

Microplastic Contamination on *Cottonii* Seaweed (*Kappaphycopsis cottonii*) Cultivated in Bomo Beach, Banyuwangi, East Java, Indonesia

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Abstract

The cultivation of *Kappaphycopsis cottonii* seaweed needs to comply with the health, waste, and sustainability in the Blue Economy standards. However, early observation showed that *K. cottonii* in Bomo Beach, Banyuwangi, East Java was potentially contaminated by microplastics. This research was performed to evaluate the density, polymer type, and contamination mechanism of microplastics in *K. cottonii* and the correlation between the planting age of *K. cottonii* and the total concentrations of microplastics in the seaweed. In this research, seaweed and seawater in the cultivated area were sampled using a simple random sampling technique every week for 5 weeks. Seaweed suspected of being contaminated with microplastics was digested using wet peroxide oxidation, followed by identification of polymer types through Fourier-Transform Infrared Spectroscopy (FTIR). The results indicated that the predominant suspected microplastics (MPs) contaminating *K. cottonii* were fibers, measuring less than 1.5 mm in size, and primarily composed of transparent polypropylene. MPs contaminate *K. cottonii* by wrapping around the thallus and being trapped by *Melanothamnus savatieri* (Hariot) Díaz-Tapia & Maggs epiphytes. The planting age of *K. cottonii* has a strong positive correlation with the number of suspected MPs contaminating *K. cottonii*. The growth of *M. savatieri* over a period of 3-4 planting weeks increases the suspected MPs on the thallus of *K. cottonii*. The results of this research can serve as a preventive measure and basis for developing strategic policies to reduce microplastic contamination.

Keywords: Cultivation; Seaweed; Microplastics; Epiphyte

1. Introduction

Bomo Beach is located on the Bali Strait on the eastern side of Java, where the seaweed species *K. cottonii* is widely cultivated. Research conducted by the Republic of Indonesia's Ministry of Maritime and Fisheries Affairs from 2015 to 2020 showed that trash originating from West Java is carried by currents east to Bali Strait and

Lombok Strait during westerly monsoons (Subandriyo, 2021). Consequently, the water off Bomo Beach is potentially polluted by microplastics (MPs). The MP pollution in Bali Strait accumulates from different directions (Bagaskara *et al.*, 2020). Several forms of MPs have been found at Bangsring Beach, including filaments (48%), fragments

(32%), films (14%), and granules (6%) (Aji, 2017). The abundance and diversity of MPs have been observed to be increasing in tandem with increasing anthropogenic activity (Govender *et al.*, 2020). In addition to being a place where seaweed is cultivated, Bomo Beach is a favorite tourist destination. Residential areas are located only 10 – 50 meters away from the littoral area. Therefore, the existence of settlements close to Bomo Beach might also contribute to MP pollution in the water. Browne *et al.* (2011) point out that residential areas often produce MP fibers. MPs also come from fishery and aquaculture equipment. Most of the people who reside around Bomo Beach are fishermen and cultivators in the fisheries sector, which uses many plastic tools. Plastic tools that are intensely exposed to UV light, ocean waves, and temperature changes can become brittle and fragmented, thus resulting in MP particles (FAO, 2017).

MPs in seawater can contaminate seaweed. Li *et al.* (2022) report that the MP contamination of seaweed is influenced by the morphology of the thallus. That is, the more structurally complex it is the greater the capacity to adsorb MPs by wrapping, attachment, entanglement, and entrapment by epibionts that live on the surface of the thallus. Seaweed contaminated with MPs can be a vector for the entry of MPs into the food chain (Gutow *et al.*, 2016).

Plastics contain additives, such as polybrominated diphenyl ether (PBDE), phthalates, nonylphenol (NP), bisphenol A (BPA), and antioxidants (Hermabessiere *et al.*, 2017), that are harmful to marine organisms. MPs can also contaminate human food, having a carcinogenic effect on the human body (Saeed *et al.*, 2023). Human exposure to MPs can cause digestive problems, endocrine disorders, and even become a vector for pathogens to infect the body (Emenike *et al.*, 2023). Pham *et al.* (2023) have examined human exposure to MPs through food in Korea, where the highest exposure was via seaweed. Therefore, it is necessary to research MP contamination in all types of food, including *K. cottonii* seaweed.

K. cottonii is the main seaweed commodity in Indonesia and is used as raw material in the carrageenan industry. Carrageenan is commonly used to form gels, thickeners, and binders in the food industry (Critchley *et al.*, 2017). Carrageenan is also used as an alternative to gelatin, which is produced from livestock (cows and pigs) (Morrison *et al.*, 1999). Unfortunately, the methane gas from livestock farming is often associated with global warming (Moumen *et al.*, 2016). This situation increases the demand for carrageenan from *K. cottonii* seaweed. Furthermore, issues regarding the hygiene in the production of Indonesian fishery products also remains the factor that cause the product to have a high rejection rate in the US and Europe (Nurkhasanah *et al.*, 2022). Therefore, research related to the contamination of MPs in *K. cottonii* is crucial as a preventive measure and a basis for developing various policies to reduce microplastic contamination.

In this research, the density, polymer type, and contamination mechanisms of microplastics in *K. cottonii*, the correlation between the planting age of *K. cottonii* and the total concentrations of microplastics on the seaweed were examined. The results of this research were used as the basis to formulate various strategies aiming to prevent *K. cottonii* products from microplastic contamination.

2. Methodology

2.1 Research Site

This research was conducted from 20 May – 17 June 2022 at Bomo Beach, Bomo Village, Blimbingsari District, Banyuwangi Regency, East Java, Indonesia. Bomo Beach was selected as the observation site as it is located in the waters of the Bali Strait, where waste carried by currents from West Java (Subandriyo, 2021) accumulate, resulting in higher possibility of microplastics contamination. Bomo Beach is classified as a sloping beach, with an area for seaweed cultivation reaching 10,000 m². The seaweed samples were taken from 9 sampling points (Table 1 and Figure 1).

Table 1. Coordinate points of seaweed *K. cottonii* sampling points

Sampling Points	Coordinate
T1, T2, T3	X = 8°22'32.97"S Y = 114°21'18.89"E
T4, T5, T6	X = 8°22'32.92"S Y = 114°21'24.98"E
T7, T8, T9	X = 8°22'32.82"S Y = 114°21'31.36"E

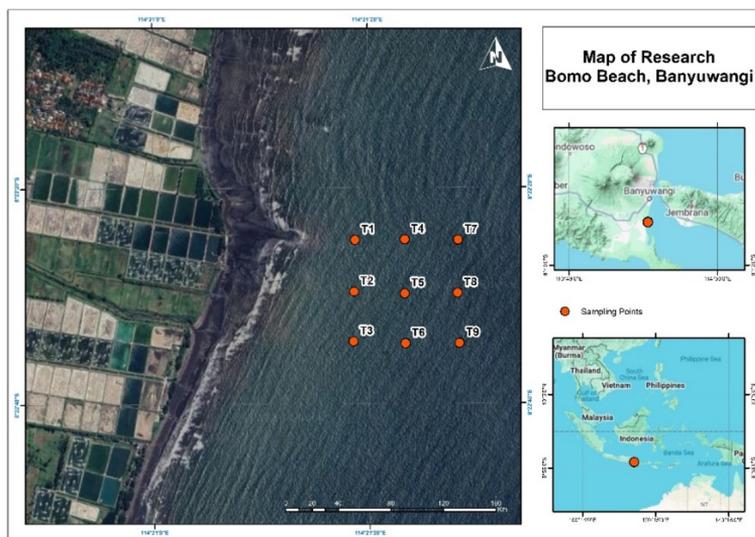


Figure 1. Sampling points in Bomo Beach, East Java, Indonesia.

2.2 A Sampling of Seawater and *K. cottonii*

At each sampling point, 1 thallus sample of *K. cottonii* was taken and put into a silicone bag. Samples of rope used for cultivation were also collected and stored in glass bottles to determine whether the rope used for cultivation is the main source of microplastics that contaminate *K. cottonii*.

Furthermore, seawater was taken vertically from each sampling point using a water sampler of 2 L (depth 20 cm). The water was transferred into a 2-L bottle and kept in cool storage for MPs analysis in the laboratory. Several environmental parameters were also measured at each sampling point, including water salinity measured using a hand refractometer, temperature measured using a thermometer, and pH level measured using a pH meter.

2.3 Visual Analysis of Suspected MPs in Seawater

Suspected MPs in seawater were collected using a sieve of 60 - mm and 250 - mm mesh sizes (Herrera *et al.*, 2018). MPs retained by the sieve then visually sorted, while suspected MPs that passed through the sieve were filtered using a glass microfiber filter (Whatman, grade GF/B) with a filtration unit on a Buchner funnel. The filter paper containing the suspected MPs was put into a petri dish for MPs count and characteristics analysis, including the form, size, and color using a microscope. The forms of microplastics were classified into fragments, fibers, films, foam and pellets. The size of microplastics is classified into small (< 1.5 mm); medium (1.6 - 3.3 mm); and large (> 3.3 mm). Meanwhile, the color of microplastics is classified into white, blue, green, red, yellow, brown, black, and transparent.

2.4 Visual Identification of Physical Interaction Between Suspected MPs and *K. cottonii*

The thallus of *K. cottonii* was placed on the microscope stage and then observed visually using a microscope. Through this observation, it was known how the thallus interacted with microplastics.

2.5 Extraction of Suspected MPs on *K. cottonii*

Suspected MPs on *K. cottonii* samples were extracted using the wet peroxide oxidation method (Masura et al., 2006). Furthermore, seaweed sample (30 g) was put into a 500 - mL Beaker glass and was added with 20 mL of 0.05 M Fe (II) as a catalyst, and 40 mL of 30% H₂O₂ to be set at room temperature for 5 minutes. The Beaker was then stirred on a hotplate using a magnetic stirrer at 75 °C for 3 minutes. The digestion solution was allowed to stand for 30 minutes and was added with distilled water to reduce the risk of overflow from the reaction. The digestion solution was then filtered using a mesh sieve and using Whatman glass microfibre filter GF/B. The filter paper was placed in a petri dish to be dried out in an oven at 60 °C for 4 hours. The suspected MPs were isolated and photographed under a stereo microscope for counting and classification based on the form (fragments, fibers, films, foam, and pellets), size (small (< 1.5 mm); medium (1.6 - 3.3 mm); and large (> 3.3 mm)), and color (white, blue, green, red, yellow, brown, black and transparent).

2.6 Identification of Suspected MPs Polymers

The identification of suspected MPs polymers was carried out using Fourier-transform infrared spectroscopy (FT-IR) Thermo Scientific Nicolet iS10 with a wave number of 4000 – 650 per cm. Not all suspected MPs were analyzed using FTIR due to small size (< 1.5 mm) and inability to completely mix with KBr. Out of 206 suspected MPs found in seawater and 125 suspected MPs found in *K. cottonii*, only 14 suspected MPs were analyzed using FTIR. In addition, *K. cottonii* samples were also analyzed using FTIR to identify the chemical

interaction between suspected MPs and *K. cottonii* tissue. The rope used for cultivating *K. cottonii* was also analyzed using FTIR to determine if the polymer that contaminates *K. cottonii* sourced from the rope used in the cultivation process. The emerging spectrum was compared with the standard spectrum using Thermo Scientific OMNIC software.

3. Results and Discussion

3.1 Types and Mechanisms of MPs Contamination on *K. cottonii*

This research identified various sizes, forms, and colors of suspected MPs that contaminated the thallus of seaweed *K. cottonii* in Bomo Beach, Banyuwangi Regency, East Java. Suspected MPs with small sizes (< 1.5 mm) mostly contaminated *K. cottonii* (92.8%; n = 116) (Figure 2). The abundance of small MPs attached to the seaweed thallus is in line with the abundance of MPs in seawater (100%; n = 206) (Figure 2). Cui et al., (2022) previously mentioned that small MPs dominated MPs contamination in the sea surface of the Western Pacific Ocean, South China Sea, and Northern South China Sea. Higher amount of small-sized MPs in thallus and seawater proves that the degradation process of plastic waste in the sea is increasing (Zhang et al., 2017). Medium and large sized microplastics were not present in the seawater where *K. cottonii* was cultivated could be due to the regular cleaning done every 2 weeks. Medium and large sized microplastics could have been caught in nets used to clean cultivation areas, while small sized microplastics escaped the nets.

The majority of suspected microplastics (MPs) contaminating *K. cottonii* were fibers (86%; n = 108), followed by fragments (14%; n = 17) (Figure 4.2). This pattern is similar to the MPs found in seawater from Bomo Beach, where fibers dominated (97%; n = 199), with smaller proportions of fragments (1%; n = 3) and films (2%; n = 4) (Figure 3).

MPs in the form of fibers commonly found on *K. cottonii* and in seawater may source from household and fishery waste in Bomo Beach area. Aji (2017) found that the microplastics found in the Bangsring

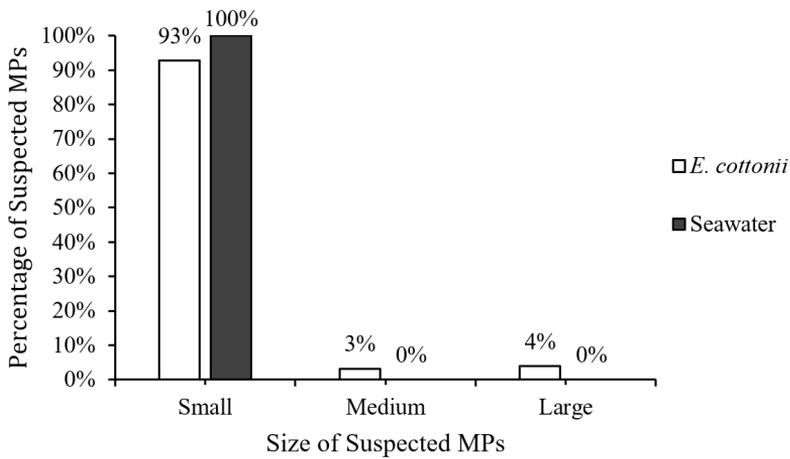


Figure 2. Size of suspected MPs that contaminated *K. cottonii* and Bomo Beach seawater (Small: < 1.5 mm; Medium: 1.6 – 3.3 mm; Large: > 3.3 mm)

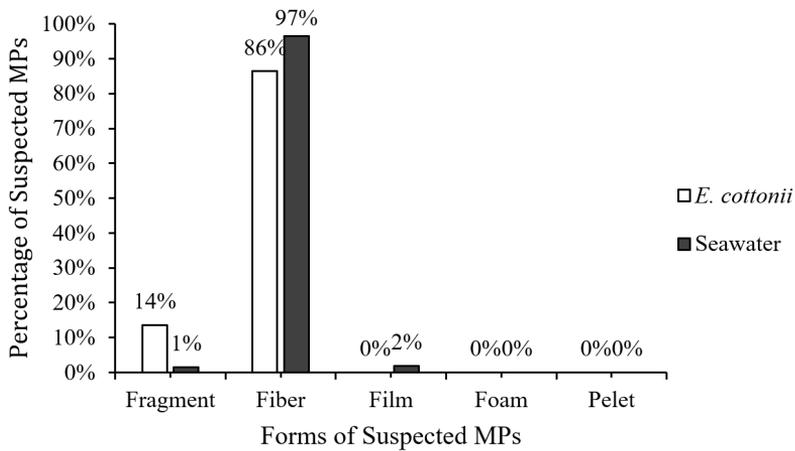


Figure 3. Forms of suspected MPs found in *K. cottonii* and Bomo Beach seawater

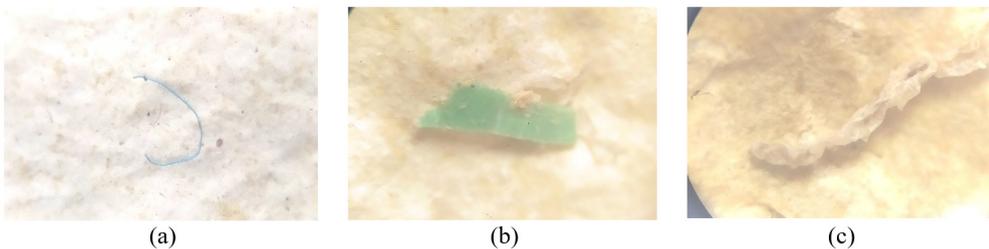


Figure 4. Examples of the form of suspected MPs found in *K. cottonii*: (a) Fiber, (b) Fragment, (c) Film

sea waters Banyuwangi in the Bali Strait were also dominated by fiber/filament forms (48%). Similarly, González-Pleiter *et al.*, (2020) and Rebelein *et al.*, (2021) stated that fibers are the most abundant in the aquatic environment. Fibers can come from household waste (laundry) as well as capture fisheries and aquaculture activities (Xue *et al.*, 2020). Bomo Beach is close to residential areas,

where local residents mostly work in the aquaculture and fisheries sector, allowing residues from household waste (washing clothes) and waste from fish farming activities to pollute the water.

The FT-IR analysis identified the polymer type that contaminated *K. cottonii*, where Polypropylene dominating (50%), followed by Polyethylene (36%) (Figure 4).

Several researchers also found similar results, including Digka *et al.*, (2018) and Takarina *et al.*, (2022) who also found that polyethylene and polypropylene dominating the sea water pollution. According to Erni-Cassola *et al.*, (2019), polyethylene and polypropylene are polymers that have low density that are often found on the surface of seawater.

The density of each form of microplastic is an important factor in determining its distribution. Fiber microplastics are lighter, making them easily transported (Acharya *et al.*, 2021). In addition, fiber microplastics have certain structure and aspect ratio that easily pass through wastewater treatment filters (Okoffo *et al.*, 2019).

Suspected MPs in *K. cottonii* contamination showed colors ranging from white, blue, green, red, brown, black, and transparent. The colors of suspected MPs found on the seaweed were transparent (28%; n = 35), black (28%; n = 35), and blue (22.4%; n = 28) (Figure 5). The color of suspected MPs found in Bomo Beach seawater was also dominated by transparent (37.86%; n = 78), black (26.21%; n = 54), and blue (16.99%; n = 35) MPs. These findings suggest that microplastics can contaminate organisms at the first trophic level of marine ecosystems, such as primary producers. Through the food chain, microplastics can then contaminate consumers at higher trophic levels, leading

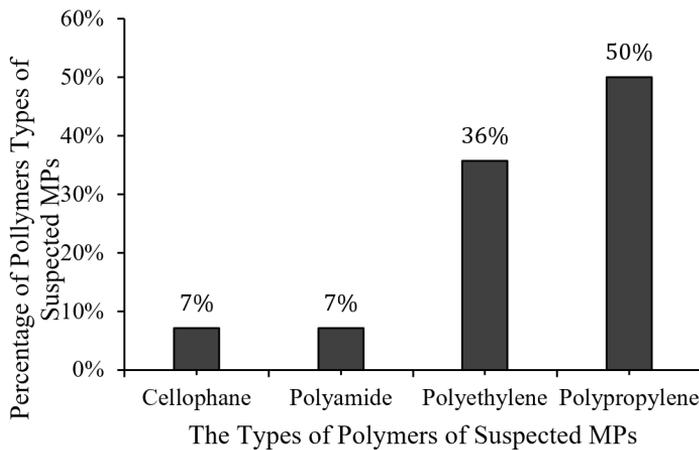


Figure 5. Polymer types of Suspected MPs that contaminated *K. cottonii*.

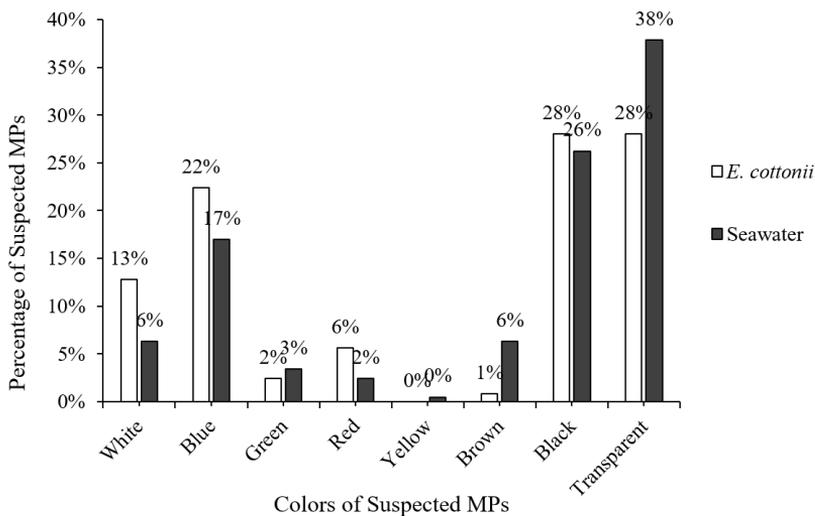


Figure 6. Colors of Suspected MPs that contaminated *K. cottonii*.

to pollution throughout the marine food chain. This supports Mariani *et al.*, (2023) who provided the evidence of trophic transfer of microplastics from producers to primary consumers.

The suspected microplastics (MPs) found on *K. cottonii* thallus and in seawater were predominantly transparent, consistent with findings by Di and Wang (2018) and Martí *et al.* (2020), which showed that most MPs in marine environments are transparent, followed by yellow, brown, and blue. The transparency of these MPs is likely due to color degradation from prolonged exposure to sunlight in the marine environment (Martí *et al.*, 2020).

K. cottonii has a hard thallus surface structure with high cellulose content, where MPs contamination might occur through an entanglement mechanism (Figure 6) and was trapped by *Melanothamnus savatieri* epiphyte (Figure 7). Zhang *et al.* (2022)

investigated the mechanism of MPs contamination on *Sargassum horneri* which also has a hard thallus structure and is rich in cellulose. The results of this research revealed that microplastic (MP) contamination in *Sargassum horneri* occurred primarily through entanglement, with no evidence of MPs adhering to or penetrating the thallus.

M. savatieri is one of the epiphytes that infects *K. cottonii* seaweed with the highest percentage (80 - 85%) among other epiphytic seaweeds. It has vertical axis body, height of 4 - 20 mm and a basal attachment system consisting of primary rhizoids and several secondary rhizoids (Vairappan, 2006).

The attachment mechanism of *M. savatieri* epiphytes to *Kappaphycus cottonii* begins with the appearance of black dots containing tetraspores on the cuticle layer surface. These dots gradually increase, causing the surface of *K. cottonii* to become rough. After 3-4 weeks,



Figure 8. *M. savatieri* living as epiphytes on the surface of the thallus of *K. cottonii*.



Figure 9. Epiphyte *M. savatieri* has grown on the surface of *K. cottonii* and trapped Suspected MPs.

the epiphytes enter the vegetative phase. Subsequently, they grow reproductively, with a density of up to 40-48 epiphytes per centimeter of the host's surface (Vairappan, 2006). *K. cottonii* cultivation usually becomes more susceptible to epiphytic infection in March – June, and September – November (Vairappan, 2006). A sampling of *K. cottonii* conducted in May – June showed relevance to the results of this study that many *M. savatieri* epiphytes infect *K. cottonii*.

M. savatieri infects almost the entire surface of *K. cottonii* thallus. The fine fiber morphology of *M. savatieri* triggers the accumulation of mud between the thalli, leading to the accumulation of mud which further strengthens the trapping of suspected MPs. Therefore, it can be assumed that the higher *M. savatieri* contamination can trigger the higher MPs contamination of *K. cottonii*.

3.2 Correlation of Planting Age of *K. cottonii* with Microplastic Contamination

The number of microplastics contaminating seaweed shows an increasing trend along with the age of seaweed cultivation (weeks 1 to 5), with the highest value reached in the 4th week (n = 40) before decreasing slightly at the 5th week (Figure 9). In the seawater, the number of MPs also showed a similar pattern (Figure 10). MPs pollution in the aquatic environment greatly affects the contamination of marine organisms. Zhang et al. (2022) showed that in an aquatic ecosystem with lower levels of plastic pollution, the MPs contamination in seaweeds is also lower. The results of the Pearson correlation test which showed a value of 0.604 ($p < 0.05$) also indicated the presence of a positive strong relationship between the

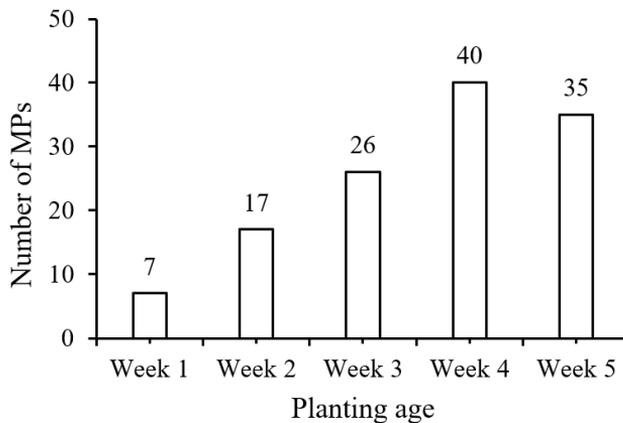


Figure 10. The number of MPs contaminating *K. cottonii* in a cropping cycle.

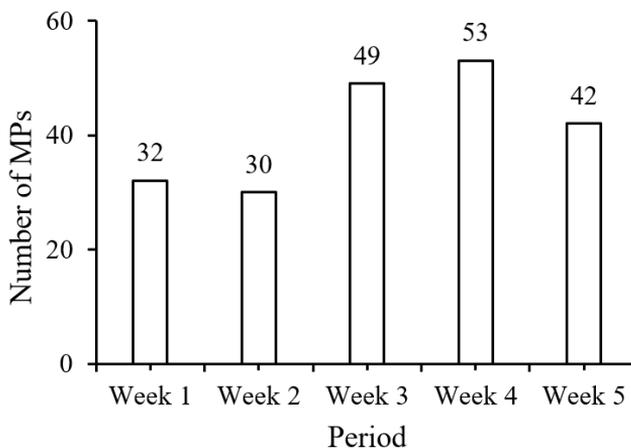


Figure 11. The number of MPs found in the seawater of Bomo Beach for 5 weeks.

planting age of *K. cottonii* and the number of MPs on the seaweed.

The presence of epiphyte *M. savatieri* affected the number of MPs trapped on the seaweed. The growth of the epiphytic *M. savatieri* naturally coincided with *K. cottonii*, using seaweed thallus as a host (Vairappan, 2006). Observations of 1-week-old *K. cottonii* thalli revealed a lower number of MPs, corresponding to the relatively low presence of attached epiphytes at that stage. After 2-4 weeks of planting, the number of MPs increased along with the increase in the rate of epiphytic infection of the adult thallus. Therefore, longer planting age is followed by higher number of *M. savatieri* growing on the surface of the thallus, thereby triggering the accumulation of MPs trapped by the epiphyte.

4. Conclusion

This research presents the results of an in-depth exploration of microplastic contamination in macroalgae *K. cottonii* at Bomo Beach, Banyuwangi. The findings indicate that microplastics contaminate *K. cottonii* due to the presence of *M. savatieri* epiphytes growing on its surface. These epiphytes facilitate the accumulation of mud and microplastics on their thalli, which subsequently attach to *K. cottonii*. Small microplastics (< 1.5 mm) in the form of fibers, and transparent in color dominated the contamination. Meanwhile, polypropylene is the most contaminating plastic polymers. Throughout the planting cycle of *K. cottonii*, the growth of *Melanothamnus savatieri* increases significantly, with densities reaching 40 - 48 epiphytes per centimeter on the thallus surface. Consequently, the accumulation of mud and microplastics trapped by *M. savatieri* also rises with the increasing age of the seaweed. These findings not only enhance our understanding of the mechanisms underlying microplastic contamination in *K. cottonii*, but they also provide a scientific foundation for formulating policies aimed at ensuring that Indonesia's leading export products remain free from microplastic contamination.

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