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Research Article

Microplastics contamination on seaweed surface at rocky shore habitat in Port Dickson beaches

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Abstract
Microplastics adhering to seaweed likely originate from the surrounding water, where they are dispersed and carried by currents. These microplastics can accumulate on the surface of seaweed over time, posing potential risks to human health through consumption. This study provides a preliminary result of microplastic presence on the surfaces of *Padina jamaicensis* (Scroll algae) and *Caulerpa lentillifera* (Seagrape) in Port Dickson beaches, in Malaysia. These species are commonly found in coastal and shallow marine environments, where microplastic pollution is often concentrated. Their distinct morphological, ecological, and physiological characteristics make them ideal for investigations into the mechanism of adherence of microplastics on the surface. The samples were collected from two locations, namely, Pantai Sri Purnama and Pantai Tanjung Biru. The samples were rinsed to collect adhered microplastics on the upper surfaces of the seaweed, and filtered with 1.2 µm glass microfibre membrane filter. Microplastics were observed under a stereomicroscope, categorized based on colour and shapes, and their polymeric composition was determined through Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy. Higher concentrations of microplastics were detected in *C. lentillifera* (4.183 items/g) as compared to *P. jamaicensis* (2.103 items/g). Fibre shapes of various colours were isolated from the samples. The analysis also identified compositions predominantly consisting of polyvinyl alcohol (PVA) and polyamide (PA) within the plastic groups. The presence of these polymers may suggest contamination from secondary plastic sources, potentially originating from water activity items such as life jackets, swimming attire, and food packaging materials, which might adhere to the surface of seaweed. This study highlights how anthropogenic activities introduce microplastics into surrounding waters, enhance their accumulation on seaweed surfaces, and pose potential health risks to consumers.

Keywords: Marine pollution, macrophytes, polymer, microplastics, coastal environment

Introduction

Plastics are readily transported over long distances from source areas and accumulate in the oceans, where they have a variety of significant environmental and economic impacts [1]. These plastics get degraded into smaller plastic particles with sizes of less than 5 mm, known as microplastics [2]. Over the last decade, microplastics have been considered as a major ocean pollutant due to their ubiquitous nature and an increasing awareness of the potential problems associated with their presence in the environment [3]. The ingestion of microplastics has been reported to have a great variety of marine organisms, including vertebrates and invertebrates. Fish, seabirds, and marine mammals often consume microplastics, mistaking them for food, which can lead to physical harm and internal blockages. Invertebrates, such as mollusks, crustaceans, and plankton, also ingest microplastics, potentially affecting their feeding, growth, and reproduction [4].

The impact of microplastics on marine organisms is dependent on a parameter that determines the position of microplastics in the water column.

Typically, high-density particles will sink and accumulate in the sediment, while low-density particles float on the sea surface, although biofouling, turbulence, and freshwater input may result in vertical mixing [5]. Additionally, results from laboratory experiments and field observations provide strong evidence of the transfer of microplastics between species from different trophic levels of the marine food web [6]. The small size of microplastics allows them to interact with marine biota across various trophic levels and habitats. Consequently, a wide range of marine organisms are vulnerable to exposure [7]. Consumption of microplastics at the millimetre and micrometre scale has become higher, and has become dominated by bulk portioning, with effects including blockages when fibres or fragments form aggregates. Besides that, at smaller size ranges, specifically at the nanometre scale, there is a potential for microplastics to cause harm to organisms [8].

Seaweeds are macroscopic marine algae that grow in shallow coastal areas. Most macroscopic marine algae or seaweeds are distinctly divided into three divisions: Chlorophyta, Phaeophyta, and Rhodophyta [9, 10]. Most of the seaweeds grow in the shallow intertidal zone throughout the world, but they can extend into the deeper subtidal zone as well [11]. Seaweeds occurring in the intertidal zone are submersed and immersed periodically or aperiodically due to tides or irregularity occurring factors [12]. The occurrence of a seaweed community in a particular intertidal shore is influenced by a wide range of environmental factors, such as nutrients, temperature, and salinity [13]. Their ability to accumulate microplastics on their surfaces makes them ideal bioindicators for accessing the levels of microplastic pollution in the marine environment. Seaweed serves as primary producers in the food chain for fish, invertebrates, and turtles, which may cause unintentional ingestion of microplastics by the organisms. Moreover, the adherence of microplastics on the surfaces of seaweed could interfere with physiological processes such as photosynthesis, nutrient uptake, and growth [14].

On rocky shores, seaweed provides habitat, food, and shelter for a diverse community of associated organisms [15]. However, the relationship of microplastics with benthic seaweeds has still not been studied. Rocky shores have been suggested to enhance the formation of microplastics from larger plastic items that are smashed against rocks and broken into small particles in the surf zone [16]. Readily polluted coastal areas may also influence the presence of microplastics in the marine organisms. So far, there have been no studies on the distribution of microplastics in Port Dickson, or on seaweeds at rocky shore habitats. This study would be a milestone for microplastics related surveys or research done at local sites of Malaysia in future. The aims of this study were to determine the presence of microplastics that adhere to the surface of seaweeds at rocky shore habitats, and to characterize the microplastics according to their shape, colour, and polymer types.

Materials and Methods

Study area

Pantai Sri Purnama, located at approximately 2.4440° N, 101.8560° E, is a rocky shore area extending up to 50 m seaward during low tide, with a gradual slope with live and dead corals interspersed with sand [17]. It is one of the largest and most popular beaches in Port Dickson, Negeri Sembilan. Consequently, this beach stretch has the greatest number of hotels, resorts, and apartments providing family accommodation. The beach gets unbelievably crowded during holidays with visitors, while the sea becomes frenzied from motorised water sports. Additionally, Pantai Tanjung Biru is located 17 kilometres from the Port Dickson town (latitude 2.4151° N, longitude 101.8560° E). This beach is also known as blue lagoon due to its geography and green scenery. Pantai Tanjung Biru is a well-managed coastal area, making it a priority for local for swimming and diving. These two beaches were selected for this study (**Figure 1**).

Sample collection and identification

Sampling was conducted in 2018. Two species of marine macrophytes (**Figure 2**), *Padina jamaicensis* and *Caulerpa lentillifera* were collected at two different beaches in Port Dickson, Negeri Sembilan, Malaysia. *Padina jamaicensis* was collected in Pantai Teluk Kemang; meanwhile, *C. lentillifera* was collected in Pantai Tanjung Biru. The seaweed species were selected due to their high abundance and widespread distribution at the sampling sites, reflecting their ecological significance and frequent exposure to surrounding water conditions. This allowed a representative comparison of microplastic levels across different pollution sources. Samples were taken during low tide by cutting the seaweed at the base of the blades and storing in a glass jar to prevent cross contamination. The identification of seaweed species was confirmed by referring to standard references of Trono [18, 19]. The morphological characteristics of the seaweeds were observed under a dissecting microscope and recorded. The weight of the samples was measured using beam balance and recorded.

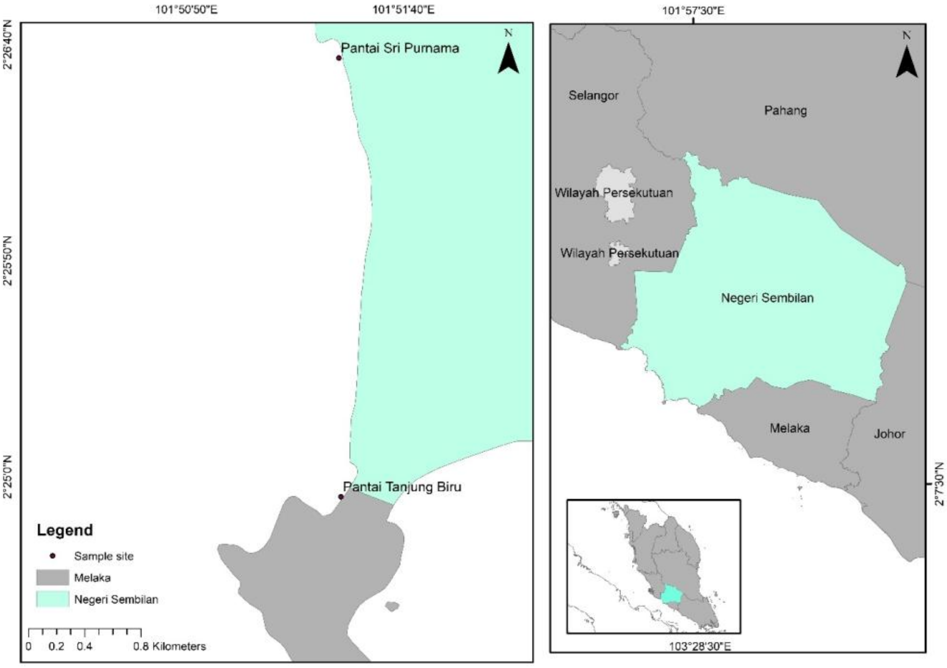

Figure 1. Two coastal areas of Pantai Sri Purnama and Pantai Tanjung Biru at Port Dickson, Negeri Sembilan, Malaysia

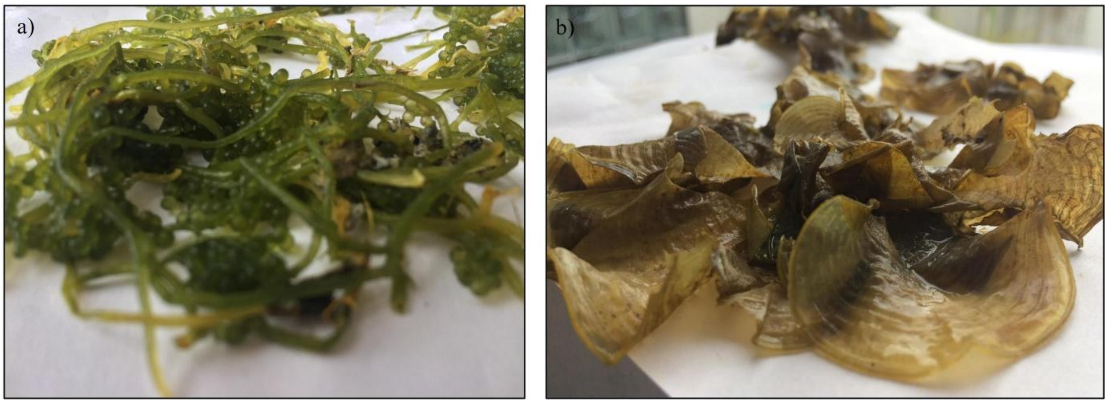

Figure 2. (a) *Caulerpa lentillifera* and (b) *Padina jamaicensis*

Microplastic quantification and identification
In the laboratory, the samples were rinsed with filtered deionized water three times in a huge glass jar, to collect microplastics adhering on the surface of the seaweeds [20]. The water samples were then passed through 1.2 µm glass microfibre membranes. The membranes were left to dry in a glass desiccator overnight before further characterization processes. Identification of microplastics was conducted using a stereo microscope (Olympus SZX7, Japan) at a magnification between 0.8x to 5.3x. Sorted microplastics were categorized by colour and shape, before being kept in glass vials. Microplastic concentrations in *P. jamaicensis* and *C. lentillifera* were measured as items per gram (items/g).

A hot needle test was done to differentiate microplastic particles from organic matter by heating a needle and placing it near the suspected microplastics particles. A reaction of curling or melting indicates the presence of microplastics, otherwise, the material is organic matter [21]. Representative microplastics were selected from the samples for analysis using attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy in the mid-IR range of 4000 cm^{-1} to 400 cm^{-1}. The spectra were compared to a polymer library (HR Aldrich Polymers) and matches of ≥ 70% were deemed acceptable.

Quality assurance and control
Strict measures were applied throughout the handling

of samples in the laboratory and in situ. Deionized water was filtered beforehand with 1.2 µm glass microfibre membranes (Whatman GF/C 1.2 µm). All glassware was pre-cleaned with filtered deionized water and dried at 90°C in the oven. Any openings of the equipment were covered with aluminium foil to prevent contamination. Additionally, blank membranes were placed nearby each process to control possible influence of contamination originating from the surrounding atmosphere. Nitrile gloves and cotton lab coat were worn throughout the process. The surface area of conducting experiments was sterilized with 80% ethanol. Moreover, standardized sampling and analysis protocols were strictly employed to ensure valid comparisons between two sites and to minimize bias. This included uniform methodology of samples collection, microplastic extraction, and contamination prevention.

Results and Discussion

Being the first evidence of microplastics adhering to macroalgae in Malaysia, the findings have shown that both species of seaweed, *P. jamaicensis* and *C. lentillifera*, tested positive for microplastics. A total concentration of 6.286 items/g of microplastics was detected across the two species of seaweed (**Figure 3**), with a total weight of 282.4 g of *P. jamaicensis*, and 40.4 g of *C. lentillifera*. Double concentrations of microplastics were observed adhering to the surface of *C. lentillifera* (4.183 items/g), compared to the blades of *P. jamaicensis* (2.103 items/g). The levels of microplastics retained on the seaweeds observed in this study were lower than those observed in *Ulva prolifera* (4.6 items/g) [22] and *Echinochloa muricata* (8.6 items/g) [23], but higher than *Pyropia yezoensis* (0.17 particles/g) [24] and *Gracilaria lemaneiformis* (0.8 items/g) [25].

The morphology of seaweed plays a big role in retaining microplastics on their surface, as seen in filamentous and non-filamentous macroalgae [26]. *Caulerpa* sp. and *Padina* sp., being non-filamentous macroalgae, exhibit relatively lower microplastics on their surface compared to levels observed in the samples collected from other regions. *Caulerpa lentillifera* collected from Semak Daun Island, Indonesia, contained an average of 4.9 particles/g [27]; meanwhile, in Samut Sakhon, Thailand, the same species had a microplastic concentration ranging from 2.57 and 9.55 particles/g [28].

Caulerpa lentillifera is commonly known as “latok” in local communities of Southeast Asia [29]. It is widely commercialized and consumed as a nutritional snack, particularly in countries such as Malaysia, Indonesia, and Thailand. A study calculated that an average person in Korea consumes approximately 24.5 particles of microplastics per week through the consumption of seaweed, higher than other commonly consumed food products such as salt, soy sauce, and fish sauce [30]. The presence of microplastics on seaweed may increase the risk of human exposure to these harmful contaminants, as microplastics could be a potential vector of pollutants and pathogens [31, 32].

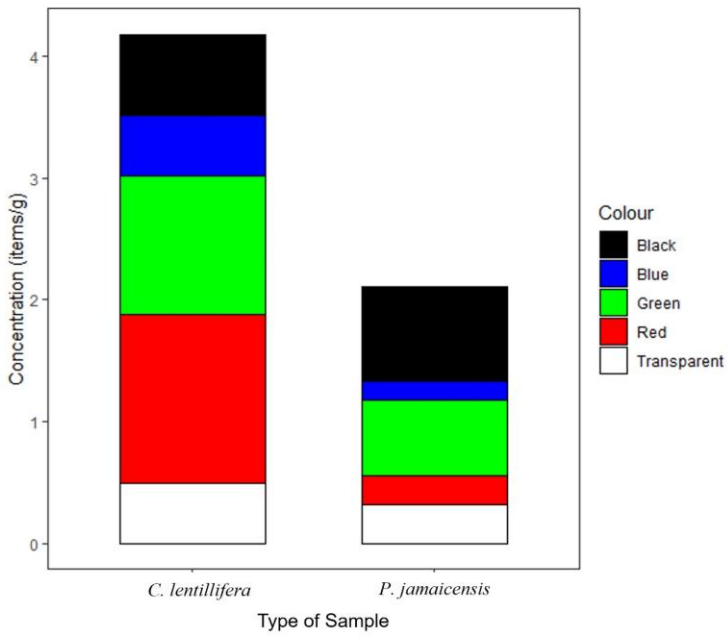

Figure 3. Concentration of microplastics retain on the surface of *C. lentillifera* and *P. jamaicensis*

The shape of the microplastics significantly influences their fate in vegetated wetlands, particularly fibres adhering to plants [33]. Elongated and small sizes of fibres make it easy to retain them on the surface, regardless of the algal morphologies [26]. The lightweight of fibres enables them to be easily transported by currents, increasing their likelihood of interacting with various marine substrates, including seaweed [34]. Furthermore, seaweed has a sticky extracellular polymeric substance (EPS) layer on the surface, which may promote the attachment of fibres, becoming embedded within the biofilm matrix [35, 36]. Fibre with different colours was the only shape found in this study (**Figure 4**), aligning with a recent study which identified 76% of the isolated microplastics as fibrous [37]. Similar shapes of microplastics were also observed in Singapore seagrass and macroalgae, where most of the particles found were fibre [38]. This is also consistent with 81% of fibre on the *Thalassia testudinum* blades, as studied by Goss et al [39]. These fibres are likely to originate from synthetic polymers used in textile, fishing, and tourism activities, where they are shed upon exposure to abrasion or friction forces [40, 41]. These are further supported by on-site tourism activities in the area, where they offer motorized watersport and other recreational activities to the public.

Several studies have investigated the mechanisms by which microplastics adhere to the surface of seaweed, highlighting the role of physical entanglement, biological adhesion, and interstitial space retention. Microplastics are prone to accumulating electric charges in the environment, leading to electrostatic interactions that enhance their physical entanglement and attachment to biological surfaces of seaweed [42]. This mechanism is particularly significant in cellulose-rich macroalgae such as *C. lentilifera*, which have a highly branched structure that may further trap particles. Previous research has also shown that microplastics can bind to plants and algal surfaces via hydrophobic interactions and electrostatic forces [43]. Moreover, the presence of biofilms and exopolysaccharides on algal surfaces can further facilitate deposition of microplastics on seaweed surfaces, potentially making seaweed an important vector for pollutant transfer within marine ecosystems [44].

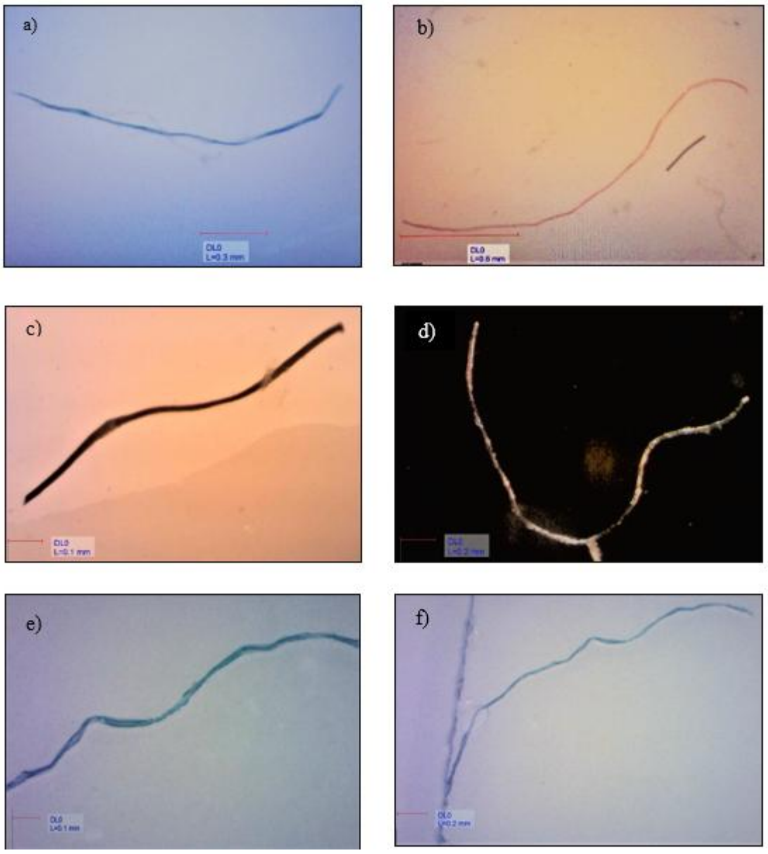

Figure 4. Five different colours of fibres were isolated in this study (a) blue, (b) red, (c) black, (d) transparent, (e) and (f) green

Polymeric characterization has detected the presence of polyamide (PA) and polyvinyl chloride (PVC) (**Figure 5**). Polyamide polymers, also known as nylon, exhibit characteristic FTIR absorption, particularly for the amide group (-CONH-), where it shows a strong peak at ν=3362 cm^{-1} due to N-H stretching, and a sharp peak at ν=1630 cm^{-1} for C=O stretching (amide I) (**Figure 5a**). The band at ν=1551 cm^{-1} corresponds to secondary amide N-H bending vibrations. The secondary amide bending of the N-H group and the stretching of C-H (ν=3050 – 2850 cm^{-1}) are less intense, while the C-N amide linkages (ν=1099 – 1023 cm^{-1}) appeared at their respective peaks, confirming the presence of polyamide polymers. The polyamide characteristics observed in this study agreed with the previous works reported by Annisa et al. [45], which describe the composition of nylon (polyamide). Nylon polymers are normally lightweight and exhibit high strength properties, primarily used in significant commercial applications in clothing textiles and fibers [46].

Additionally, the FTIR spectra of PVA in this study exhibited all major peaks corresponding to hydroxyl and acetate groups (**Figure 5b**), which were consistent with the pure PVA spectra documented by Nafisyah et al. [47]. The broad and overlapping bands observed at ν= 3338 – 3300 cm^{-1} are attributed to the stretching of O–H from intermolecular and intramolecular hydrogen bonds, suggesting the presence of strong hydrophilic forces among the PVA polymeric chains [48]. The sharp vibrational band at ν=2924 – 2858 cm^{-1} corresponds to the stretching of C–H from alkyl groups. The carbonyl (C=O) groups, which can be randomly distributed along the alkyl chain, are indicated by the band at around ν=1714 cm^{-1}, and are due to the stretching of C=O from the residual acetate groups in PVA [49]. It thus indicates the amorphous and crystalline nature of PVA. PVA is commonly used in textiles and packaging films due to its high tensile strength [50]. It is also frequently utilized in detergents, pharmaceutical coatings, and water-soluble film packaging, making it prevalent in water environments [51, 52].

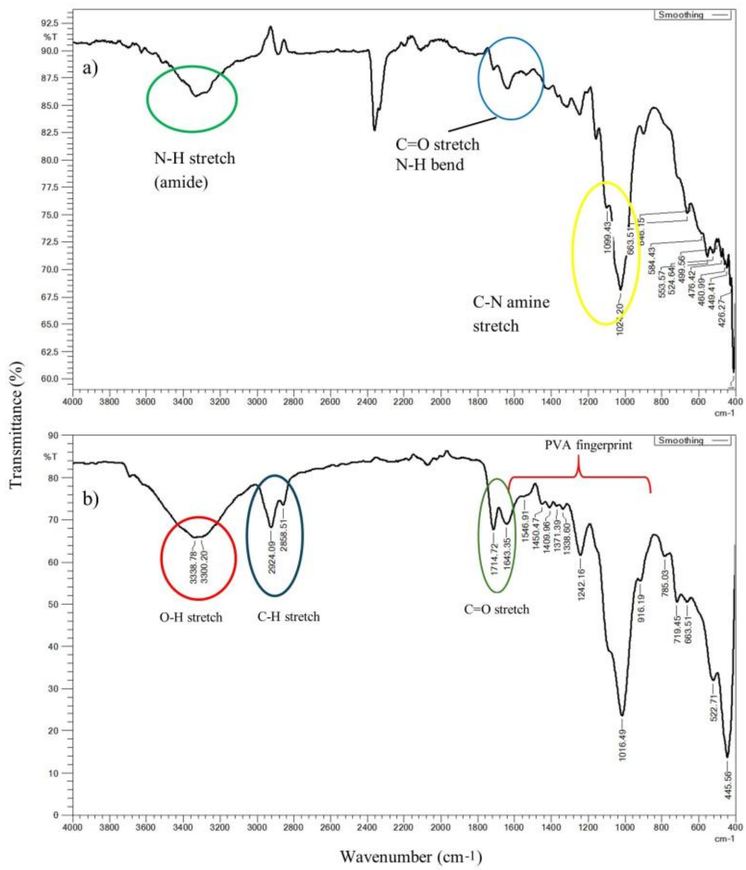

Figure 5. Two different polymers obtained from the polymeric characterization including (a) polyamide and (b) polyvinyl alcohol

The presence of microplastics adhering to seaweed indicated that the intertidal areas of Port Dickson coastal waters are also polluted with microplastic particles. In general, seaweed from Pantai Sri Purnama was more contaminated, at a concentration of 2.536 particles/g, compared to Pantai Tanjung Biru (2.001 particles/g). Pantai Sri Purnama is a tourist hotspot, as it is accessible from the town, compared to Pantai Tanjung Biru, which is a bit far from the town. Port Dickson's coastal waters are heavily polluted with microplastics, as studied by Zainuddin et al., with most particles being fibre-shaped particles [53]. This could be attributed to the heavy water activities in the area, including motorized watersport and other recreational activities. This indicates that human activities directly impact microplastic distribution in the coastal waters, leading to interactions with marine organisms such as seaweed.

Conclusion

The accumulation of microplastics on seaweed surfaces highlights the critical role of the species as a passive microplastic sink in coastal environments. *Caulerpa lentillifera* trapped higher microplastics compared to *P. jamaicensis*. Fibre-shaped microplastics were the only particles isolated from both species collected on two beaches of Port Dickson. This signifies that the coastal waters of Port Dickson are heavily polluted with microplastics. The prevalence of polymer types such as polyamide, polyvinyl alcohol, and polytetrafluoroethylene, associated with industrial and domestic waste, further emphasizes the importance of reducing plastic discharge from tourism and urban activities. Understanding the mechanism of microplastic accumulation on the surface of seaweed is a crucial step towards mitigating contamination in marine food chains. Future efforts should consider integrating microplastic contamination assessments into routine environmental monitoring, to protect marine resources and public health.

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