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Abundance and distribution of microplastics in seawater, sediment, and macroalgae sea grapes *Caulerpa racemosa* from Semak Daun Island, Jakarta Bay, Indonesia

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Abstract. *Patria MP, Kholis N, Anggreini D, Buyong F. 2023. Abundance and distribution of microplastics in seawater, sediment, and macroalgae sea grapes* Caulerpa racemosa *from Semak Daun Island, Jakarta Bay, Indonesia. Biodiversitas 24: 3424-3430.* Microplastic pollution in marine ecosystems poses a significant global concern, specifically regarding the security of food derived from the sea. Macroalgae, as a food source from the sea, can be susceptible to contamination by microplastics. This research assessed the abundance and forms of microplastics in macroalgae sea grapes (*Caulerpa racemosa* (Forssk.) J.Agardh) collected from Semak Daun Island within the Seribu Island National Park. Additionally, the impact of washing and stirring on microplastic abundance was examined in macroalgae. The average number of microplastics and their identification showed that the washed, stirred, and NaOH-treated macroalgae samples contained an average abundance of 3.28±0.31 particles/g, 5.06±0.59 particles/g, and 2.0±0.81 particles/g, respectively. Furthermore, microplastics were detected in seawater and sediment samples, with an average abundance of 8.2±2.19 particles/L and 15,200±4,932 particles/Kg, respectively. Fragmented microplastics were the predominant form in macroalgae, while fibrous microplastics dominated seawater and sediment samples. Significant differences were observed between the washed and stirred samples (p = 0.009), where the stirred sample exhibited a higher reduction percentage. Microplastics in edible macroalgae indicated the potential hazards of exposure and subsequent accumulation in the human body.

Keywords: Macroalgae, microplastics, Semak Daun Island, stirring, washing

INTRODUCTION

Microplastics' contamination of marine ecosystems and their impact on marine life has become a pressing global concern (Vegter et al. 2014; Bonanno and Orlando-Bonaca 2020). This issue is related to the vital role of marine ecosystems in providing food resources for humans, specifically through fishing activities. Oceans represent a vast reservoir that significantly contributes to increasing and sustaining food production. However, estimates indicate that approximately 51 trillion plastic particles, weighing around 268,940 tons, are floating in the world's oceans and progressively decomposing into microscopic microplastics (Eriksen et al. 2014). Microplastics in marine ecosystems can affect human health through the food web (Barnes et al. 2009; Saley et al. 2019). These particles can be directly consumed by organisms or embedded on the surfaces of marine biota, including microalgae (Zhang et al. 2017; Ng et al. 2022).

Macroalgae are prominent marine organisms found along the coastal areas of Indonesia and are traditionally consumed as sea vegetables in Asian countries (Pangestuti et al. 2021). The increasing global interest in a healthy diet has boosted the popularity of edible macroalgae, particularly *Caulerpa*, commonly called 'sea grapes' (Paul et al. 2014). *Caulerpa racemose* (*C. racemosa*) (Forssk.) J.Agardh, a species of green macroalgae, is consumed by humans in its natural form or after processing (Sinurat et al. 2022). Sea grapes such as *C. racemosa* are commercially grown in several Southeast Asian countries, specifically Vietnam and the Philippines, due to their ease of cultivation, rapid growth rate, and potential health benefits (Nagappan and Vairappan 2014; Paul et al. 2014; Pangestuti et al. 2021).

Macroalgae play a crucial role in marine ecosystems by serving as habitats and food sources for various marine biotas such as crustaceans, mollusks, echinoderms, and small fish (Murphy et al. 2017; Lucía et al. 2018; Carrasco et al. 2019). They are predominantly found in littoral and sublittoral zones, with sufficient sunlight for survival. Environmental factors, including pressure and anthropogenic factors such as local human activities and tourism, influence their distribution in water bodies. From an anthropological perspective, macroalgae can also be used as a food ingredient, industrial raw material, and practical material in laboratory settings, such as wet preservation (Mateos-Cárdenas et al. 2021).

Previous research has demonstrated microplastics in macroalgae *Ulva prolifera* O.F.Müll. and *Sargassum horneri* found in the Yellow Sea (Gao et al. 2020; Zhang et al. 2022). Additionally, investigations conducted on two species of macroalgae off the coast of California showed a microplastic abundance of 2.34±2.19 particles/g in the brown algae *Pelvetiopsis limitata* and 8.65±6.44 particles/g in the red algae *Endocladia muricata* (Endlichter) J.Agardh

(Saley et al. 2019). These observations indicate the potential for microplastic contamination in macroalgae to affect other living things, including humans, through the food web (Gao et al. 2020).

This research aims to assess the abundance and density of microplastics in *C. racemosa* compared to the values in seawater and sediment found at the sampling site. Furthermore, persistent microplastics will be identified to determine the percentage of microplastics retained in macroalgae after washing and stirring. López-Rosales et al. (2022) reported that both processes were the most common procedure conducted before the consumption of macroalgae, so the findings in this study can support the previous studies.

MATERIALS AND METHODS

Research area and samples collection

Semak Daun Island is situated in the Seribu Islands District of *Daerah Khusus Ibukota* (DKI - the Special Capital Region) Jakarta, Indonesia and belongs to Panggang Island Village, with geographic coordinates ranging from 5°43'46"-5°43'49.12" South Latitude and 106°34'12.98"-106°34'19.38" East Longitude (PST 2021:1). Covering an area of 0.75 ha, it primarily serves as a camping site for tourists and not inhabited by permanent residents. Also, the environment is characterized by scattered litter, including fishing nets and plastics discarded indiscriminately by tourists. Previous research has found 85 scattered macro debris in the subtidal and intertidal beach of Semak Daun Island, which is 98% plastic (Utami and Sujatmiko 2021).

The investigation on the abundance of microplastics in sea grapes, seawater, and the surrounding sediment was conducted at Semak Daun Island, located within the Seribu Island National Park. Sampling was carried out in February 2022 at the South Station containing sea grapes, with coordinates of 5°43'48.17"S Latitude and 106°34'18.20" E Longitude, measured using the Global Positioning System (GPS) as shown in Figure 1. The abundance of microplastics in each sample was analyzed at the Marine Biology Laboratory, Department of Biology, University of Indonesia, Depok, and concluded in April 2022.

Sea grapes samples were collected directly into five glass jars and transported from the site to the laboratory. Subsequently, they were weighed using a 10-gram analytical balance and stored in each glass jar filled with 100 mL of distilled water. A shovel collected 300-400 g sediment sampled approximately 5-10 cm below the sediment surface in the sea grapes vicinity and stocked them in 1,000 g glass jars. Seawater samples were obtained from the surrounding macroalgae population by filtering 30 L of seawater with a 300 μm diameter plankton net until 100 mL of seawater remained. The nets designed for plankton collection were used for microplastic sampling. Plankton nets generally facilitate the sampling of large volumes of water from the surface to the bottom. Furthermore, 30 liters of water is used as a standard in filtering using a plankton net to increase the number of filtered microplastics (Uurasjärvi et al. 2021). The filtered contents were transferred into a 1,000 mL glass jar. All samples were securely stored in glass jars, placed in cool boxes, and transported to the Marine Biology Laboratory.

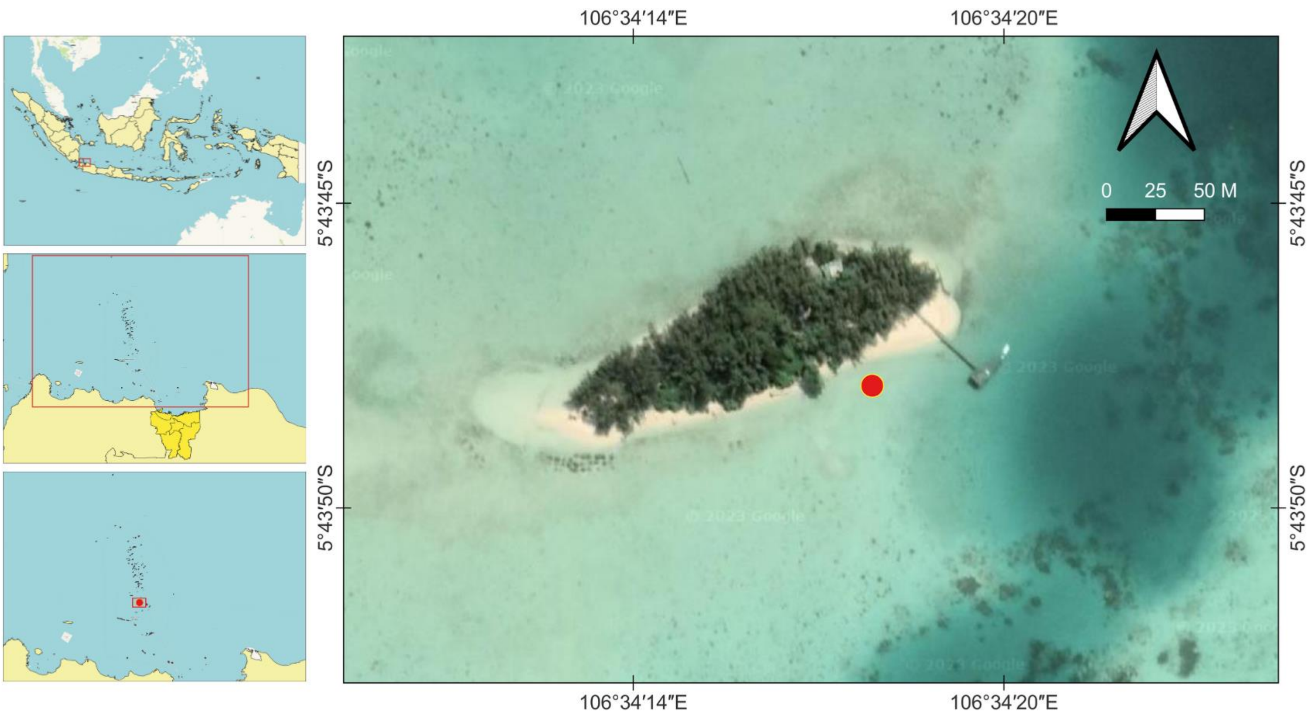

Figure 1. Sampling location at Semak Daun Island, Seribu Islands National Park, Jakarta, Indonesia

Microplastics extraction and identification from macroalgae

The extraction of microplastics from macroalgae involved a three-step process. Firstly, macroalgae samples weighing 10 grams were rinsed with 100 mL of distilled water, and the rinse water was filtered using 90 mm diameter and 2.5 µm pore size Whatman's Grade 42 Ashless Cellulose-based filter paper. The filtered contents were subsequently examined under a Zoom Stereo Microscope Olympus SZ61-ILTS with 45 times maximum magnification.

Next, the previously rinsed macroalgae sample was placed in a 250 mL beaker glass and washed with 100 mL of distilled water. The sample was stirred using a metal magnetic stirrer at 150 rpm for 15 minutes. The solution was filtered with 90 mm diameter and 2.5 µm pore size Whatman's Grade 42 Ashless Cellulose-based filter paper and examined under a Zoom Stereo Microscope Olympus SZ61-ILTS with 45 times maximum magnification.

The last step involved extraction through a destruction process using NaOH. Macroalgae samples were dried at 60°C for 48 hours, dissolved with 50 mL 6 M NaOH, and incubated in a water bath at 60°C for 72 hours (Hamzah et al. 2021). Therefore, to reduce the concentration of NaOH, distilled water was added to the sample at a ratio of 1:2. The sample was then filtered with 90 mm diameter and 2.5 µm pore size Whatman's Grade 42 Ashless Cellulose-based filter paper and examined under a Zoom Stereo Microscope Olympus SZ61-ILTS with 45 times maximum magnification.

Microplastics extraction and identification from seawater and sediment

Microplastic extraction from seawater involved filtering 100 mL of seawater through 90 mm diameter and 2.5 µm pore size Whatman's Grade 42 Ashless Cellulose-based filter paper. The filter paper was subsequently examined under a Zoom Stereo Microscope Olympus SZ61-ILTS with 45 times maximum magnification to identify the form and quantity of microplastics (Kanhai et al. 2017).

For sediment samples, they were first dried at 60^{O}C for 48 hours. Then, 25 grams of the dried samples were dissolved in a 26% NaCl solution at a ratio of 1:5. The mixture was stirred for 2 minutes and left to settle for 24 hours (Cozzolino et al. 2020). From the surface, 20 mL of the solution was transferred to a test tube using a glass pipette for homogenization. The solution was filtered using 90 mm diameter and 2.5 µm pore size Whatman's Grade 42 Ashless Cellulose-based filter paper and examined under a Zoom Stereo Microscope Olympus SZ61-ILTS with 45 times maximum magnification.

Data analysis

A two-sample paired t-test was performed using Paleontological Statistics Software (PAST) version 4.03 to determine the differences in microplastic reduction between the washed and stirred extraction steps, with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Microplastic abundance in macroalgae

Table 1 and Figure 2 present the average number and identification of microplastics. The washed, stirred, and NaOH-treated macroalgae sample had an average microplastic count of 3.28±0.31 particles/g, 5.06±0.59 particles/g, and 2.0±0.81 particles/g, respectively. The most dominant forms of microplastics varied as 38% fiber, 38% fragments, and 35% foam among the samples, respectively, as indicated in Figure 3. A significant difference was observed between the washed and stirred samples (p = 0.009), where the stirred showed a higher reduction percentage (mean = 49%).

Microplastic abundance in seawater and sediment

The average number and identification of microplastics in seawater and sediment samples showed an average abundance of 8.2±2.19 particles/L and 15,200±4,932 particles/Kg, respectively, as presented in Table 2. The most dominant microplastic form found in both seawater and sediment samples was fiber, with a percentage of 46% and 39%, respectively, as presented in Figures 4 and 5.

Table 1. Abundance (particles/g) and forms of microplastics found in the water after macroalgae were washed, stirred, and treated with NaOH

Forms	Washed	Stirred	NaOH	Total
Fiber	1.26±0.46	1.42±0.24	0.48±0.17	3.16±0.51
Fragment	1.00±0.28	1.92±0.31	0.64±0.31	3.56±0.64
Film	0.36±0.12	0.60±0.11	0.18±0.18	1.14±0.21
Foam	0.66±0.21	1.12±0.19	0.70±0.42	2.48±0.55
Total	3.28±0.31	5.06±0.59	2.00±0.81	10.34
%	31.72	48.94	19.34	100.00

Table 2. Abundance and forms of microplastics in seawater and sediment

	Seawater (particle/L)	Sediment (particle/kg)
Fibre	3.80 ± 1.65	5,920 ± 3,704
Fragment	1.13 ± 0.40	2,400 ± 1,131
Film	0.67 ± 0.37	2,080 ± 815
Foam	2.60 ± 0.53	4,800 ± 1,011
Total	8.20 ± 2.19	15,200 ± 4,931

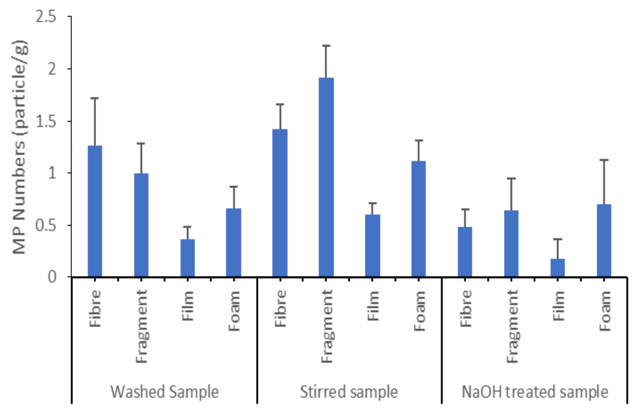

Figure 2. The abundance of microplastics in macroalgae *C. racemosa* based on treatment

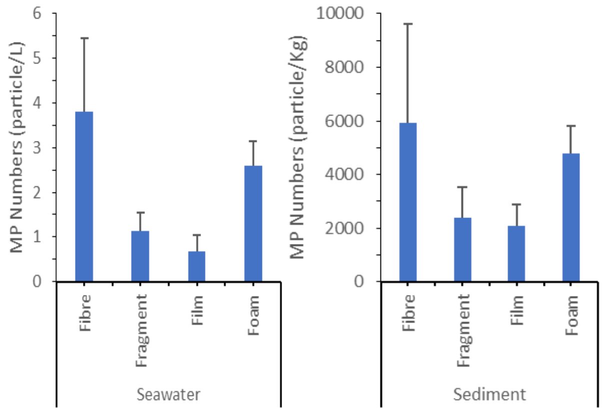

Figure 4. Microplastic abundance in seawater and sediment is based on the number of particles

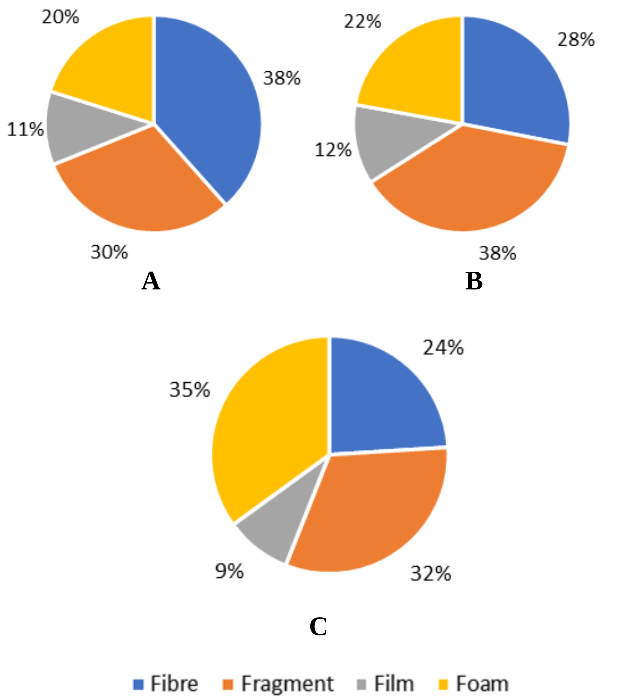

Figure 3. The percentage of microplastics forms in *C. racemosa* from the: A. Washed, B. Stirred, and C. NaOH-treated samples

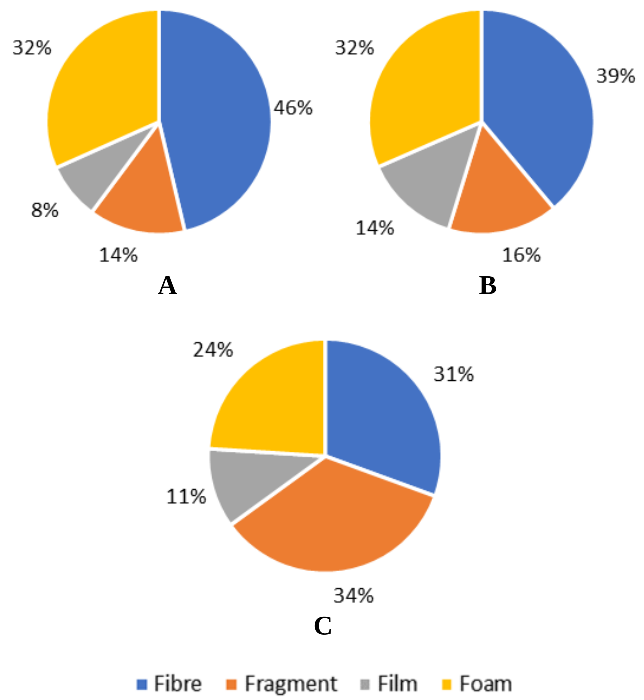

Figure 5. The percentage of microplastics forms in: A. Seawater, B. Sediment, and C. Macroalgae *C. Racemosa*

Discussions

Microplastic abundance in macroalgae

The average abundance of microplastics in macroalgae was eighty times higher than in previous research (current study = 10.34 particle/g; Zhang et al. (2022) = 0.119 particle/g) (Zhang et al. 2022). This could be attributed to the high human activity in the vicinity of the sampling location, which serves as the main source of microplastic pollution in marine ecosystems. Panggang Island, the closest inhabited island to the sampling location, had a population of around 4,200 people in 2016 and a waste piling problem contributing to marine pollution (Suryadjaja and Astuti 2021). The increased tourism activity and the influx of plastic wastes from land and rivers also contribute significantly to the higher abundance of microplastics in macroalgae.

The presence of microplastics in macroalgae can originate from entanglement and embedding processes. Generally, fiber forms are more prone to entangling and embedding, leading to their dominance in quantity (Esiukova et al. 2021; Zhang et al. 2022). The thin and elongated structure of fiber microplastics facilitates their easy entanglement. However, this research discovered fragmented microplastics as the most dominant form; they were also reported by Li et al. (2020) to be the second most prevalent type found on the surfaces of macroalgae. This can be attributed to the irregular shapes and rough edges of fragmented microplastics, which provide a greater tendency to adhere to surfaces. In contrast, fibers, film, and foam seemingly possess smoother and streamlined shapes, reducing their propensity to stick to surfaces. The research by Utami and Sujatmiko (2021) also observed that around 30% of macroplastics at Semak Daun Island were plastic cups and bottles, which can be a source of fragmented microplastics.

The morphological structure of microalgae influences the entangled microplastic forms (Ng et al. 2022; Zhang et al. 2022), leading to variations in those found among different macroalgae species. All types of macroalgae can accumulate microplastics; hence, they are valuable bioindicators of microplastic pollution in aquatic ecosystems (Sfriso et al. 2021).

Microplastics also become embedded and encased within macroalgae (Li et al. 2022). Research on *Ulva prolivera* showed the possibility of this process through air sacs found in macroalgae structures (Feng et al. 2020). The specific surface area and softer surface structure facilitate the entry of microplastic particles under external pressure (Zhang et al. 2022). Chia et al. (2020) reported that microalgae decompose microplastics in the oceans through their usage as a carbon source. Although the absorption of microplastics as a carbon source has not been extensively investigated, already obtained results can potentially be applied to macroalgae research.

Even so, there are some limitations to this study. Using NaOH solution and high temperature for samples from the environment could reduce the amount of extracted micro-plastics. However, using NaOH proved nondestructive, especially for Polystyrene (PS), and 60°C temperature showed no degradation in morphology (Gulizia et al. 2022). In addition, polymer analysis also was not conducted, so contamination from the natural polymer is possible.

Microplastic abundance in seawater and sediment

The abundance of microplastics in seawater greatly affects their presence in marine biota, while sediment serves as the final sink for microplastics in the oceans (de Smit et al. 2021; Sfriso et al. 2021). Microplastics predominantly originate from floating plastic waste carried away by water currents. Once the plastic waste decomposes and becomes denser particles than seawater, they sink and accumulate in sediment (Esiukova et al. 2021).

The predominant forms of microplastics in seawater and sediment differ from those in macroalgae. This variation may be due to the specific ability of *C. racemosa* to entangle fragmented microplastics. Fibre microplastics are the most dominant in macroalgae, some float in seawater, while others accumulate in sediment. They can originate from the degradation of fishing equipment composed of fibrous materials and human activities (Welden and Cowie 2017; Aiguo et al. 2022; Lam et al. 2022). A study by Rasyid et al. (2022) on Pramuka Island's seagrass beds showed the same findings, with an abundance of 20-440 particles/Kg, which predominated by fiber microplastic. That indicates the same forms predominated the microplastics found around Seribu Island District.

Persistent microplastics and potential health hazards

The NaOH destruction method employed on *C. racemosa* samples showed the presence of persistent microplastics. Approximately 19% of microplastics in macroalgae were detected in the NaOH-treated sample, as indicated in Figure 1. Rinsing with water and stirring only removed around 81% of the microplastic particles found in *C. racemosa*. The results were still lower than the percentage of microplastics lost through rinsing in *Fusus vesiculosus*, reaching 94.5% (Sundbæk et al. 2018). These indicated the presence of embedded microplastics within macroalgae that do not dissolve in water during rinsing and stirring. The rinsing and stirring could not remove all microplastic particles adhering to macroalgae (López-Rosales et al. 2022). These observations are crucial, considering that *C. racemosa* is often consumed in Asia.

Caulerpa racemosa is commonly used as a food source in Southeast Asia and Japan. Furthermore, it has a high carbohydrate and protein content and antimicrobial activity (Nagappan and Vairappan 2014). *Caulerpa racemosa* can serve as an alternative nutritious food (Hao et al. 2019) due to the content of minerals and fatty acids (Paul et al. 2014; Pangestuti et al. 2021; Sinurat et al. 2022). The presence of microplastics reduces the value as a nutritious food source; consuming *C. racemosa* contaminated with microplastics increases the potential for human exposure to these particles. As persistent organic pollutants, microplastics can pose serious health problems once consumed and accumulated in large quantities (Andrady 2011; Gassel et al. 2013). Their abundance in microalgae is more closely associated with location than species (Sfriso et al. 2021). Therefore, the consumption of macroalgae from areas with high levels of plastic and microplastic contamination must be avoided to reduce the risk of exposure.

In conclusion, fragmented microplastics were the most dominant form in macroalgae, while fiber prevailed in seawater and sediment. A significantly higher reduction percentage was observed in the stirred sample than in the washed sample (p = 0.009), with a mean reduction of 49%. The rinsing and stirring methods effectively removed approximately 81% of microplastic particles attached to macroalgae. That suggests the consumption of *C. racemosa* originating from the waters of Semak Daun Island may still pose a risk of microplastic exposure to humans if not processed further. Therefore, developing more efficient methods for removing embedded microplastics from edible macroalgae is crucial to ensure their safety for human consumption.

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