IRJIEST 10 (1) 2024 16-24
Available online at https://ojs.batstate-u.edu.ph
International Research Journal on Innovations in Engineering, Science and Technology

Microplastic contamination in seaweed along Verde Island Passage: Implications for marine biodiversity and ecosystem health

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ABSTRACT
Microplastics are one of the global threats to marine ecosystems. Numerous studies have confirmed their presence and their implications in aquatic organisms. However, studies on microplastic contamination in seaweeds are very scarce, especially in the Philippines. In this study, the researchers aim to determine the presence of microplastics in *Caulerpa chemnitzia* var. *turbinata* locally known as *"Lato"* or *"Ar-arusip"* in the country. The researchers collected 6 kg of wet-weight of *C. chemnitzia* var. *turbinata* on the west coast of Calatagan, Batangas, along the Verde Island Passage marine corridor through random sampling. A total of 113 pcs of microplastics were detected under a stereomicroscope and compound microscope. The researchers identified and classified microplastics according to their size, shape, and color. The most abundant type of microplastic is fiber (84.07%), while the least kind of microplastic is film (6.19%). These 16 pcs of microplastics are probably degraded from textiles, plastic containers, and plastic bags transported through air, wastewater, and water currents. Furthermore, the ceaseless use and disposal of microplastics result in lethal and devastating impacts on marine biodiversity. This study calls for effective policies and management to mitigate the impact of microplastics on the marine environment.

Keywords: contamination, macroalgae, marine ecosystem, microplastics, seaweeds

Received: July 26, 2024 **Revised:** September 16, 2024 **Accepted:** October 4, 2024

1. Introduction

The Philippines is the heart of the Coral Triangle, which is recognized as the global center for marine biodiversity [1]. In 2018, a total of 1.48 million MT, or 4.56% of the total world production of 32.39 million MT of aquatic plants (including seaweed), were produced in the Philippines, making it the fourth-largest producer in the world [2,3]. Conforming to [1], conservationists advocated for the country to focus on marine conservation efforts. Nevertheless, despite these efforts [4], as cited in [5], the country ranked third out of 192 countries in estimates of mismanaged plastic waste production in 2010, generating 0.28–0.75 million metric tons of marine plastic annually [6]. It concludes that plastic pollution remains persistent in the country's marine ecosystems [7].

Plastic production has increased rapidly and continuously since the 1950s, from nearly 60 million tons in 1980 to approximately 368 million tons in 2019 [8]. By 2030, it is estimated that 99 million tons of plastic waste will end up in the environment if improvements and measures of plastic waste management are not implemented [9]. as stated by [10] annual production is expected to reach 590 million metric tons by 2050. For this reason, our oceans are increasingly impacted by anthropogenic activities. Hence, people demand to be cognizant of the potentially harmful effects of plastics in the oceans [11].

The Philippines generates approximately 2.7 million tons of plastic waste annually, of which about 20% ends up in the ocean [12]. Microplastic contamination studies in the Philippines published in the last five years revealed the presence of microplastics in surface water, marine sediments, and marine biotas such as fish, mussels, and oysters [13].

Plastics with a diameter of less than 5 mm that are not visible to the naked eye are termed "microplastics" (MPs) [14] and are identified as a crucial component of the global environmental crisis [15]. MPs generated by anthropogenic activities are carried out via winds, currents, tides, and waves, causing them to travel long distances from their origin and end up entering beaches or coastal waters, which are now considered significant sinks MPs [16,17]. Estimates indicate that 80% of the plastics in marine water come from land-based sources, travel down rivers, and end up in the ocean [18].

Studies show that marine organisms can consume MPs, so there are concerns regarding the environment and human health. The edible seaweed nori (*Pyropia* spp.) from two local markets in China demonstrates the attachment of MPs and concludes that seaweeds can adsorb it [19]. MPs entering the food chain can be passed on to the higher trophic levels and humans, respectively [20].

MP contamination is becoming more challenging as plastics generated from anthropogenic activities are escalating. Even more, studies in the Philippines remain comparatively few [21]. In recent decades, plastic pollution has received the attention it needs [22].

This study aims to detect the presence of MPs in *Caulerpa chemnitzia* var. *turbinata*, which can be found on the west coast of Calatagan, Batangas, along the Verde Island Passage marine corridor. Furthermore, the result of the study

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will provide information about the existence of MPs in *C. chemnitzia* var. *turbinata* seaweed from the municipal water of Calatagan, Batangas, which will benefit the aquaculturists and fishermen in managing their livelihood through the awareness and management of plastic contamination in the marine location. Finally, it will provide data for future reference about MPs and seaweeds that may serve as a guide to discover new techniques for the convenience of seaweed cultivation and subsistence strategies.

2. Materials and methods

2.1. Research design

The study comprised a descriptive research design to utilize a qualitative and quantitative approach in investigating the MPs' occurrence in *Caulerpa chemnitzia* var. *turbinata*, found in the municipal water of Calatagan, Batangas. MPs were classified to investigate the quantity and detect its abundance in *C. chemnitzia* var. *turbinata*.

2.2. Research locale

The *C. chemnitzia* var. *turbinata* samples were collected in the west coastal area of Calatagan, Batangas (Figure 1), along the Verde Island Passage marine corridor, known as "the world's center of the center of marine biodiversity" [23]. Under the socio-economic profile of Calatagan [24], the Tagalog term "*latag*" is the origin of the word Calatagan, synonymous with "*kapatagan*," which denotes a large area of open plain lands where the shoreline is bounded by reef and comparatively shallow water in the presence of low fish corals. The municipality is located 125 km by road from Manila and 93 km from Batangas City.

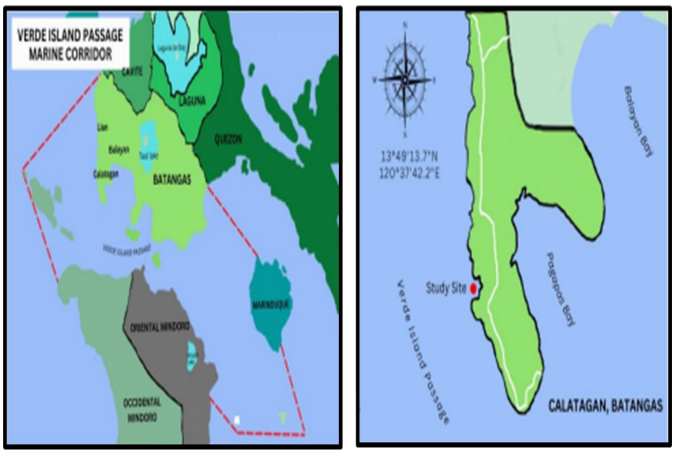

Figure 1. Map of Calatagan, Batangas, Philippines.

The study site as shown in Figure 1 is bounded north by the municipalities of Balayan and Lian, on the south by the Verde Island Passage, on the east by Balayan and Pagapas Bay, and the west by South China Sea, located at 13° 49'13.7" N latitude and 120°37'42.2" E longitude [24]. The municipal water of Calatagan has a total surface area of approximately 112 km^2, an average depth of about 3 m, and an elevation of 6 m to 347 m. Conversely, the pH level has an average of 9.07, and the temperature has an average of 28℃ [25]. *C. chemnitzia* var. *turbinata* was collected at a distance of 1.7 km from the shoreline to offshore with a depth of approximately 3-4 ft during low tide and 7 ft during high tide.

2.3. Population sampling

A simple random sampling was used to collect *C. chemnitzia* var. *turbinata* in their natural habitat. The researchers collected a 6 kg wet-weight seaweed sample [26] from *C. chemnitizia* var. *turbinata*. After receiving approvals from the municipality of Calatagan, Batangas, the sample collection was carried out with the assistance of three local fishermen.

The collected samples, having the same weight of 200 g wet-weight, were stored in aluminum foil bags, which acquired 30 samples, and placed into an ice cooler box at room temperature for processing in the laboratory.

2.4. Research instruments

The researchers prepared the materials such as aluminum foil bags, ice cooler boxes, nylon filter disks (5.0 µm, diameter 47 mm), and deionized water. Fenton's reagent was used for oxidative digestion, a simple, low-cost method, and widely used in isolating MPs from organic matter [27]. Compound microscopes (40–100x) and stereomicroscopes (10x) were used to observe and collect the potential MPs. The suspected MPs were isolated using stainless steel dissecting needles and a micro-scoop spatula [28,29]. A calibrated compound microscope (100x) was used to measure and identify the length of MPs, as well as their shape and color. Moreover, android phones were used to photograph the MPs.

Personal protective equipment (PPEs) like face masks, disposable nitrile gloves, hairnets, safety goggles, and laboratory gowns were worn by researchers to avoid any possible contamination and to protect the researchers from chemicals. Laboratory instruments such as analytical balance, petri dishes, weighing scale, graduated cylinder, and 1000 mL, 500 mL, and 250 mL beakers were used during the experiment.

2.5. Sample processing

2.5.1. Sample collection

The *C. chemnitzia* var. *turbinata* samples were collected on the west coast of Calatagan, Batangas, along the Verde Island Passage marine corridor. The researchers asked for the assistance of the local fishermen in the collection of fresh seaweed of no more than 6 kg of wet weight [26]. The researchers collected *C. chemnitzia* var. *turbinata* on October 27, 2022, and December 8, 2022.

The entire plants of *C. chemnitzia* var. *turbinata*, except the rhizoids, were collected and quickly placed in aluminum foil bags to minimize plastic contamination [30,31]. Each aluminum foil bag contained 200 g of thalli for each sampling. which amounted to 30 samples. The samples were then kept in a cooler for laboratory treatment [31].

2.5.2. Species identification

The researchers submitted a biological specimen identification service request form to the University of the Philippines Los Baños Museum of Natural History, and sent the samples for species identification. The results of the species verification were received later, and the researchers performed laboratory experiments immediately after the specimen was verified (Figure 2).

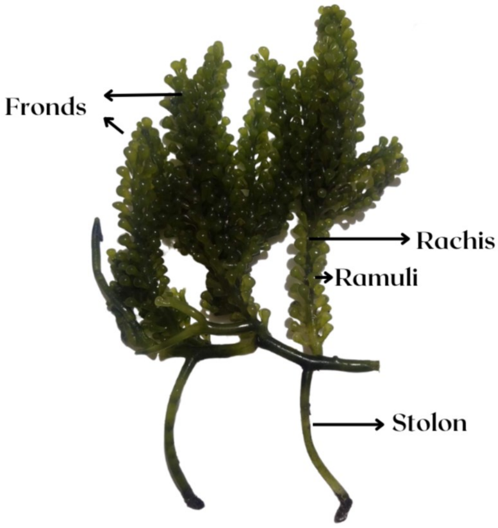

Figure 2. The anatomical structure of *C. chemnitzia var. turbinata* found in Calatagan, Batangas, Philippines.

2.5.3. Sample preparation

The *C. chemnitzia* var. *turbinata* were rinsed using deionized water. The rinse water for each sample was collected and filtered on a nylon filter disc (5.0 µm, diameter 47 mm). The filtered samples were observed under the compound microscope (40–100x) and stereomicroscope (10x) to see if MPs were released from the samples when rinsed, as every MP that was found should be considered. The surface water of the samples was removed using an absorbent tissue. The samples were weighed afterward, stored in aluminum foil bags, and put into an ice cooler box at room temperature as storage for the preparation of oxidative digestion [29,31].

2.5.4. MP isolation

Oxidative digestion protocol was utilized based on combined recommendations by [29,31,27]. The researchers followed the oxidative digestion protocol to extract the MPs from organic contents. Precautionary measures were taken to reduce contamination in the research setting such as reducing the number of people assisting with the experiment, rinsing the laboratory equipment with deionized water 6-8 times, scrubbing the hands and forearms of the researchers three times before the process, and requiring to wear white laboratory gowns, disposable nitrile gloves, and masks throughout the process.

The natural organic contents of the seaweed were degraded using the process of oxidative digestion to isolate MPs from the *C. chemnitzia* var. *turbinata* samples. Each sample was placed into a 500 mL or 1000 mL beaker and digested by 100-120 mL Fenton's reagent consisting of 30% (v/v) hydrogen peroxide (H_2O_2) and iron catalyst solution, ferrous sulfate (20 g of $FeSO_4$-$7H_2O$ in 1 L of filtered RO water). To enhance the dissolution of the ferrous sulfate granules and optimize the degradation of the seaweed, the iron catalyst solution was adjusted to pH 3.0 using concentrated sulfuric acid. Oxidative digestion of the samples was performed in an improvised oscillation incubator and then set at 40 °C for 24 h. Procedural blanks for each sample were included to observe the extent of MP contamination in the laboratory. Each mixture from oxidative digestion was filtered on a nylon filter disc (5.0 µm, diameter 47 mm) using an improvised vacuum filtration setup, and the collected MPs were transferred to a clean petri dish with lids.

2.6. Data collection

Examination of the samples under the microscope was performed at the laboratory of the College of Arts and Sciences at Our Lady of Fatima University, Quezon City Campus. The researchers identified and isolated the suspected MPs from *C. chemnitzia* var. *turbinata* using a stereomicroscope (10x) and compound microscope (40–100x). The physical characteristics, such as size, color, and shape of the extracted MPs, were examined under the calibrated compound microscope (100x) [28]. For proper examination and accurate measurements, the researchers carefully followed the standard procedure by calibrating the microscope and the microscope stage micrometer with the assistance of a laboratory technician.

In this study, MPs were identified with a size <5mm and were classified into five shapes or morphologies consisting of beads, fragments, fibers, films, and foam [32]. The color classification of plastics by [33] was employed as [34] recommended. The criteria for the classification and quantification of MPs by [35,33,32] were used. Finally, photographs of the MPs observed under the compound microscope were taken using android phones.

2.7. Data analysis

In describing the fundamental characteristics of the MP data, a descriptive design and analysis were used. The size (length), type (based on shape), and color classification and quantification for MPs were as described by [33,32,35]. Microsoft Excel was used for the computation of the abundance of MPs.

3. Results and discussion

3.1. Classification and quantification of MPs

Classification and quantification of MPs revealed differences in terms of color, type, shape, and size. Its presence has not been observed in 30 procedural blanks, which denotes that the quality control is valid. Figure 3 shows that 16 out of 30 rinse waters from fresh seaweed samples were contaminated with MPs. A total of 45 MPs were subsequently examined from the samples. MPs found in rinse water suggest that some adhered to seaweed were released during the rinsing process, thus indicating the presence of MPs in the seaweed. Furthermore, MPs were not detected in 14 rinse water including sample numbers 1, 2, 3, 4, 5, 6, 7, 9, 10, 13, 17, 19, 22, and 29.

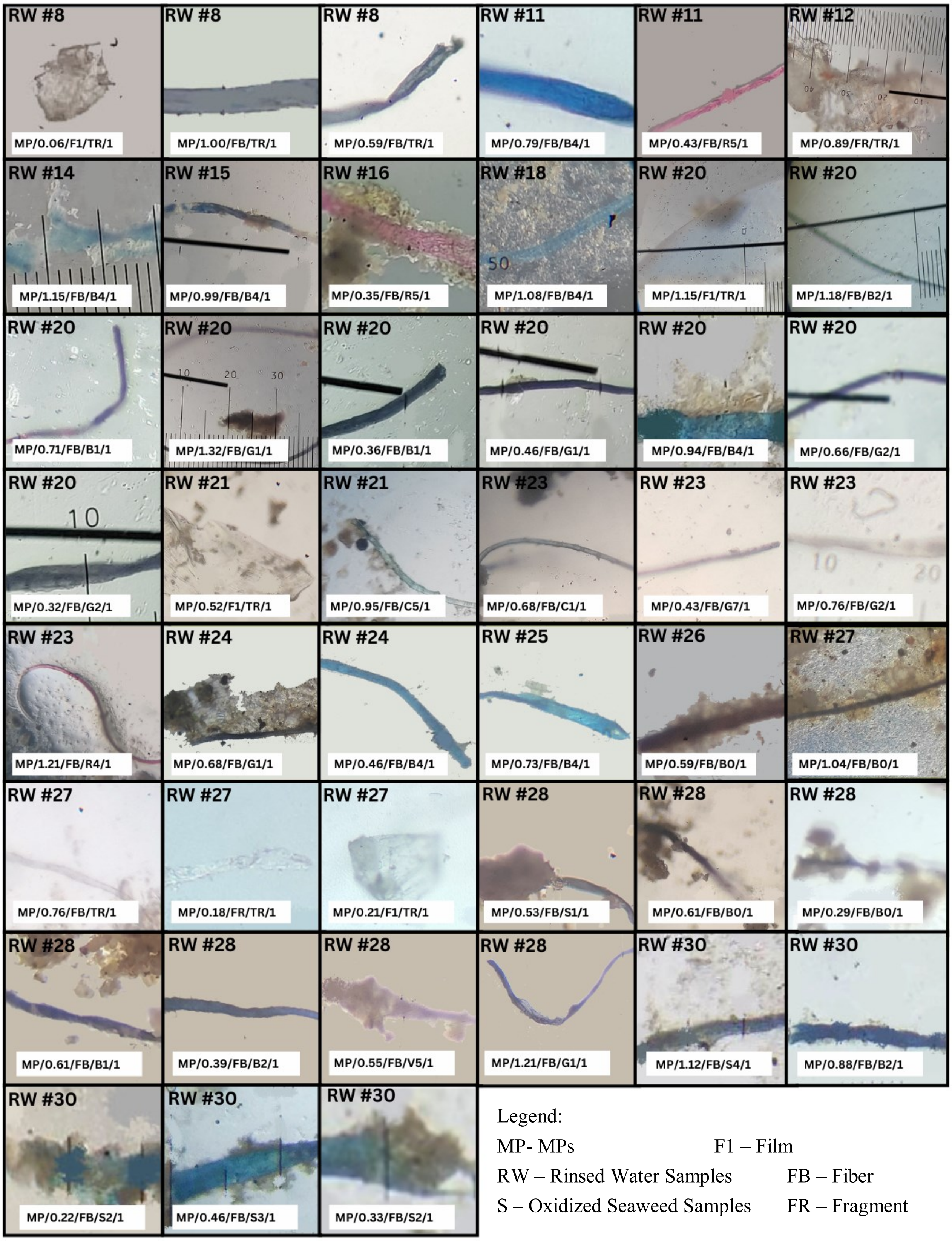

Figure 3. Classification and quantification of MPs obtained from rinse water.

MPs with different colors (transparent, sky, blue, magenta, violet, gray, turquoise, cyan, orange, and black), types based on shape (fiber, film, fragment), and sizes in length have been assessed (Figure 4). These categories were considered for quantifying the MPs collected per rinse water. The differences between MPs in these categories yielded different codes accordingly. Only three types of MPs were obtained from the rinse water, which are mostly fiber (FB), fragment (FR), and film (Fl). Other common types of MPs, such as beads and foam, were not found. Finally, the majority of MPs obtained from rinse water are blue, and sizes range from 0.06 mm to 1.32 mm.

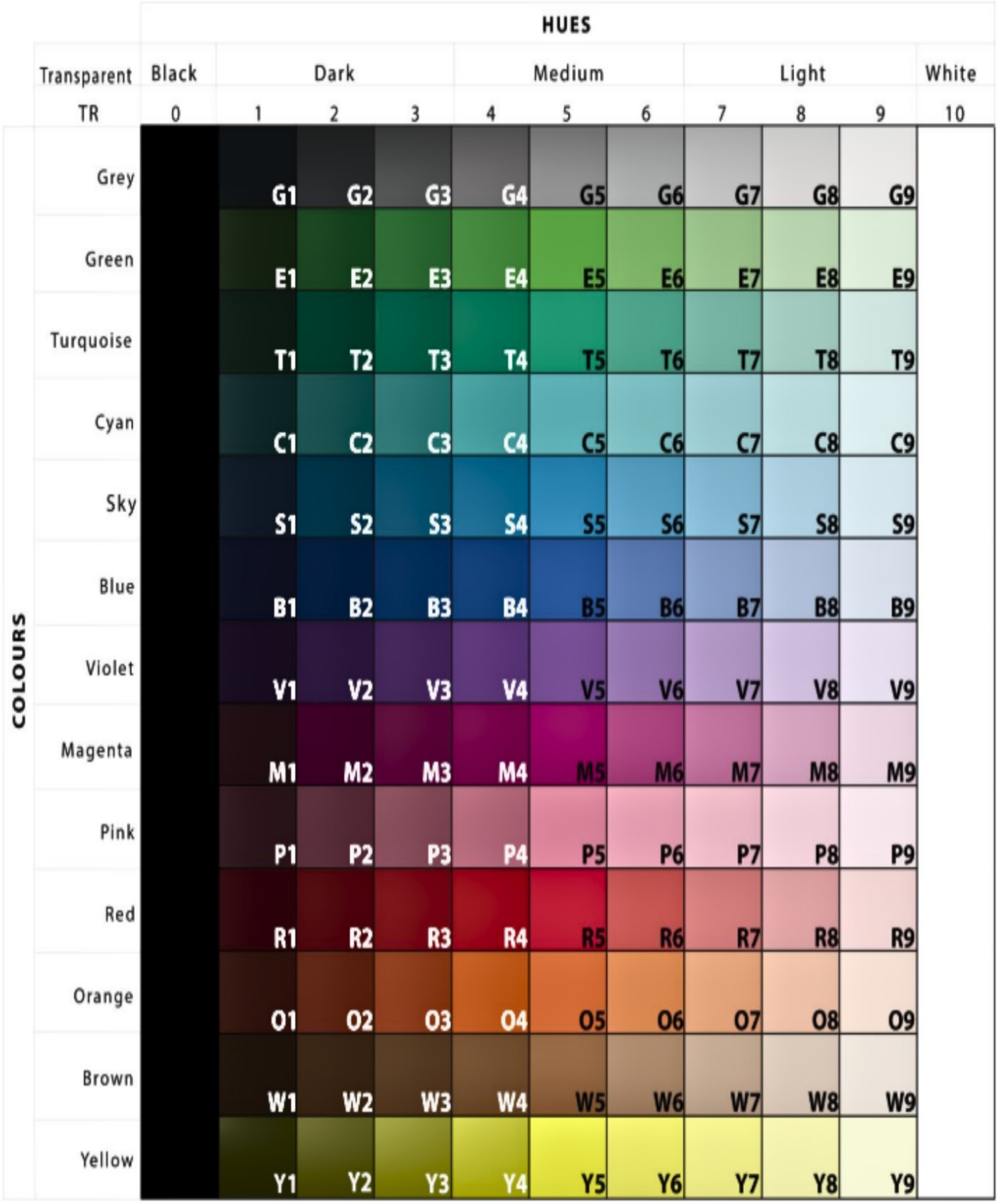

Figure 4. Color palette used for the characterization of MPs.

Out of the 30 oxidized seaweed samples, 23 have been observed to be contaminated with MPs (Figure 5). A total of 68 pcs MPs were detected from 23 oxidized seaweed samples. MPs were not detected in the other 7 oxidized seaweed samples, including sample numbers 6, 8, 10, 13, 27, 28, and 30. Different colors (transparent, sky, blue, magenta, violet, gray, cyan, black, brown, pink), types based on shape (fiber, film, fragment), and sizes in length were observed, and these categories were considered for the quantification of potential MPs. Different codes were made due to the differences in categories between the MPs per sample.

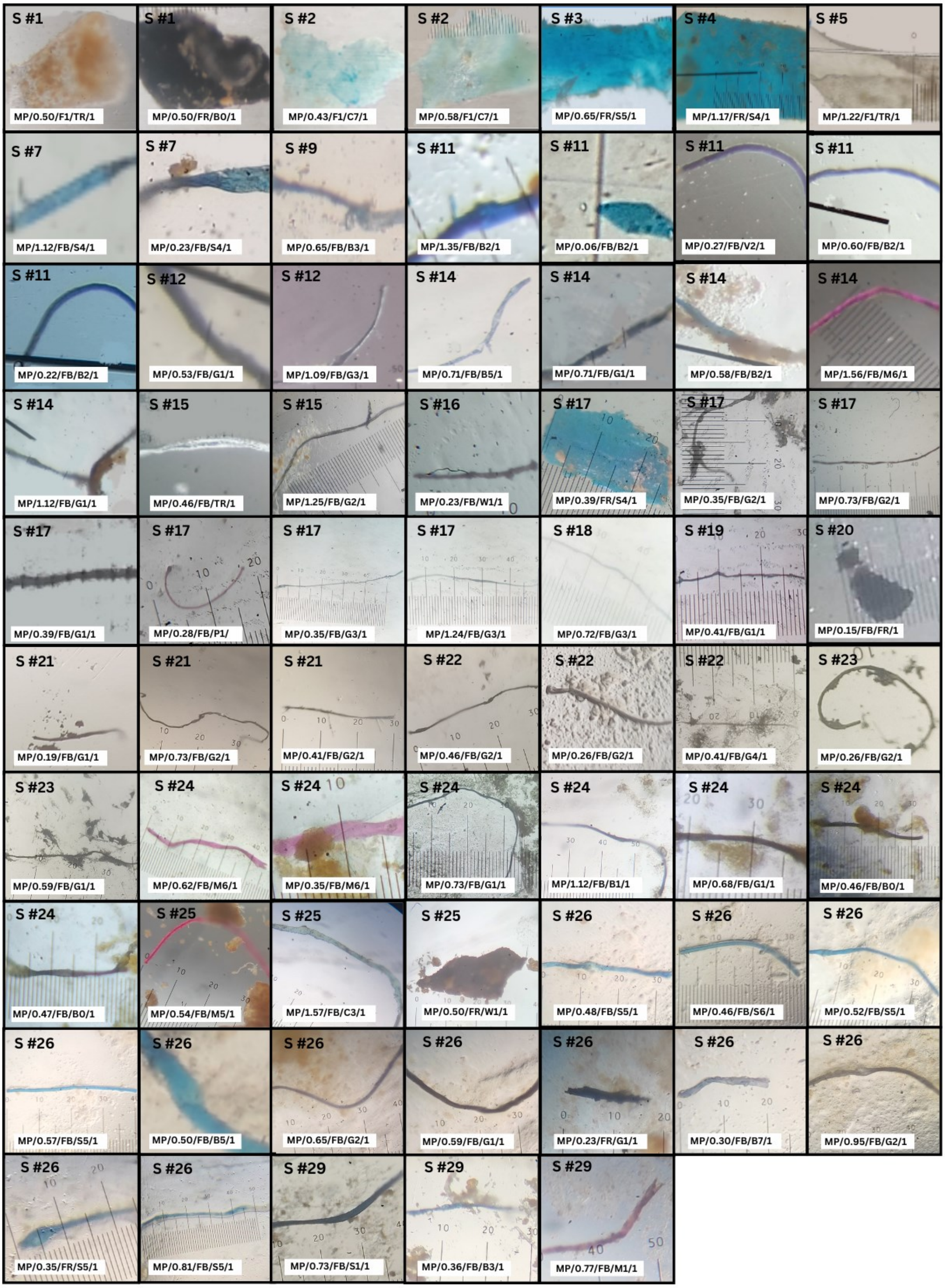

Figure 5. Classification and quantification of MPs obtained from the oxidized seaweed samples.

Furthermore, out of the five most common types of MPs, only three types were obtained from the oxidized seaweed samples, which are mostly fiber (FB), followed by fragment (FR), and film (Fl). Other types of MPs, such as beads and foam, were not found. Sizes of MPs obtained from the samples range from 0.06 mm to 1.57 mm. The majority of the MPs discovered in oxidized seaweed samples are gray. Finally, observations that MPs are possibly from the breakdown of larger plastic items suggest that the MPs discovered are secondary MPs.

3.2. The abundance of MP types

MPs discovered in the rinse water of 16 fresh seaweed samples comprised 3 films (6.67%), 1 fragment (2.22%), and 41 fibers (91.11%) (Table 1).

Table 1. The abundance of MP types obtained from rinse water

Type of MPs	Quantity/No. of MPs	Percentage
Film	3	6.67%
Fragment	1	2.22%
Fiber	41	91.11%
Total of MPs	45	100%

MPs collected from oxidized seaweed samples comprised 4 films (5.88%), 10 fragments (14.71%), and 54 fibers (79.41%) (Table 2).

Table 2. The abundance of MP types obtained from oxidized seaweed samples

Type of MPs	Quantity/No. of MPs	Percentage
Film	4	5.88%
Fragment	10	14.71%
Fiber	54	79.41%
Total of MPs	68	100%

Table 3 shows the total abundance of MPs obtained from the seaweed samples. A total of 113 MPs were obtained from 30 samples of *C. chemnitzia* var. *turbinata,* with a total of 68 MPs found on oxidized seaweed and a total of 45 MPs found in the rinse water. Overall, MPs discovered from the targeted species samples include 95 fibers (84.07%), making it the most abundant MPs type, 11 fragments (9.73%) the second most abundant MPs 253 type, and 7 films (6.19%), the least abundant MPs type.

Table 3. The abundance of MP types in seaweeds (rinse water + oxidized seaweed samples)

Type of MPs	Quantity/No. of MPs	Percentage
Film	7	6.19%
Fragment	11	9.73%
Fiber	95	84.07%
Total of MPs	113	100%

Based on the observations in the sampling site and interviews with the local fishermen, fishnets may be the primary source of MPs, followed by plastic ropes, and fishing gear, since these were used in their daily lives. Textiles and other types of plastics, such as plastic bottles, plastic bags, and plastic containers, may also be the source of MPs in the location since it is also a tourist spot.

Accordingly, plastic litter is distributed, from its source to its end location, which is a crucial aspect of MP studies. Several studies have shown that anthropogenic activity is primarily responsible for the presence of MPs in various bodies of water. Drainage systems and wastewater could be the potential sources of these MPs. Consequently, coastal waters and estuaries are prone to MP contamination. Fibers are derived from textiles, ropes, and fishing nets [36]. Approximately 60% of the material that makes up clothes is in various forms of plastic, such as polyester, nylon, acrylic, and other synthetic fibers. [35] pointed out that clothes washed in washing machines could release thousands of fibers, and thereby be discharged by wastewater treatment plants. Meanwhile, the degradation of ropes and nets used in maritime or fishing activities could release numerous MPs. In addition, fragments and films are probably from degraded disposable plastic containers, plastic bags, plastic bottles, jars, gallon flakes, and fast food packaging. However, more research on MPs' source is needed to specifically identify their origin or history of degradation in the ocean [37].

The presence of MPs in oxidized samples indicates that MPs are retained on the *C. chemnitzia* var. *turbinata* surface after fresh samples are rinsed with deionized water, denoting that adsorption of MPs in our samples has occurred. Adsorption of MPs has been demonstrated on several seaweed specie,s namely, *U. prolifera* [38,31,39,40], *Cladophora* sp. [29,39], *F. vesiculosus* [41,30], *S. horneri*, *U. pinnatifida*, *U. pertusa* [39], *P. yezoensis* ([40,39], *Padina* sp., *S. ilicifolium* [42], *C. prolifera* [43], *G. lameniformis*, *C. ocellatus*, *U. lactuca*, and *S. japonica* [40]. The proposed pathways by which MPs are adsorbed to seaweeds include wrapping, embedment, attachment, entanglement, and entrapment by epibionts [40] as cited in [44]. Moreover, several components that aid in MP adsorption include polysaccharides of the cell wall [41] and carbohydrates in macroalgae such as pectin, cellulose, rhamnose, and xylose because of its high charge density, molecular size, and amphiphilic character [45] as cited in [29].

4. Conclusions

This study confirmed the presence of MPs in *C. chemnitzia* var. *turbinata* found on the west coast of Calatagan, Batangas, along the Verde Island Passage Marine Corridor. Types of MPs were determined based on shapes, colors, and sizes. This study revealed that fibers are the most abundant type, which suggests that it is probably from the degradation of plastic-made materials such as fishnets, plastic ropes, fishing gear, textiles, etc. Numerous MPs were released during rinsing, thus the presence of MPs was detected in the rinse water. The presence of MPs detected in both rinse water and *C. chemnitzia* var. *turbinata* samples proved the adherence of MPs to seaweed species.

Although numerous studies have demonstrated and validated the existence of MPs, more research is still needed to understand exactly how MPs affect the ecosystem and every organism with which they come into contact, since its contamination is ubiquitous.

Acknowledgment

The researchers would like to acknowledge and credit to Research Development and Innovation Center of Our Lady of Fatima University (RDIC) for guiding the researchers; To Municipal Environment and Natural Resources Office of

Calatagan (MENRO) for helping the researchers to collect their samples; To Bureau of Fisheries and Aquatic Resources (BFAR) - Provincial Fisheries Office (PFO-Batangas) for allowing the researchers to collect the samples; and to all the people who sacrificed their time and effort for the researchers to finish their research paper.

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