MICROPLASTIC CONTAMINATION IN *S. cucullata* AND ASSOCIATED HEALTH RISK ASSESSMENT IN THE STRAIT OF MALACCA

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Abstract

Saccostrea cucullata, also known as rock oysters, are chosen as the targeted organisms on rocky shores to elucidate the pathway of microplastics in sessile organisms. Eight rocky shores along the Strait of Malacca located on the coastal waters of the Malaysian states of Johor, Melaka, and Negeri Sembilan were selected. A total of 65 particles were discovered, of which 58.5 percent was identified as polymers. The microplastic abundance was between 0.0302 to 0.3586 microplastic items/wet weight and 0.1053 to 0.6000 microplastic items/individual *S. cucullata*. The microplastics found was typically filament-shaped, black in colour, and ranging in size from 107.85 μm to 14614.43 μm. With the exception of Teluk Kemang, the hazard quotient (HQ) values for all of the sampling sites were unacceptable and exceeded 1. The information gathered could serve as the starting point for further research into microplastics contamination of the marine environment and its inhabitants in Malaysia.

Keywords: Microplastics; *Saccostrea cucullata*; FTIR analysis; Health risk analysis

1. Introduction

Microplastics have been classified as a contaminant because they persist in the marine environment (Kolandhasamy et al., 2018; Qu et al., 2018). According to Ajith et al., (2020), microplastics are any solid synthetic organic polymers with a size range of 0.001 mm to 5 mm. In order to better understand the bioavailability of microplastics in marine organisms, microplastics monitoring must be conducted to assess the environmental and health risks associated with them (J. Li et al., 2016). In determining the baseline of microplastics concentrations in the marine biota for the purpose of monitoring microplastic pollution, field evaluations of bivalves can be helpful (Bråte et al., 2018; Digka et al., 2018; Foekema et al., 2013; Foo et al., 2022; Hamaguchi et al., 2014; Kolandhasamy et al., 2018). Studies using bivalves and molluscs have been carried out worldwide, and they were frequently used in microplastics research due to their instant exposure to the microplastics in the marine environment which allows them to act as a local-scale indicator of microplastics in the water column along the coastline (Digka et al., 2018; Kolandhasamy et al., 2018; J. Li et al., 2016; Phuong et al., 2019). Furthermore, bivalves and moluscs have been used successfully in the biomonitoring of marine environments due to their wide geographic distribution, ease of accessibility, and high tolerance to a wide range of salinities (Fuad et al., 2013; Fuad Miskon et al., 2016; J. Li et al., 2015; Nik Ali et al., 2019).

In this study, *Saccostrea cucullata* was selected as a representative bivalve for monitoring microplastic bioavailability in marine organisms as it can be found in nearly all rocky shorelines in Malaysia and on any rigid substrate (Fuad Miskon et al., 2016; Nik Ali et al., 2019; Rahman et al., 2016). It is also a representative of benthic filter feeders and can be used for microplastics biomonitoring (Li et al., 2018). Previous studies on microplastics monitoring in Malaysia covered surface water, sediment, and organisms such as zooplanktons, blood cockle and fish and with different study areas and digestion methods (Hamzah et al., 2021; Ibrahim & Mat Noordin, 2020; Ibrahim et al., 2016, 2017; Karbalaei et al., 2019; Khalik et al., 2018; Najihah et al., 2020; Sarijan et al., 2018; Yang Hwi et al., 2020). Moreover, microplastic monitoring data are invaluable to monitor the long term effect of microplastics entering our food chain, especially with Malaysia being ranked as the eighth most poorly managed plastic waste country in the world (Ibrahim & Mat Noordin, 2020). Thus, knowing the pathway of microplastics is important for future mitigation. Previous studies have reported numerous organisms, sediment, sand, and water samples to contain microplastics in China, Vietnam, the Philippines, South Korea, France, the United States, and British Columbia, (Davidson & Dudas, 2016; Martinelli et al., 2020; Mathalon & Hill, 2014). However, research on microplastics contamination of Malaysian marine organisms is currently lacking, especially that of rock oysters in the Strait of Malacca. The Strait of Malacca is the name of the waterway that separates Sumatra, Indonesia, from Peninsular Malaysia. It is one of the busiest and most vital waterways in the world, acting as the main shipping route between the Pacific and Indian Oceans and is vulnerable to pollution. Thus, it is crucial to assess the microplastic contamination there. Therefore, this study sought to examine the microplastic contamination in rock oysters, and to predict the health risks related to long-term consumption of microplastics in shellfish.

2. **Methods**

2.1. *Materials and chemicals*

Potassium iodide (KI) and potassium hydroxide (KOH) pallets were purchased from Merck Sdn. Bhd. The pellet was dissolved in filtered, deionized water to create solutions of KOH (10% w/v) and KI (50% w/v). All of the distilled water and chemical solutions used in the research were filtered using Whatman GF/C microfiber filter membranes (1.2 m pore size, 47 mm diameter).

2.2. Sample collection

A chisel and hammer were used to randomly select 218 similar-sized *S. cucullata* specimens (Arkhipkin et al., 2017). Samples were placed in an aluminium foil bag and placed inside a vacuum-sealed container. During sample collection, samples were kept chilled with ice in an ice box. Once at the lab, samples were stored at a temperature of -20 °C until further use (Su et al., 2018). Figure 1 shows a map of the sampling locations while Table 1 lists the sampling site's coordinates along the Strait of Malacca's coastal waters.

Table 1

Figure 1

2.3. Extraction

Soft tissue was removed from the shells of the *S. cucullata* samples using stainless steel dissecting tools after they had thawed at room temperature. The soft tissue was then placed on a glass petri dish (Covernton et al., 2019). Samples were wrapped in aluminium foil and the wet weight of the soft tissue was recorded. This laboratory procedure used six replicates with five individuals per digestion, which resulted in the use of 30 individuals, and an addition of procedural blank and the positive blank. Soft tissue samples that had been pre-weighed were covered in aluminium foil and dried in the oven for 24 hours at 60 °C (Phuong et al., 2018). After obtaining the dry weight of the soft tissue, the dried soft tissue samples were put in a conical flask and prepared for digestion. Table 2 shows the compiled *S. cucullata* biometric data (mean and standard deviation) for all sites, including shell length, shell width, wet weight, and the dry weight of soft tissue.

Table 2

Soft tissue samples were dried in an oven at 60 ^{o}C before being put in an Erlenmeyer flask with 200 ml of a 10% KOH solution (Baechler et al., 2020; Bråte et al., 2018; Phuong et al., 2019). Then, digestion solutions were put in an oscillating shaker oven that was kept at 60 ^{o}C and 80 rpm for 24 hours (Dehaut et al., 2016). Following the breakdown of soft tissue, the digestion mixture was put into a separating funnel and allowed to stand for eight hours. Two separate densities were separated. Glass filtering sets were used for the filtration process, and the filter paper was kept in pre-cleaned petri dishes and dried at 60 ^{o}C for 24 hours until achieving a constant dry weight that was then recorded before its visual examination (Phuong et al., 2018; Renzi et al., 2018).

2.4. Visual examination

Visual examination of the filter paper was done using a Nikon SMZ745 stereoscopic microscope (Bråte et al., 2018; Covernton et al., 2019; Ibrahim et al., 2016). Images of microplastic samples were taken using an eyepiece-mounted microscope camera attached to a desktop with a visualization application (ImageView software). Presumed microplastics were physically identified based on their shape and size and colour, which were all recorded. A total of 65 particles were assumed to be microplastics based on this visual examination, and ATR-FTIR was used to further characterize the polymers (Ibrahim et al., 2016).

2.5. ATR-FTIR analysis

Presumed microplastic particles obtained from the visual examination were marked, and samples were subjected to polymer characterization using an ATR-FTIR (Perkin Elmer FTIR Spectrometer Frontier) equipped with a diamond crystal sensor and PerkinElmer Spectrum software, with wavenumbers ranging from 4000 cm^{-1} to 600 cm^{-1} (Ibrahim et al., 2016; Jaafar et al., 2021; Khalik et al., 2018; Renzi et al., 2018; Zahari et al., 2022; Zainuddin et al., 2022). Sixteen scans were the minimum amount that were carried out on each sample. The samples were kept in an oven at 60 ^{o}C until they were analysed because particles must be dehydrated before polymer characterization. All particles were subjected to baseline correction using OriginLab 2021 before the library search in order to improve the quality of the spectra. With a search score greater than 0.65 and characterization of the polymer, 37 particles were determined to be microplastics (Razali et al., 2022).

2.6. Contamination prevention

Strict guidelines on contamination control from the literature review to avoid contamination from the workspace's surroundings and a contamination protocol have been followed throughout the experimental process. Thus, the experiment was carried out in a closed space, with the equipment used for the entire laboratory procedure was either made of glass or aluminium (Davidson & Dudas, 2016; Li et al., 2015; Qu et al., 2018; Su et al., 2018), and all lab apparatus was rinsed with filtered distilled water. Before conducting the experiment, the tools and working space were kept dry and spotless. To prevent fibre contamination from synthetic clothing, the filter paper containing microplastic samples was kept in a sealed petri dish at room temperature while laboratory researchers always wore an outer lab coat.

2.7. Health risk assessment

Assessment of health risks is part of a comprehensive health risk assessment (HRA) (US EPA, 2018). Using quantitative HRA, we calculated both carcinogenic and non-carcinogenic health hazards. By the end of the research, the hazard risk at each site was determined by calculating the hazard quotient (HQ) for each dominant polymer at the sample site. This study only focused on quantitative HRA. The estimated daily intake (EDI) needs to be calculated before the Hazard Quotient (HQ) can be pinpointed. Using the EDI, we were able to estimate how much microplastic polymers that will be ingested after eating the rock oysters. To calculate the EDI, the following formula was used (Sharif et al., 2016; US EPA, 2018):

$$EDI = \frac{(C \times IR \times EF \times ED)}{(BW \times AT)} \quad (1)$$

Where,

C (mg/g) is the concentration of microplastic polymer in the edible tissue of shellfish obtained from the analysis; R (ingestion rate) is the rate of consumption of shellfish a day (160 g/day/person) (Agusa et al., 2007); EF is the exposure frequency, and it is estimated at 52 days for those who eat shellfish once a week over a year; ED is the duration of exposure (ED is calculated for a period of a minimum of 40 years); BW is the average body weight (70 kg), AT is the average exposure to non-carcinogens for 365 days/year multiplied by ED (14600 days).

The hazard quotient (HQ) is a ratio used to characterise non-carcinogenic risk and determine whether a given risk would have an effect. If an HQ is below 1, it is deemed optimal, while anything above that value indicates an undesirable situation. We used a formula developed from a study by Sharif et al., (2016):

$$HQ = \frac{EDI}{RfD} \quad (2)$$

Where,

EDI (mg/kg/day) is the intake of respective microplastic polymer through the consumption of shellfish and RfD (mg/kg/day) is the reference dose, which is the estimated safe limit intake of microplastic polymer. The RfD value used for microplastic polymer was obtained from US EPA, (2018).

The following assumptions are applied while evaluating the HQ: (a) the dose of microplastics consumed is equivalent to the dose absorbed; and (b) microplastics are unaffected by cooking (Md. Abdul Mottalib et al., 2018). However, there is a lack of RfDs for microplastic polymers because of the limited research into the dangers that microplastic pose to human

health. Therefore, this study used the respective microplastic monomers' RfDs to identify the primary polymer's HQ value.

2.8. Data Analysis

All data were subjected to descriptive analysis using OriginLab 2021 and Microsoft Excel (Ding et al., 2020).

3. Results

Figure 2 shows that there are different amounts of microplastics per wet weight at each of the eight sampling sites, with S8 recording the lowest amount (0.0302 microplastic item/w.w.) and S5 the most (0.3586 microplastic item/w.w.). The data showed that the amount of microplastic varied depending on the location of the sampling site and the primary coastal activities carried out in the vicinity. According to the data, the microplastic item/individual abundance ranged from 0.1053 microplastic item/individual in S8 to 0.6000 microplastic item//individual in S5.

Figure 2

Over half the sampled locations were found to have microplastics in the shape of a filament. Figure 3 shows the various shapes of the sampled microplastics. Microplastics in filament, fragment, and bead form were all discovered in the soft tissue of *S. cucullata* in all of the sampling sites. The beads had a tough exterior that cracked under pressure, exposing a white and hard substance on the inside. In bead-shaped samples, there were two distinct classes of polymers present: the outer layer was usually cellulose triacetate (CTA), and the inner layer was polyvinlidene fluoride (PVDF).The microplastics samples extracted from *S. cucullata* came in a wide variety of hues. With the exception of site S8, where blue and red microplastics particles predominated, black was found to be the most prevalent colour. Black microplastics made up 48% of all microplastics found across all sites, followed by brown (11%), red (11%), colourless (10%), blue (8%), orange (6%), grey (2%), and white (2%) (Figure 3). The microplastics concentrations at the Strait of Malacca sampling sites are shown in Figure 4. Overall, most microplastics were determined to be smaller than 5000 µm in size, with a range of 107.85 µm to 14614.43 µm (Figure 5). Except for locations S2, S4, and S5, the microplastic size distribution was < 5000 µm at all other sites (Figure 4). Overall, eight different types of polymers were detected in the soft tissue of *S. cucullata* from coastal waters in the Strait of Malacca, and the FTIR spectral analysis verified 37 items as microplastics. CTA (54%), PCT (19%), PVAc (8%), PBT (5%), PP (5%), and PVDF, PET, and PS (3% each) were the most

common polymers. All sampled microplastics were positively identified to be polymeric. The CTA polymer was the most common at all sites except for S2.

Figure 3

Figure 4

Figure 5

Figure 6

4. Results and Discussion

The amount of microplastics varied depending on the sample site location and the primary coastal activities carried out in their vicinity. The range of microplastic abundance in the west of Malaysia was 0.03 to 0.40 microplastic item/w.w., which is close to reported amount from Italy (0.05 - 0.12 microplastic item/w.w.) (Bonello et al., 2018), the Salish Sea, USA (0.02 - 0.3 microplastic item/w.w.) (Martinelli et al., 2020), and New Zealand (0 - 0.48 microplastic item/w.w) (Webb et al., 2019). Another study similarly found that microplastics contamination in fish in Pantai Remis, Selangor also exhibited variable abundance depending on location (Jaafar et al., 2021). Sites surrounding urban areas and human activity tend to have a greater abundance of microplastics. The area is heavily inhabited, with locals flocking there on weekends and public holidays. Recreational and tourism activities could increase plastic waste on-site that can enter coastal waterways due to wind or tidal waves. Sites with comparable features, such as S5, had a high level of boating and tourism activity and are heavily populated, with the sampling sites being at the river mouth where all the effluent from upstream end up moving into the ocean.

Previous studies on microplastics in Malaysian coastal waters indicated that fibres or filaments were the most often detected forms of microplastics (Hamzah et al., 2021; Jaafar et al., 2021; Taha et al., 2021; Zainuddin, Aris, Zaki, et al., 2022). Filaments or fibres are common forms observed in the sampled organism, and are believed to originate from clothing fibres from washing machines effluent that finally ends up in the ocean, or were derived from abandoned fishing nets or lines used by fishermen (Jaafar et al., 2021; Khalik et al., 2018). Meanwhile, plastic fragments are often derived from commercial plastics discharged as a result of human activities or poor waste management in the sampled regions (Jaafar et al., 2021; Kershaw & Rochman, 2016). The shape of the microplastic discovered matched the data acquired from several worldwide microplastic investigations that sampled bivalves, in which the majority of the types discovered were fibres, fragments, pallets, and foam (Ding et al., 2020; J. Li et al., 2016; Qu et al., 2018; Zhu et al., 2019). The dominance of microplastic with a black hue in our study is similar to that of the Northeast Atlantic Sea (Lusher et al., 2017), Northern Tunisia (Abidli et al., 2019), and the Skudai River in Malaysia (Sarijan et al., 2019). Figure 3 shows a collection of different hues of microplastics. The size of microplastic can affect its impact on living organisms since smaller-sized plastics can interact with a wider spectrum of species (Ding et al., 2020; Jaafar et al., 2021). Toxic pollutants such as plasticizers, polybrominated diphenyl ether (PBDE), heavy metals, and polychlorinated biphenyls (PCBs) could also interact with microplastics, producing toxicity, particularly at the cellular level (Jaafar et al.,

2021; Webb et al., 2019). Furthermore, polymers with a 1000 µm diameter can pass through the cell barrier and be delivered to other organs. This process referred as adherence which the mechanism through which cells respond to oxidative stress and inflammation (Ding et al., 2019, 2020; Kolandhasamy et al., 2018).

Polymer composition can aid in determining the origin of microplastics. For example, microplastics derived from the alkyl polymer group detected in ocean surface water came from ship paint (Song et al., 2014). According to the findings in Figure 4, the predominance of fibres identified as CTA can be derived from natural sources or man-made products. CTA is used to make cigarette filters, textile fibres, photographic films, surface coatings, polymers, and membranes in various separation processes (Fei et al., 2017). Polyester polymer groups such as PET, PCT, and PBT were also dominant, reflecting the numerous activities carried out in the area particularly to single-use plastic goods. According to Figure 4, microplastics with the PE, PS, and PP polymers were frequently detected in the *S. cucullata* biomarkers in Malaysian waters, implying that the Malaysian marine ecosystem has already been polluted by microplastics.

In this study, the majority of polymers detected in *S. cucullata* were low-density polymers (PP, PS), which typically float in seawater, (Digka et al., 2018). Meanwhile, PET and other high-density microplastics are essentially non-existent on the sea surface. Nonetheless, other microplastics isolated from *S. cucullata* included PET and its variants, such as PCT and PBT. The methodology demonstrated that all samples could be collected from soft tissue, since both high-density and low-density polymers could be removed from *S. cucullata* soft tissue.

The microplastics polymers were characterised using ATR-FTIR, and the FTIR spectra for the polymer were assembled in a graph as shown in Figure 7. The transmittance intensity for CTA was 3337 cm^{-1} (-OH stretching of unacetylated cellulose), 2972 cm^{-1} (C-H stretching of CH_2 or CH_3), 1738 cm^{-1} (C=O stretching of the acetyl group), 1435 cm^{-1} (C-C stretching), 1369 cm^{-1} (C-C-O), 1218 cm^{-1} (C-O stretching of the acetyl group), and 1027 cm^{-1} (C-O-C of the (Fei et al., 2017). The transmittance intensity for PET was 2959 cm^{-1} (medium C-H stretching of aromatic and aliphatic), 1712 cm^{-1} (strong C=O stretching of carboxylic acid compound class), 1244 cm^{-1} (strong C-O stretching of ester group), 1098 cm^{-1} (strong C-O stretching of methylene group), and 752 cm^{-1} (aromatic C-H out-of-plane bend) (Chen et al., 2013; Jung et al., 2018). PBT had a transmittance intensity of 2965 cm-1 (medium C-H stretching of alkane compound class), 1715 cm^{-1} (strong C=O stretching of carboxylic acid compound class), 1245 cm^{-1} (strong C-O stretching of alkyl aryl ether compound class), 1094 cm^{-1} (strong C-O stretching of aliphatic ether), and 726 cm^{-1} (aromatic C-H out-of-plane bend) in FTIR spectra

(Chen et al., 2013). FTIR spectra for PCT had wavenumbers of 2918 cm^{-1} (medium C-H stretching of alkane compound class), 1722 cm^{-1} (strong C=O stretching of carboxylic acid compound class), 1562 cm^{-1} (medium C=C stretching of cyclic alkene), 1451 cm^{-1} (C-C stretching), 1167 cm^{-1} (strong C-O stretching of ester polymer class), and 719 cm^{-1} (Jung et al., 2018) (Jung et al., 2018). The transmittance intensity for PP was 2918 cm^{-1} (C-H stretch), 1457 cm^{-1} (CH_2 bend), 1375 cm^{-1} (CH_3 bend), 1169 cm^{-1} (CH bend, CH_3 rock, C-C stretch), 998 cm^{-1} (CH_3 rock, CH3 bend, CH bend), 975 cm^{-1} (CH_3 rock, C-C stretch), and 843 cm^{-1} (CH_2 rock, C-CH_3 stretch) (Jung et al., 2018). FTIR spectra of PVDF had wavenumbers of 2924 cm^{-1} (CH_2 stretching), 1447 cm^{-1} (CH_2 deformation), 1040 cm^{-1} (strong C-F stretching), and 855 cm^{-1} (strong CH bending) (Nallasamy & Mohan, 2005). The transmittance intensity for poly (vinyl acetate) (PVAc) was 3329 cm^{-1} (strong and broad O-H stretching), 2970 cm^{-1} (medium C-H stretching), 1739 cm^{-1} (strong C=O stretching), 1374 cm^{-1} (medium C-C bending), 1232 cm^{-1} (C-O stretching), and 1016 cm^{-1} (strong C-O stretching) (Dibbern-Brunelli et al., 1998). The transmittance intensity for PS was 3028 cm^{-1} (aromatic C-H stretching), 2846 cm^{-1} (C-H stretching), 1603 cm^{-1}, and 1493 cm^{-1} (aromatic ring stretching), 1452 cm^{-1} (C-C bending), 1028 cm^{-1} (aromatic C-H bending), 755 cm^{-1} (aromatic C-H out-of-plane-bending), and 696 cm^{-1} (aromatic ring C-H out-of-plan (Jung et al., 2018).

Figure 7

Numerous research have been carried all over the world to explore the environmental damage that microplastics cause to the marine environment and marine creatures. Microplastics exposure from shellfish intake has the potential to harm human health. Thus, an attempt was undertaken in this work to estimate the possible human health consequences of eating microplastics in *S. cucullata*. Ingestion was the most prevalent route for microplastics to reach the human body, and the first evidence of microplastics identified in human faeces came from its unintentional ingestion by a human (Ibrahim et al., 2021). Another study found that the microplastics detected in human excrement was identical to those found in bivalves, suggesting that people might have consumed microplastics through food (Ding et al., 2022). As a result, it is critical to examine the microplastics danger to humans caused by eating fresh *S. cucullata* fresh without removing the guts. The most prevalent polymer in each survey site was chosen as the major contaminant, which were CTA (4 sites), PCT (2 sites), PBT (1 site), and PS (1 site). The study revealed that the top two sample locations with a high concentration of microplastic polymers in edible tissue were S4 which contained 17.791 mg/g PCT, and S3, which contained 15.160 mg/g CTA. The EDI range for all sample locations was 0.009 mg/kg/day in S8 to 5.793 mg/kg/day in S4. The model assumed a daily consumption rate of

160 g/person, with the community consuming it just once each week for nearly 40 years. The most abundant monomer connected to the polymer obtained from the microplastics was employed as guidance for oral RfD. Because there is a lack of studies on the impact of polymers on human health, the monomers' RfD values were utilised. Table 3 displays the polymers with the highest RfD of the most prevalent monomer.

Table 3

The HQ values for all sampling sites are shown in Table 4. Except for S8, all locations exceeded the HQ of 1. A HQ of more than 1 indicated possible detrimental health effects. Notably, even though the S8 sampling site is Port Dickson's most popular beach destination, and is home to the majority of hotels, resorts, and family flats, it was well-managed and free of tourist litter. The chosen rocky shore at S8 was located 1 km south of Pancing Port PD World Marina and Admiral Marina and Leisure Club, next to Pusat Ikan Hiasan Teluk Kemang. However, the *S. cucullata* abundance was low and sparse at S8, despite the continuous rocky shore, with only 18 samples taken at this site.

Dimethyl terephthalate (DMT) is the most prevalent monomer in PET, PBT, and PCT. The RfD of DMT was determined from previous animal studies, and an uncertainty factor of 1000 was used to account for uncertainties in the extrapolation of experimental animal data to people, the range of human sensitivity, and extrapolation from a lowest-observed-adverse-effect level (LOAEL) to a hypothetical no observed adverse effect level (NOAEL). It is also known as the modifying factor of one (US EPA, 2002). Assuming that the community consumes shellfish once per week for more than 40 years, at a rate of 160 g/day/person S1, S2, and S4 are at high risk of acquiring a non-carcinogenic health consequence associated to chronic kidney inflammation due to having a HQ greater than 1.

Meanwhile, the most prevalent monomer in the CTA polymer is 1,4-dioxane. Previous human and animal studies were used to develop the RfD. Hemorrhagic nephritis and centrilobular necrosis of the liver have been linked to occupational exposure to 1,4-dioxane (US EPA, 2010). The uncertainty factor of 300 was utilised in the RfD for 1,4-dioxane, which was obtained from the NOAEL of 9.6 mg/kg-day. The uncertainties included the extrapolation of experimental animal data to people, the spectrum of human sensitivity, and the lack of a multigeneration reproductive toxicity research, which was assigned a rating of 3 (US EPA, 2010). Assuming the community consumes shellfish once per week for over 40 years, at a rate of 160 g/day/person, S3, S5, and S7 are at high risk of acquiring a non-carcinogenic health consequence related to liver and kidney damage due to having a HQ greater than 1.

The monomer for the PS polymer is styrene, and this polymer is only detected at S6. The RfD of styrene was determined from previous animal studies, and an uncertainty factor of 1000 was used to account for uncertainties in extrapolating experimental animal data to people, the spectrum of human sensitivity, and extrapolation from a NOAEL. It is also known to have a modifying factor of one (US EPA, 1987). Assume the community near S6 has been eating shellfish once a week for over 40 years, at a rate of 160 g/day/person they have a high risk of acquiring a non-carcinogenic health consequence involving red blood cells and liver damage. Previous studies found reduced packed cell volume and haemoglobin, increased iron deposits, liver dysfunctions due to exposure to styrene (US EPA, 1987). Although the HQ values in most sampling sites surpassed 1, the results should be regarded with caution because the model was based on the RfD value of the monomers rather than the polymers. Table 5 summarises the microplastics discovered in the Strait of Malacca.

Table 4

Table 5

5. Conclusion

Microplastics were found in the soft tissue of *S. cucullata* from sites along the Strait of Malacca, leading to the conclusion that the Strait of Malacca and its rocky shore habitats are contaminated with microplastics. This finding also reflected the availability of microplastics in seawater, which *S. cucullata* accumulates through its feeding capacity, as well as the possibility of microplastics adherence from its surroundings on the soft tissue. The research also shows that *S. cucullata* can be used as a bioindicator to provide detailed information on microplastics, such as their quantity, shape, colour, size, and polymer composition. Futhermore, the polymer characterization study identified 8 polymers and their health risk assessment was calculated using the hazard quotient (HQ). Based on the obtained EDI and RfD values, the majority of sampling sites showed possible detrimental health consequences to humans after consuming *S. cucullata*.

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Table 1 Table shows the sampling site along the Strait of Malacca

Station	Sampling Site	Coordinate	
		Latitude	Longitude
S1	Tanjung Laboh, Batu Pahat, Johor	1.7390 ° N	102.9927 ° E
S2	Tanjung Telaga, Pantai Minyak Beku, Batu Pahat, Johor	1.7955 ° N	102.8887 ° E
S3	Puteri Beach, Tanjung Kling, Melaka	2.2170 ° N	102.1542 ° E
S4	Tanjong Bidara, Melaka	2.2920 ° N	102.0860 ° E
S5	Kuala Linggi, Melaka	2.3842 ° N	101.9689 ° E
S6	Pasir Panjang Beach, Negeri Sembilan	2.4126 ° N	101.9424 ° E
S7	Blue Lagoon, Negeri Sembilan	2.4152 ° N	101.8226 ° E
S8	Teluk Kemang, Negeri Sembilan	2.4620 ° N	101.8482 ° E

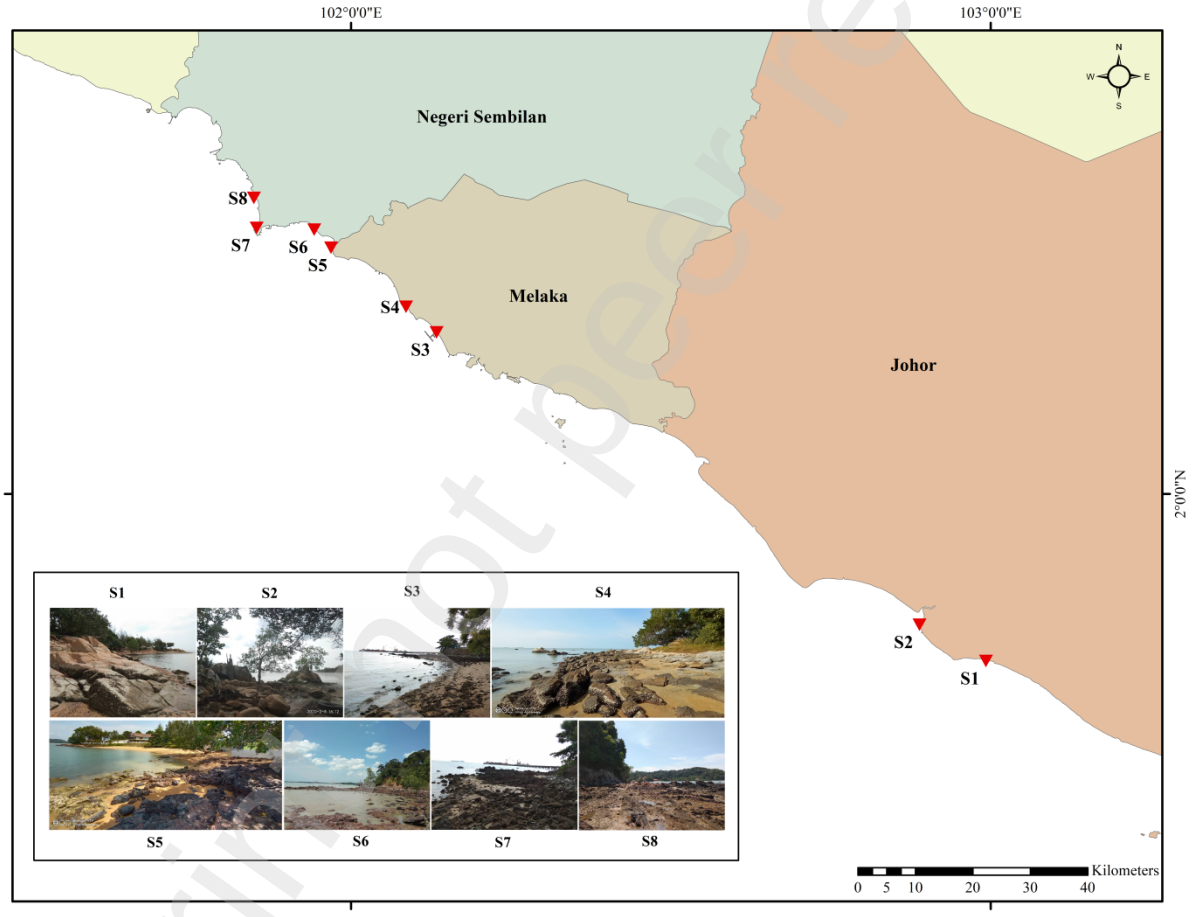

Figure 1 The map of the sampling site along the Strait of Malacca

Table 2 Table shows biometric data (mean and standard deviation) of *S.cucullata* for all sampling sites

Station	Shell length (cm)	Shell width (cm)	Wet weight (g)	Dry weight (g)
S1	4.8±0.5	4.1±0.6	1.1177±0.2438	0.3462±0.1117
S2	4.4±0.5	3.7±0.6	1.0954±0.3241	0.3619±0.0683
S3	3.4±0.6	3.3±0.6	0.7160±0.2734	0.3415±0.1067
S4	4.3±0.5	3.8±0.6	1.2416±0.4247	0.3438±0.0629
S5	4.7±0.7	4.1±0.7	1.6721±0.6379	0.4971±0.2158
S6	4.0±0.5	3.3±0.4	1.2855±0.4200	0.4136±0.1566
S7	4.9±1.4	4.1±1.5	2.1463±1.8918	0.7097±0.8344
S8	6.6±1.2	4.3±1.2	3.4884±2.0514	1.0840±0.7105

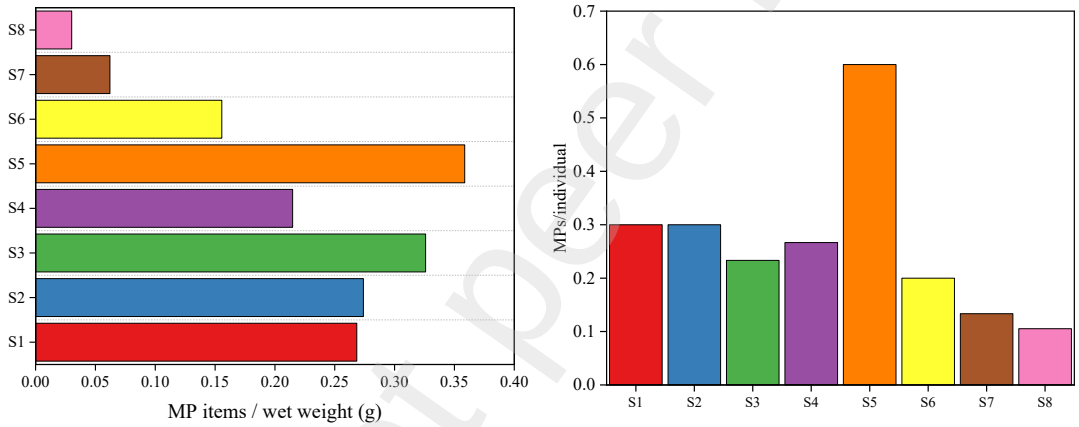

Figure 2 Abundance of MPs according to sites

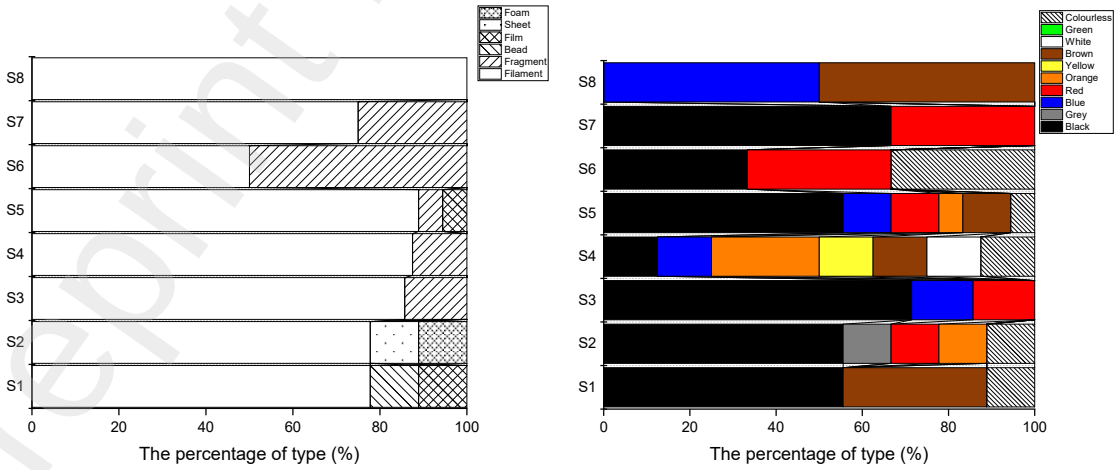

Figure 3 Distribution shape, and color according to sites

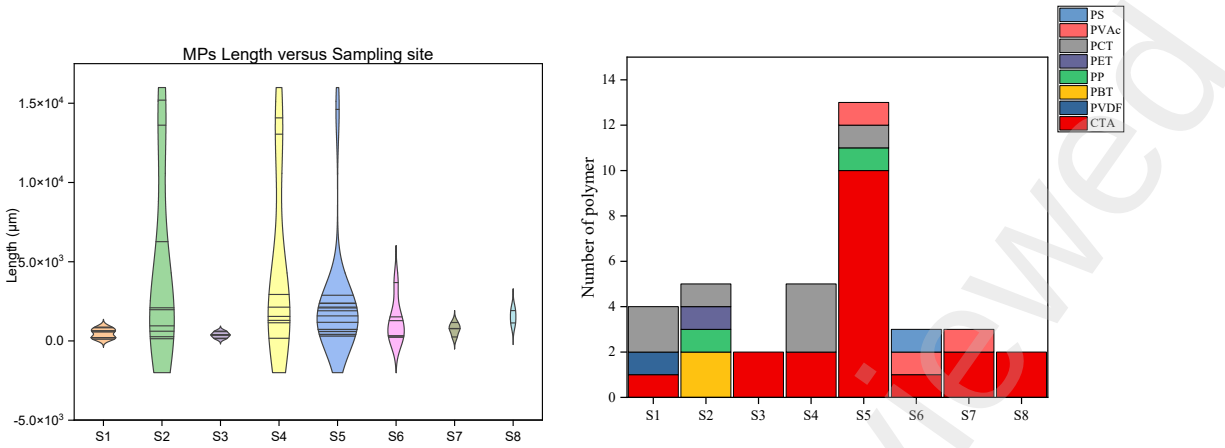

Figure 4 Distribution of size, and microplastic polymer according to sites

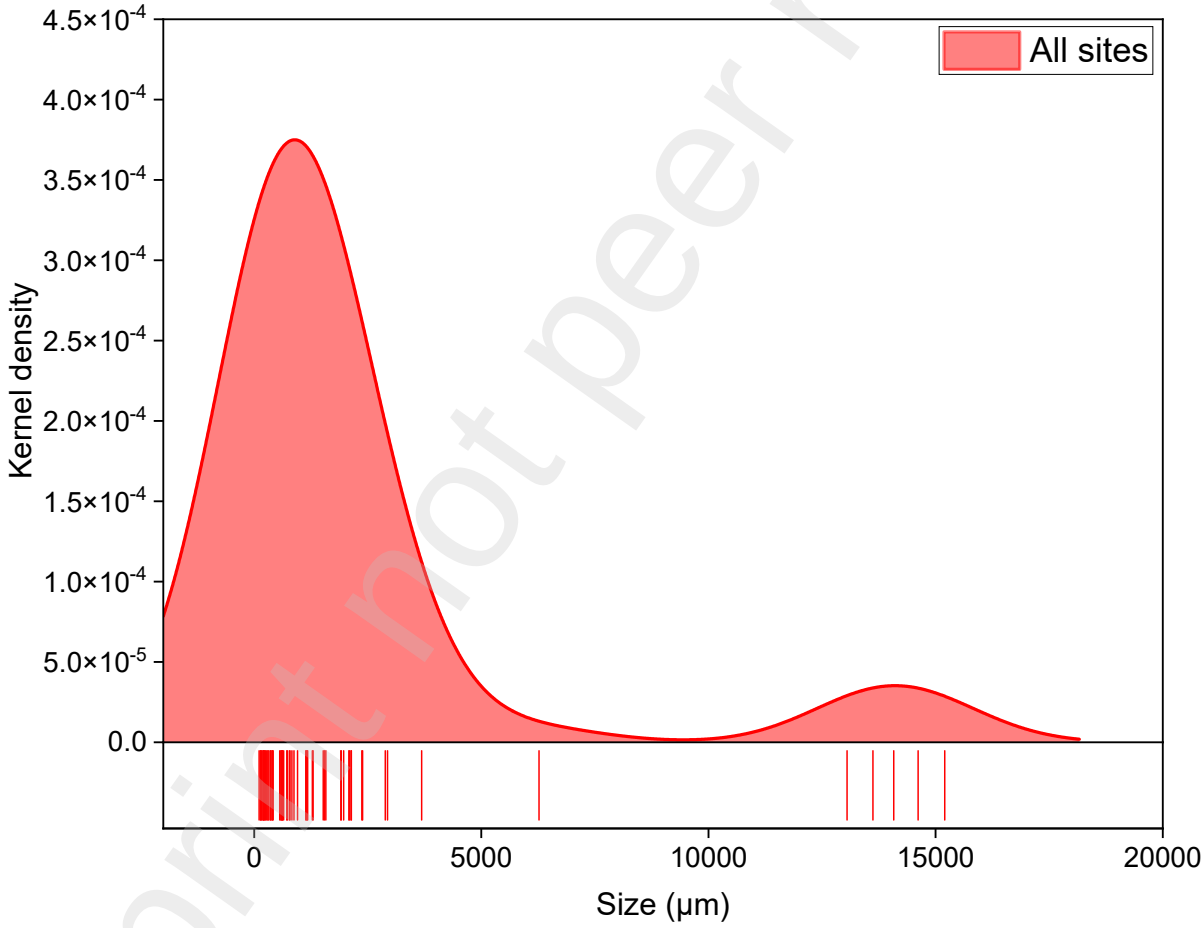

Figure 5 Distribution of microplastic size range in the Strait of Malacca

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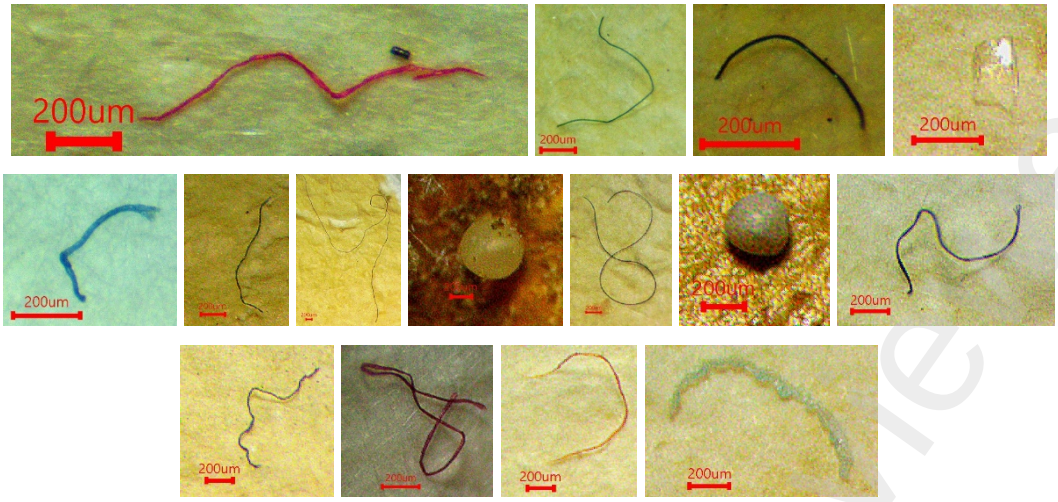

Figure 6 Compilation of pictures of microplastic collected in the study

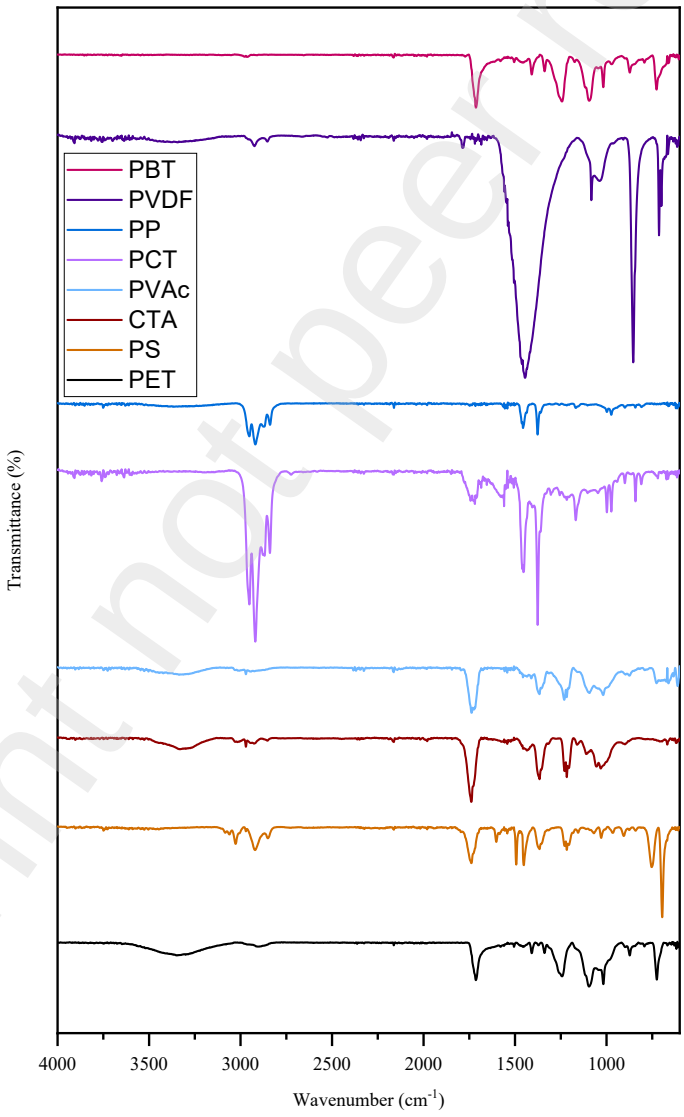

Figure 7 Compilation of polymer spectra produced from microplastic identified in *S. cucullata*

Table 3 List of MPs monomers with RfD

Polymer	Monomer	RfD (mg/kg-day)
CTA	1,4-Dioxane	0.03
PVDF	1,1-Difluoroethane	NA
PET	Dimethyl terephthalate (DMT)	0.1
PBT		
PCT		
PS	Styrene	0.20
PVAc	Vinyl acetate	NA
PP	NA	NA
Note: RfD values for each polymer were obtained from IRIS US EPA webpages *NA: Not Available, as the oral RfD had not been evaluated due to limited research		

Table 4 HQ calculations for all sampling sites

Site	Sampling Site	Pollutant	EDI	RfD	HQ	Health Effects
S1	Tanjung Laboh, Batu Pahat, Johor	PCT	2.048	0.10	20.478	Urinary
S2	Tanjung Telaga, Pantai Minyak Beku, Batu Pahat, Johor	PBT	2.593	0.10	25.927	Urinary
S3	Puteri Beach, Tanjung Kling, Melaka	CTA	5.083	0.03	169.436	Hepatic, urinary
S4	Tanjong Bidara, Melaka	PCT	5.793	0.10	57.933	Urinary
S5	Kuala Linggi, Melaka	CTA	0.601	0.03	20.043	Hepatic, urinary
S6	Pasir Panjang Beach, Negeri Sembilan	PS	0.964	0.20	4.818	Hepatic, hematologic
S7	Blue Lagoon, Negeri Sembilan	CTA	0.262	0.03	8.744	Hepatic, urinary
S8	Teluk Kemang, Negeri Sembilan	CTA	0.009	0.03	0.297	Hepatic, urinary
*EDI(mg/kg/day); RfD(mg/kg/day); NE: Not evaluated, as the value of RfD for the monomer was not available *All the health effects were taken from the IRIS US EPA website from the previous research						

Table 5 Table shows the summary of microplastic in each site

Site	Item / w.w.	Item / individual	Shape	Colour	Size range (µm)	Polymer found	HRA
S1	0.2684	0.3000	Filament, beads, film	Black, brown, colourless	107-874	CTA/PVDF, CTA, PCT	>1
S2	0.2739	0.3000	Filament, sheet, foam	Black, grey, orange, colourless	138-15198	PCT, PBT, PP, PET	>1
S3	0.3259	0.2333	Filament, fragment	Black, blue, red	146-617	CTA	>1
S4	0.2148	0.2667	Filament, fragment	Black, blue, orange, brown, colourless	166-14078	PCT, CTA	>1
S5	0.3586	0.6000	Filament, fragment, film	Black, blue, red, orange, brown, colourless	300-14614	CTA, PCT, PP, PVAc	>1
S6	0.1556	0.2000	Filament, fragment	Black, red, colourless	228-3687	PVAc, CTA, PS	>1
S7	0.0621	0.1333	Filament, fragment	Black, red	251-1164	CTA, PVAc	>1
S8	0.0302	0.1053	Filament	Blue, brown	1134-1914	CTA	<1