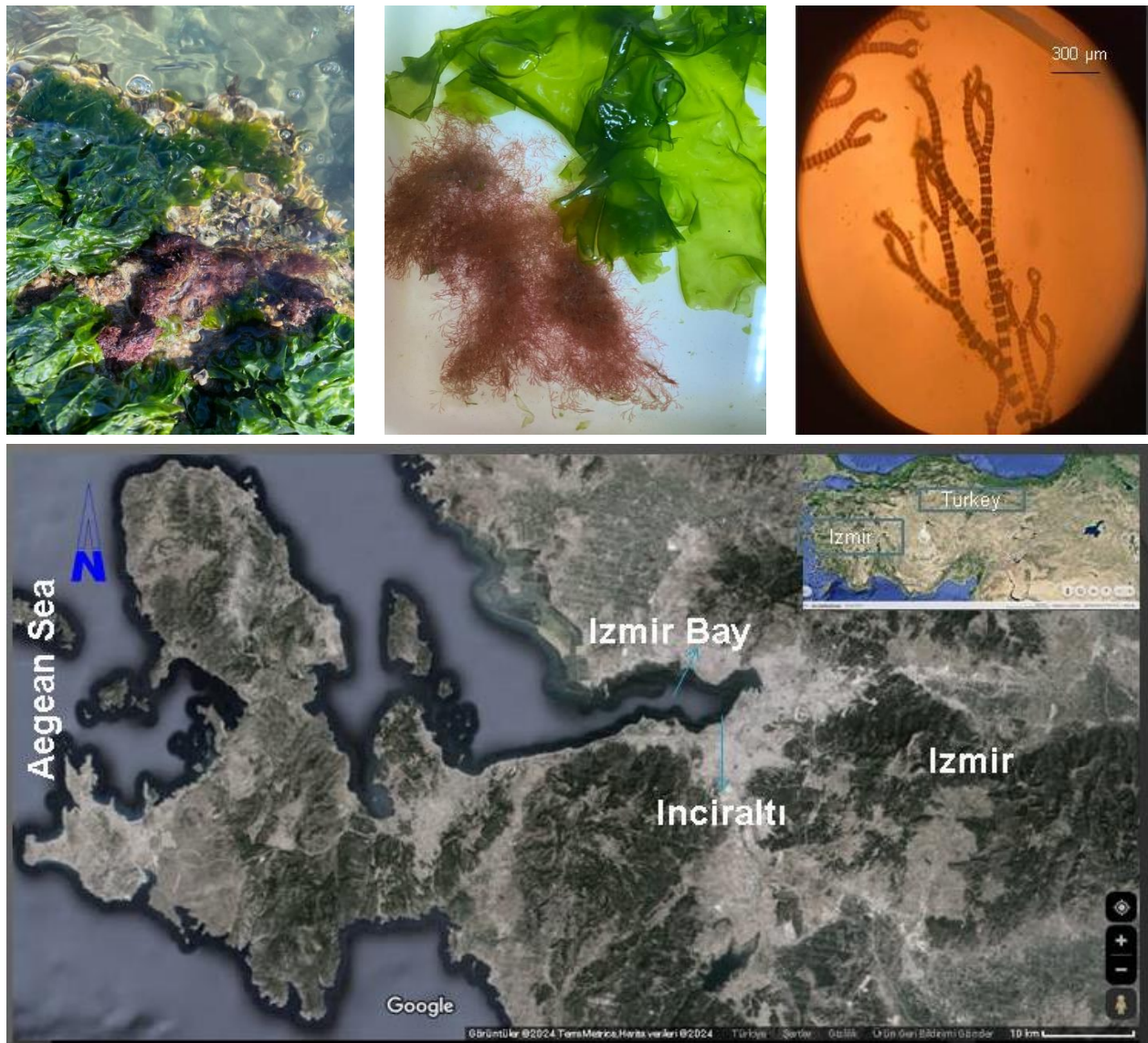


Figure 1. Study area, sampling location and organisms.

Morphological characteristics of MPs (shape and size) were obtained using inverted and binocular microscopy.

Analysis

Photosynthetic Pigment Analysis (mg g^{-1})

For pigment analysis, 0.1 g fw of macroalgae samples were homogenized in 10 mL of 96% (v/v) acetone solution into the capped glass tube equal to the number of experimental samples. The filter paper obtained from the total biomass analysis was folded untouched using forceps. It was cut into small pieces with scissors and placed in tubes. Pigment extraction was completed by leaving it in a dark environment of $25 \pm 1^\circ\text{C}$ for 24 hours. At the end of the period, the filter papers kept in acetone were filtered and measured in the spectrophotometer (Varian Cary 50 UV-Vis SPM, Agilent Technologies, USA) at wavelengths of 665, 652

and 470 nm (Parsons & Strickland 1963). Chl-*a* and carotenoid values were calculated with the absorbance values obtained. Chl-*a* and carotenoid values were calculated as follows:

$$\text{Chl-}a \text{ (mg g}^{-1}\text{)} = (16.74 \times A_{665}) - (9.16 \times A_{652})$$

$$\text{Total carotenoid (mg g}^{-1}\text{)} = (1000 \times A_{470} - 1.63 \times \text{Chl-}a) / 221$$

Lipid (Malondialdehyde-MDA) Analysis (nmol g^{-1})

MDA content that is the indicator of lipid peroxidation was determined using the thiobarbituric acid method (Draper et al. 1993). 0.5 g (fw) of macroalgae samples was taken and homogenized in 20% trichloro acetic acid (TCA) and 5% thiobarbituric acid (TBA) (3 mL in total). The homogenate was incubated at 95°C for 30 minutes and then placed in ice to stop the reaction. The samples were centrifuged at 10000 rpm

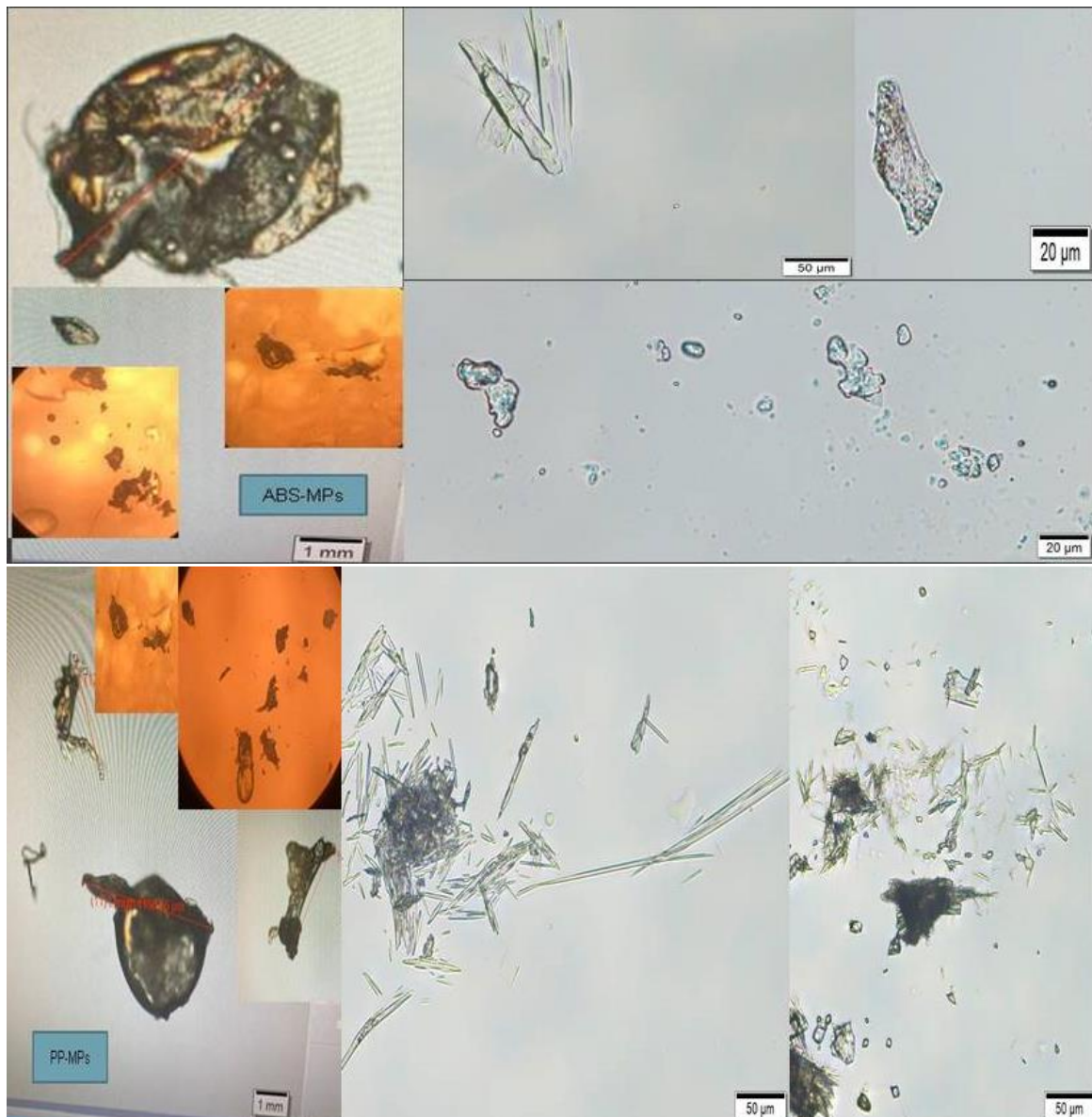


Figure 2. Image of pure ABS and PP microplastics under an inverted microscope (Olympus DP25 camera attached to Olympus IX71 DIC microscope with an objective magnification of 40 \times).

for 10 minutes and after the supernatant was removed. Then, absorbance values were determined at 532 and 600 nm wavelength in the spectrophotometer (Heath & Packer 1968). A unit of MDA is determined as 1 nmol product per g (fw) protein. MDA was calculated as follows (Rao & Stresty 2000):

$$MDA (nmol g^{-1}) = \left(\frac{A_{532} - A_{600}}{155} \right) \times 10^6$$

Analysis of the Total Carbohydrate Contents (mg mL⁻¹)

Total carbohydrate contents were measured using the simple and rapid colorimetric method of phenol-sulfuric acid assay and the d-glucose concentration scale was used to construct the standard curve by DuBois et al. (1956). Briefly, 5% (w/v) phenol solution was prepared in distilled water. 0.1 g (fw) of test samples and 5 mL of concentrated sulfuric acid were added to 1 mL of phenol solution. The mixture was stirred for 30 min. 1 mL aliquots of the cultures were used to quantify spectrophotometrically at 490 nm against a blank of 0.2 mL 5% w/v phenol, 1 mL concentrated sulfuric acid, and 0.2 mL of de-ionized water.

Determination of Fresh Weight

Absorbent paper was used to remove the seawater on the surface of macroalgae. Fw was measured using a precision electronic balance (Mettler Toledo AL 204, Switzerland, Zurich).

Growth Rate (RGR)

The growth of macroalgae was estimated by fw changes. The RGR was calculated as follows:

$$RGR (d\%) = \left(\frac{\ln M_t - \ln M_0}{t} \right) \times 100$$

M₀ is the initial fresh algal biomass. M_t is the fw after t days of cultivation. t is the exposure time (day) (Glenn & Doty 1992).

Statistical Analyzes

To investigate the presence or absence of significant differences between biochemical factors and the abundance of microplastics in macroalgae samples, one-way ANOVA statistical method was used in Minitab-19 package program and Microsoft Excel 2010 was used to draw the graphs. The difference between the data with a significance level of P<0.05 was determined using the least significant difference. All the measurements were carried out in triplicate (n=3) to demonstrate the statistical difference. Entire data is presented as mean ± standard deviation to express the results.

Results

Effect of Microplastics on the Growth Rate Of Macroalgae

Laboratory experiments were conducted to investigate the impacts of MPs on physiological performance and growth rate of *U. lactuca* and *C. diaphanum* and the tolerance of these plants to high concentrations of MPs. Statistical analysis showed that ABS-MPs did not affect relative growth rate of *U. lactuca* until it reached the extremely high concentration (75 and 100 mg L⁻¹, P<0.05) while no inhibitory effect of PP-MPs on the RGR (P>0.05, Figure 3). Compared with the control, when exposed to high concentration of ABS-MPs, the RGR of *U. lactuca* was inhibited by 28.11% approximately. On the other hand, the relative growth rates of *C. diaphanum* were significantly (P<0.05) inhibited by two types of microplastic particles. Compared with the control, when exposed to ABS and PP-MPs, the growth of *C. diaphanum* was inhibited by 6.69% and 6.95% at 100 mg L⁻¹ MPs exposure, respectively.

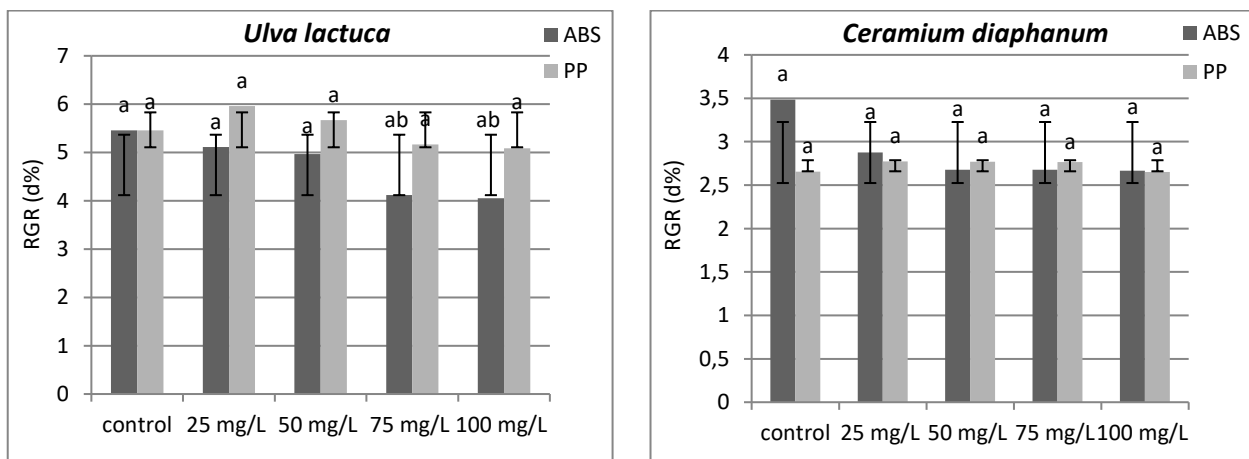


Figure 3. In the column chart, abundance (average ± SD) of RGR (d%) is shown (n=3). Different letters in each data set are significantly different (P<0.05).

Effect of Microplastics on the Photosynthetic Pigment of Macroalgae

As a result of exposure to different concentrations of ABS and PP microplastics, the amount of chl-*a* of *U. lactuca* and *C. diaphanum* is shown in Figure 4. Control chl-*a* values of *U. lactuca* and *C. diaphanum* were measured as 7.6725±0.015 mg g⁻¹ fw, and 0.2190±0.023 mg g⁻¹ fw, respectively. The total carotenoid amount of the control group of *Ulva* and *Ceramium* was measured as 0.0269±0.004 mg g⁻¹ fw and 4.0461±0.005 mg g⁻¹ fw, respectively.

Under the 25 mg L⁻¹ ABS and PP-MPs culture condition of, the chl-*a* content of *U. lactuca* decreased by 22.66% (P<0.05, Figure 4) and the carotenoid significantly increased by 87.20% compared with the control (P<0.05, Figure 5). However, under the PP-MPs culture condition, there is no significant difference in chl-*a* content of *U. lactuca* (P>0.05).

For *C. diaphanum*, ABS-MPs and PP-MPs have significantly effect on the chl-*a* value (P<0.05). The chl-*a*

content of *C. diaphanum* was significantly increased under the low concentration of ABS (25 mg L⁻¹), increasing by 81.56% compared with the control while the amount of chl-*a* decreases (32.05%) in parallel with the increasing concentration of PP-MPs. On the other hand, high concentrations of ABS microplastics had negative impacts on the chl-*a* of *Ceramium*. Under the condition of 100 mg L⁻¹ ABS-MPs, the chl-*a* content of *C. diaphanum* decreased by 70.27% compared with the control. Similarly, the carotenoid content of *C. diaphanum* was significantly decreased under the 100 mg L⁻¹ concentration of ABS and PP-MPs, increasing by 31.81% and 20.82% compared with the control, respectively (P<0.05).

Effect of Microplastics on the Lipid and Carbohydrate Contents of Macroalgae

The control total carbohydrate (CH) was 0.4257±0.01 g 100 g⁻¹ fw in the species of *Ulva lactuca*. The results indicated that the CH content of *U. lactuca*

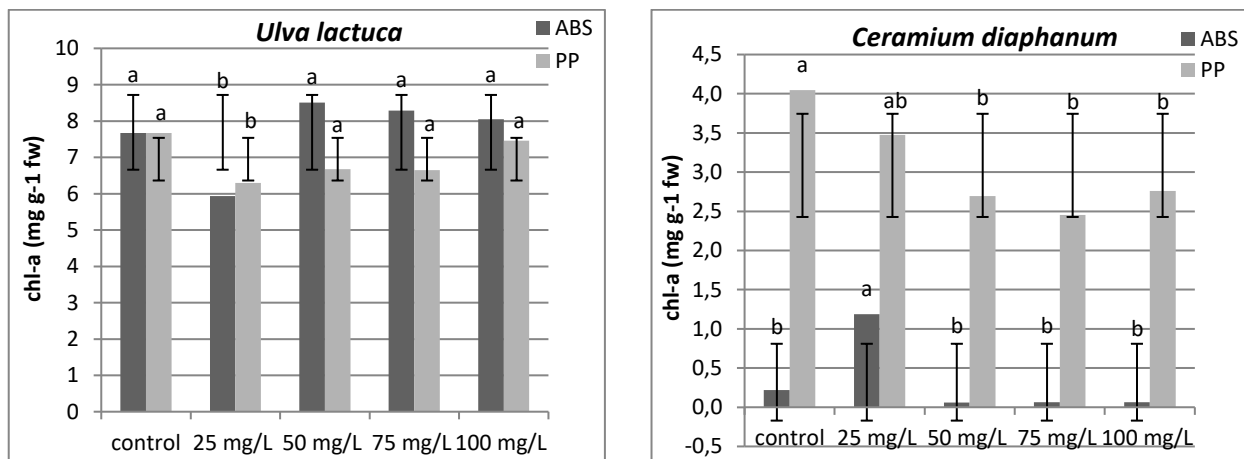


Figure 4. The chl-*a* contents of *U. lactuca* and *C. diaphanum* exposed to different MPs concentrations. Means and standard deviation are shown (n=3). Different letters denote a significant difference in values (P<0.05).

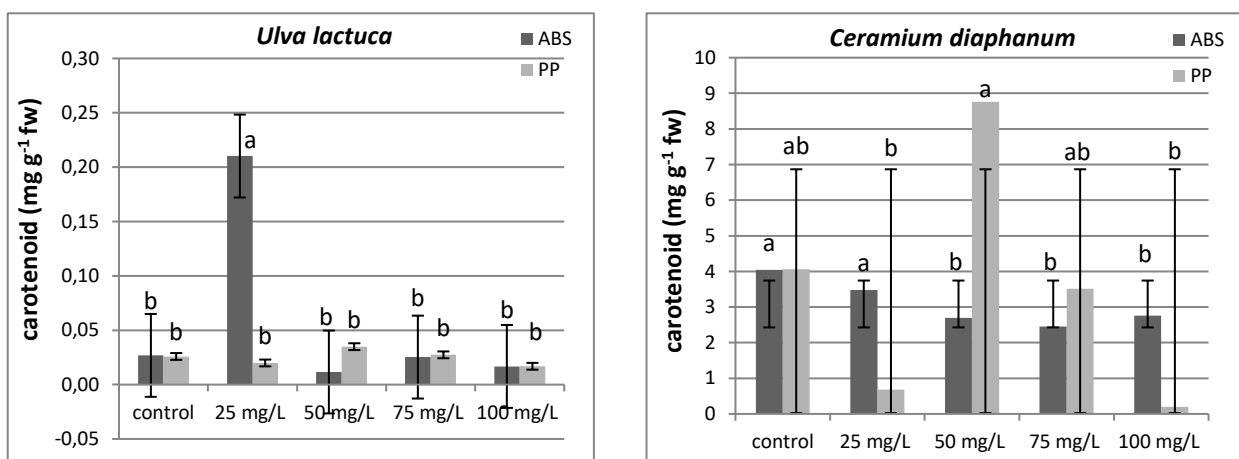


Figure 5. The total carotenoid contents of *U. lactuca* and *C. diaphanum* exposed to different MPs concentrations. Means and standard deviation are shown (n=3). Different letters denote a significant difference in values (P<0.05).

was reduced significantly after microplastic concentration increase ($P < 0.05$). The lowest total CH was 0.2713 ± 0.002 and 0.2606 ± 0.001 g 100 g⁻¹ fw under the high concentration of ABS-MPs and PP-MPs (100 mg L⁻¹), and the CH content significantly decreased ($P < 0.05$) by 36.26% and 37.12% compared with the control, respectively. The control total CH of *C. diaphanum* was 1.6069 ± 0.04 mg g⁻¹ fw. As similar to *U. lactuca* macroalgae, *C. diaphanum* CH content first increased and then decreased with the increasing microplastic concentration (at 100 mg L⁻¹, especially). The lowest total CH was 1.0074 ± 0.001 and 1.3301 ± 0.001 mg g⁻¹ fw under the high concentration of ABS-MPs and PP-MPs (100 mg L⁻¹), decreasing by 37.30% and 19.31% compared with the control, respectively (Figure 6). Microplastics have no significantly effect on the carbohydrates content of macroalgae ($P > 0.05$) on *C. diaphanum*.

The control lipid of species *U. lactuca* and *C. diaphanum* was measured as 2.5159 ± 0.05 and

0.4226 ± 0.004 g 100 g⁻¹ fw, respectively. The total lipids contents of two algal species analysed are presented in Figure 7. The results indicated that the total lipid content of *U. lactuca* were reduced significantly in parallel with the increase in microplastic concentration ($P < 0.05$). The *C. diaphanum* lipid content ranged from 0.3263 ± 0.02 (100 mg L⁻¹ ABS and PP MPs) to 0.7245 ± 0.03 g 100 g⁻¹ fw (ABS and PP-MPs at 25 mg L⁻¹ exposure) total lipids. ABS and PP-MPs have significantly effect on the *Ceramium* lipid content ($P < 0.05$).

Discussion

Effect of Microplastics on the RGR

Microplastics (MPs) pollution has gained significant attention due to its widespread presence and potential implications especially in aquaculture areas. The relative growth rate of the macroalgae are shown in Table 1. Compared with the control group, the fresh weight of

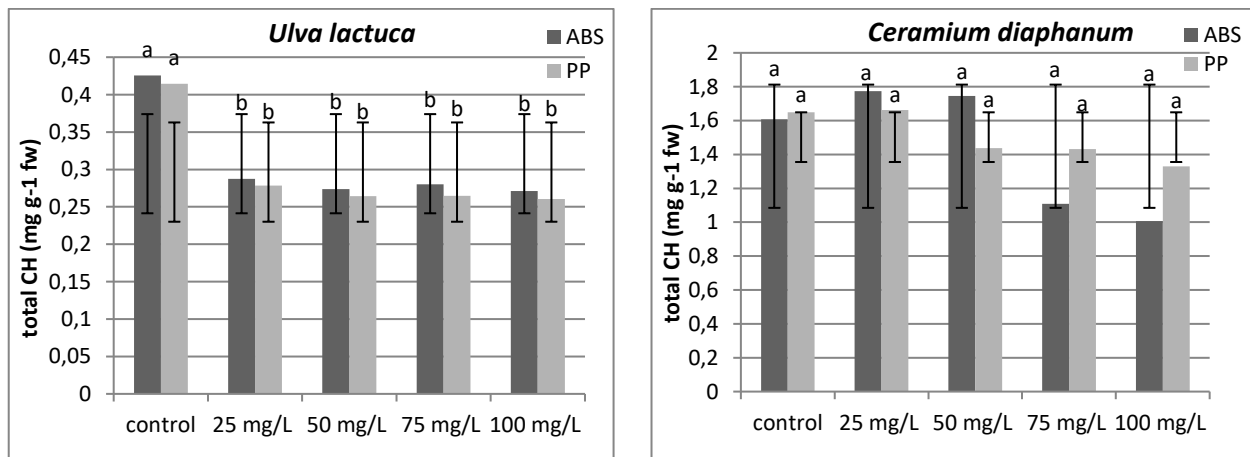


Figure 6. The total CH contents of *U. lactuca* and *C. diaphanum* exposed to different MPs concentrations. Means and standard deviation are shown (n=3). Different letters denote a significant difference in values ($P < 0.05$).

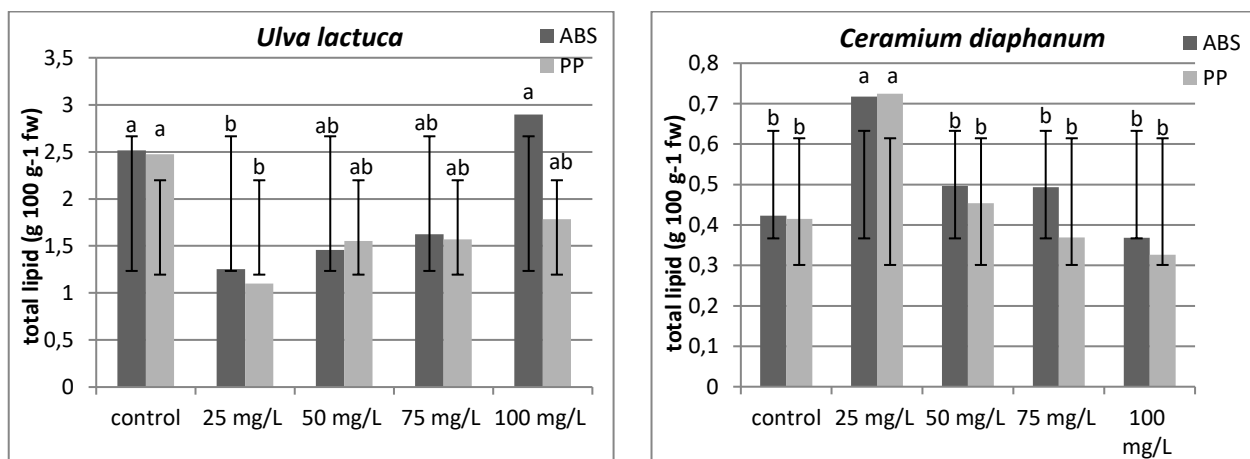


Figure 7. The lipid contents of *U. lactuca* and *C. diaphanum* exposed to different MPs concentrations. Means and standard deviation are shown (n=3). Different letters denote a significant difference in values ($P < 0.05$).

the *C. diaphanum* significantly decreased in the two MPs and all concentration MP exposure ($P < 0.05$).

The similar result was found in previous study that relative growth of macroalgae was inhibited by microplastic particles (Zhang et al. 2017; Feng et al. 2020; Khandare et al. 2022). Studies have shown a decrease in average growth rate compared to control groups due to exposure to microplastics. A study conducted by Li et al. (2023) revealed a significantly ($P < 0.05$) inhibited by PS particles (54.56%) while determined that PA fibers had no inhibitory ($P > 0.05$) effect on the growth of *Caulerpa lentillifera* and *Gracilaria tenuistipitata*.

For *U. lactuca*, the RGR was significantly dependent on concentration and the microplastic type. *Ulva* was negatively affected by high concentration of ABS-MP in relative growth and polypropylene (PP) had no effect on the RGR parameters of *Ulva* thallus. According to the data in the table, we can say that the *Ulva* parenchymatic thallus (non filamentous) is more resistant to microplastic toxicity than *Ceramium* thallus (filamentous) in growth rate. This low growth rate varies depending on the algal species, the type, size and concentration of microplastic. The most accepted reasons for this decrease as a result of exposure to microplastics are the inhibition shadowing effect, increased turbidity of the environment, internalization of microplastics and their adhesion to the cell wall (Hazeem et al. 2020; Wang et al. 2020; Yang et al. 2020).

In studies conducted within 96 hours, it was determined that the growth of algae exposed to high

microplastic concentrations decreased and this inhibitory effect increased with exposure time (Gao et al., 2022; Ni et al., 2023; Zheng et al., 2023). In contrast, Yang et al. (2021) revealed that the inhibitory effect on growth gradually weakened as the exposure time progressed to 21 days. Based on these results, we can say that algae adapted to the environment through self-regulation and gained resistance to toxicity.

Effect of Microplastics on the Photosynthetic Pigment

The content of chl-*a* and total carotenoid of *U. lactuca* was not significantly affected by the presence of two microplastics ($P > 0.05$). On the other hand, it was observed that the ABS-MPs had a negative concentration-dependent effect on the chl-*a* and carotenoid content ($P < 0.05$) of *C. diaphanum* except at a low concentration (25 mg L^{-1}). Although chl-*a* content was increasing in 25 mg L^{-1} concentration ABS and PP-MP groups, there were significant inhibition in comparison with the control group, and the inhibition rate increased with the increase of microplastics concentration. This indicated that ABS microplastics could exert a strong negative impact on algal photosynthetic efficiency as compared to PP on *C. diaphanum*. Similarly, this negative effect of microplastics on algae had also been reported by Ansari et al. 2021. Some studies have shown that when algae were stressed, chl-*a* in algae cell was also affected to some extent (Patil et al. 2017; Metzler et al. 2018; Alahverdi & Savabieasfahani 2012) (Table 2).

Table 1. Growth inhibition ratio (%)^a of the *U. lactuca* and *C. diaphanum* macroalgae under different MPs treatments

	<i>Ulva lactuca</i>			<i>Ceramium diaphanum</i>		
	RGR	g fw	RGR (%) ^a	g fw	RGR (%) ^a	
	Control		5.4567	9.13	3.484	9.68
ABS exposure levels (mg L^{-1})	25	5.1152	6.83	2.8765	2.47	
	50	4.967	9.79	2.676	6.48	
	75	4.1194	26.75	2.6759	6.48	
	100	4.0514	28.11	2.6655	6.69	
PP exposure levels (mg L^{-1})	25	5.9633	10.13	2.7721	4.55	
	50	5.6697	4.26	2.7697	4.60	
	75	5.1688	4.03	2.7671	4.65	
	100	5.0854	2.36	2.6524	6.95	

Table 2. The effect of different MPs treatments on algae. + and - indicate the decrease and increase mean values compared to the control group. Asterisk (*) expressed as inhibition average growth rate (IGR).

MPs	Organism	Concentration (mg L^{-1})	IGR (%) [*]	MDA	Chl- <i>a</i>	Ref.
PS-PA	<i>C. lentillifera</i>	100	54.56	59.89 (+)	39.79 (+)	Li et al. 2023
PS-PA	<i>G. tenuistipitata</i>	100	30.62	91.79 (+)	66.04 (+)	Li et al. 2023
PP	<i>A. obliquus</i>	250	37.7	-	-	Ansari et al. 2021
PE	<i>L. sativa</i>	100	16.68	18.76 (+)	9.39 (-)	Gao et al. 2019
PP	<i>Chlorella pyrenoidosa</i> and <i>Microcystis flosaquae</i>	5-500	-	-	-	Wu et al. 2019
PET and PP	<i>Spirulina</i> sp.	300-550	75	-	-	Khoironi et al. 2019
PVC	<i>Skeletonema costatum</i>	5-10	39.7	-	20 (-)	Zhang et al. 2017
PVC	<i>C. pyrenoidosa</i> and <i>Microcystis flos-aquae</i>	5-500	-	-	55.23 (-)	Wu et al. 2019
ABS	<i>U. lactuca</i>	25-100	17.87	38.14 (-)	35.34 (+)	this study
PP	<i>C. diaphanum</i>	25-100	5.19	29.87 (+)	19.04 (-)	this study

As can be seen from the results, under the condition of 100 mg L⁻¹ ABS-MPs, the chl-*a* and total carotenoid content of *C. diaphanum* decreased by 70.27% and 31.81% compared with the control, respectively. The results obtained show that *Ceramium* is sensitive to especially ABS-MPs exposure and is negatively affected with the increase of microplastic particles concentration in terms of chl-*a* and total carotenoid synthesis. These findings may be related to the shadowing impact of ABS fibers or fragments. Previous study has confirmed that macroalgae can enrich microplastics and may reduce chlorophyll synthesis by reducing light penetration into the thallus. A decrease in the pigment amount of macroalgae is observed due to the decrease in light intensity (Feng et al. 2020; Gao et al. 2020; Li et al. 2020; Peller et al. 2021; Zhang et al. 2022). Additionally, studies show that as the size of microplastic increases, the negative impact on algae may increase (Chae et al. 2019; Gao et al. 2021). The fact that the size of ABS (100-3500 µm) is much larger than PP (10-1500 µm) may also explain this negative effect.

Effect of Microplastics on The Lipid and Carbohydrate Contents

The lipid content of *C. diaphanum* was significantly increased under the 25 mg L⁻¹ concentration of ABS and PP-MPs, increasing by 41.12% and 42.67% compared with the control, respectively. The similar result was also found in previous study, where the photosynthetic efficiency of *Chlamydomonas reinhardtii* increased distinctly by 33.3% under 25 mg L⁻¹ of polystyrene microplastics at 72 h (Li et al., 2020). When algae are exposed to pollutants, they can produce reactive oxygen (ROS) and the substances can accumulate within the organism. Increased ROS production can break down antioxidant defenses (SOD, CAT and POD enzymes) and as a result, cell organelles are damaged (Li et al. 2016; Zhang et al. 2018). As can be seen from the Figure 7 results, the effects of ABS and PP microplastics on the lipid activity of *C. diaphanum* were investigated in terms of the MDA (product of membrane lipid peroxidation) content. Malondialdehyde content exhibited a positive response to two microplastics at low concentrations on *Ceramium*. Excessive levels of ROS could promote the lipid peroxidation of the cell membrane, which was commonly manifested by an increase in MDA content (Deng et al. 2014; Gu et al. 2017). Therefore, the increase in MDA was considered to be the main biomarker of oxidative stress intensity. *C. diaphanum* may be particularly susceptible to ABS-MPs because of its special feeding system which is explained by algal gene express (Zhang et al. 2019; Sun et al. 2020; Song et al. 2020).

The authors observed an increase in lipid (MDA) levels at a low microplastic concentration (0-25 mg L⁻¹), and a decrease in MDA levels in high microplastic concentration (>25 mg L⁻¹), versus the control conditions

on macro or microalgae (Yan et al. 2021). They also observed that there was a significant dose-effect relationship for the MDA content, which increased with the increase in the MPs concentration (Zhang et al. 2022; Ni et al. 2023).

Conclusion

This study investigated the effects of two MPs (ABS and PP microplastics) on two typical commercial macroalgae (*Ulva lactuca* and *Ceramium diaphanum*) in Inciralti-Izmir/Turkey. Compared these two algae, it can be concluded that the two MPs will greatly inhibit the RGR of *Ceramium* algae. The results obtained show that *Ceramium* is sensitive to PP-MPs and is negatively affected with the increase of PP particles concentration in terms of chl-*a* and total carotenoid synthesis especially, while there is no significant difference in chl-*a* and total carotenoid content of *U. lactuca*. Compared these two MPs, it could be concluded that PP-MPs will significantly inhibit the pigment synthesis of *Ceramium*, especially. Microplastics had no significantly effect on the CH content of macroalgae on *C. diaphanum* while significantly decreasing under the low and high concentration of ABS-MPs and PP-MPs on *U. lactuca*. The results showed that two macroalgae, especially *U. lactuca*, were not affected at low MPs concentrations. As a result, our findings contribute to improving the current understanding of the biochemical mechanism risks of two microplastics in laboratory conditions on *Ulva* and *Ceramium* macroalgae. In addition, according to the results obtained, we can say that *Ulva* is a durable alternative organism to reduce microplastic pollution in the aquatic environment, as it is more resistant to the toxic effects of microplastics than the *Ceramium* algae. *Ulva* has shown higher tolerance than other organisms to microplastic exposure at concentrations much higher than typical environmental microplastic levels. Considering the results of the above studies, further research is needed to understand the self-regulatory mechanisms that relevant algae have developed against microplastic toxicity and the species that are more likely to develop this mechanism. Besides given the large number of studies conducted so far, further development of approaches such as quality assurance and quality control (QA/QC) to assess the risk of microplastic exposure, develop appropriate organisms for biodegradation, and compare them would further contribute to the analysis of data.

Ethical Statement

This study does not require ethics committee permission or any special permission.

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This study was not funded.

Author Contribution

This study is performed by single author.

Conflict of Interest

The author declares 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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