Microplastics contamination in bivalves off the island in the strait of malacca and its potential health risks

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Abstract. The widespread presence of microplastics in the ocean is a significant threat to marine life and humans. A study was conducted to investigate the extent of microplastic contamination in the coastal waters of Langkawi and Penang, situated on the northern coast of Peninsular Malaysia. Rock oysters (*Saccostrea cucullata*) were utilized as bioindicators due to its availability in all sampling sites to evaluate microplastics, by considering its abundance, types, polymer composition, and potential health risks related to consumption. Soft tissues were digested with 10% KOH, and the resulting microplastics were examined using a stereo microscope and microplastics polymer were identified through ATR-FTIR. Kok Beach and Penarak Beach exhibited notably higher microplastic abundance, mainly in the form of filaments with predominant black and red colours. The most common polymer types were cellulose triacetate (CTA) and polycyclohexanedimethylene terephthalate (PCT). Hazard Quotient values, indicating potential health risks from consuming *S. cucullata*, surpassed a critical threshold at all locations. The study's findings suggest that it serves as a fundamental reference for future research on microplastic contamination in the islands along the northern coast of Peninsular Malaysia.

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1 Introduction

The persistent presence of microplastics in the environment can be attributed to the worldwide increase in plastic production [6,54,55,60]. Microplastics, which range in size from 0.001 mm to 5 mm [1,31,58], pose a threat to a variety of marine organisms due to their small dimensions. There is evidence indicating that microplastics have the ability to release contaminants, such as metals and additives, act as carriers for hydrophobic pollutants, and create new habitats for microorganism colonization [27,30,38]. Preliminary examinations of microplastics within organisms are crucial, as they establish a basis for comprehending how these particles are absorbed from the environment into living organisms [4,11,14-16,20,27,34,36,48,62]. Earlier assessments in Malaysia focused on microplastics in surface water, sediment, and organisms like fish, molluscs, and zooplankton. However, challenges arise in comparing data due to variations in locations and digestion methods [15,24,26,32,43,44,59]. Notably, previous studies in Langkawi and Penang concentrated solely on sea surface water, neglecting the pathway of microplastics into marine organisms [32]. Our research involved a comprehensive survey of microplastics in the coastal waters of Langkawi and Penang, with a specific focus on quantifying the diversity of microplastics in rock oysters (*Saccostrea cucullata*), which served as bioindicators. *S. cucullata* was readily available across all sampling sites and could attach to hard substrates. Consequently, it was present throughout all sampling sites. This encompassed evaluating microplastic abundance, shapes, colours, polymer composition, and assessing potential health risks associated with consuming contaminated rock oysters.

2 Methodology

2.1 Sample collection and laboratory preparation

Nine sites along the rocky shore were chosen for investigation (refer to Table 1), considering the presence of *S. cucullata* on rocks and the accessibility of the sites. These sites were distributed across 4 locations in Penang and 5 locations in Langkawi (see Figure 1). A total of 258 individual *S. cucullata*, each approximately the same size (± 4 cm), were randomly gathered using a chisel and hammer [2]. Only samples with intact shells were selected and placed in aluminum foil bags, then sealed in a vacuum container. To maintain freshness, the samples were preserved in ice during transport and stored at -20 °C in the laboratory until further analysis [47].

Table 1. Coordinates of sampling sites.

| Site ID	Sampling Site	Coordinate	
		Latitude	Longitude
S1	Teluk Nangka, Miami Beach, Penang	5.4781 °N	100.2673 °E
S2	Pasir Panjang Beach, Penang	5.3006 °N	100.1842 °E
S3	Sungai Batu Beach, Teluk Kumbar, Penang	5.2791 °N	100.2404 °E
S4	Teluk Mat Inca, Teluk Bahang, Penang	5.4637 °N	100.2271 °E
S5	Pasir Tengkorak Beach, Langkawi, Kedah	6.4307 °N	99.7267 °E
S6	Tengah Beach, Langkawi, Kedah	6.2713 °N	99.7309 °E
S7	Penarak Beach, Langkawi, Kedah	6.3044 °N	99.8614 °E
S8	Pasir Hitam Beach, Langkawi, Kedah	6.4357 °N	99.7985 °E
S9	Kok Beach, Langkawi, Kedah	6.3657 °N	99.6754 °E
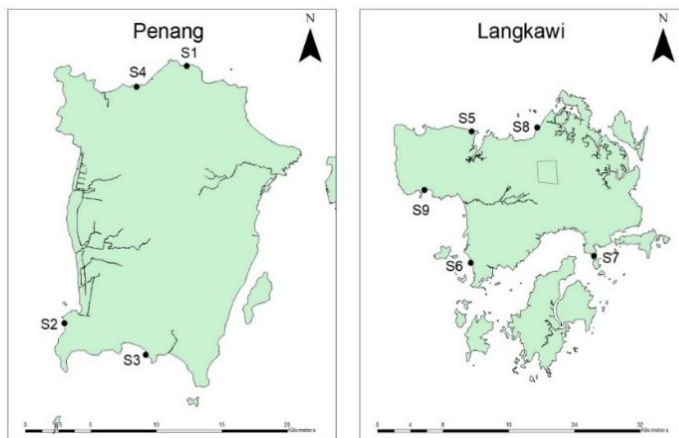
Fig. 1. Sampling sites along Penang and Langkawi.

At each rocky shore site, 30 samples were randomly organized into six groups, each containing five *S. cucullata* individuals for digestion. Within each batch, one procedural blank and one positive blank were included. After thawing at room temperature, soft tissues were separated from the shells using dissecting tools, and the samples were placed in a petri dish [7]. Following this, the samples were subjected to a 24-hour drying process in an oven set at 60 °C [37]. The wet and dry weights of the soft tissue were recorded, and the samples were then moved to a 250 mL Erlenmeyer flask containing a 10% KOH solution [3,4,35]. The flask, covered with aluminium foil, was placed in an oscillator shaker operating at 80 rpm and 60 °C for 24 hours [10]. Density separation of the digested solution was achieved by adding 50 mL of 50% KI for 8 hours [15,23]. The resulting solution underwent filtration using a 47 mm diameter Whatman GF/C filter paper disc with a 1.2 µm pore size and was subsequently dried at 60 °C for 24 hours until a constant dry weight was reached [35]. The filter paper discs were stored in petri dishes, and the dry weights of the filter paper discs before and after Fourier-transform infrared spectroscopy (FTIR) were recorded [40].

2.2 Physical Identification and Polymer Identification of Microplastics

The Whatman GF/C filter paper discs were examined under Nikon SMZ745 stereo microscope featuring a magnification range from 0.67x to 5x [4,7,17]. A microscope camera attached to the eyepiece of the stereo microscope was connected to a computer, and the ImageView software facilitated the capture of presumed microplastic images for size estimation. Validation of polymer characterization was conducted through ATR-FTIR [17]. Presumed microplastics were identified based on their shape, with recorded observations of shape, size, and colour aiding in subsequent polymer identification [40,60]. Particles marked during pre-assumed physical identification underwent polymer characterization using an ATR-FTIR (Perkin Elmer FTIR Spectrometer Frontier) equipped with a diamond crystal sensor and PerkinElmer Spectrum software, covering wavenumbers from 4000 cm-1 to 600 cm-1. The samples were kept in an oven at 60°C until analysis, and prior to library search, all particles underwent baseline correction using OriginLab 2021 to enhance spectrum quality. With a search score exceeding 0.65 and successful polymer characterization, 37 particles were identified as microplastic.

2.3 Quality assurance and quality control

To mitigate atmospheric microplastic contamination, a closed chamber was utilized, and all surfaces underwent acetone wiping before analysis. Glassware and equipment were thoroughly washed with filtered distilled water and shielded with aluminum foil [8,29,38,47]. Chemical solutions used in the experiment were dissolved in filtered distilled water and further filtered using Whatman GF/C filter paper [56]. Each procedural step was accompanied by both negative and positive blanks to track contamination throughout the procedures [42]. No damaged to microplastics in positive blank, and no microplastics detected from negative blank.

2.4 Calculation of Hazard Quotient

The hazard quotient (HQ) serves as a ratio to assess risk in non-carcinogenic scenarios, helping estimate the significance of a particular risk [52]. An HQ below 1 is considered acceptable, while an HQ exceeding 1 indicates an unacceptable condition. The formula used to calculate HQ is derived from the study conducted by Sharif et al. [45].

$$HQ = \frac{EDI}{RfD} \qquad (1)$$

Here, EDI (mg/kg/day) represents the intake of the specific microplastic polymer through shellfish consumption, and RfD (mg/kg/day) stands for the reference dose, which is the estimated safe limit for microplastic polymer intake.

3 Result and discussion

3.1 Biometric data

Table 2 displays the biometric data, including mean and standard deviation, for *S. cucullata* across all sites, covering shell length, shell width, wet weight, and dry weight of soft tissue. To ensure data comparability between sites, *S. cucullata* specimens with similar shell sizes were collected during sampling [57]. Allometric data for bivalve species are recognized to correlate with factors like substratum nature, salinity, temperature, and other chemical characteristics of the water, influencing dimensional relationships [41]. Samples obtained from areas near water sources discharged from land, such as estuaries or riverine zones, exhibited higher wet weight yields. This trend was observed in Teluk Nangka (S1), Sg Batu (S3), and Teluk Mat Inca (S4). In contrast, locations with low yields of wet tissues were observed in isolated areas like Pasir Panjang (S2), Pasir Tengkorak (S5), and Pasir Hitam (S8). Penarak (S7) recorded the lowest wet tissue yield due to a densely populated rock oyster environment attached to the substrate. This led to a competitive environment, resulting in oysters stacking on top of one another and, consequently, reducing the oyster size and soft tissue yield.

Table 2. Biometric data of sample collected in each site.

Station	Shell length (cm)	Shell width (cm)	Wet weight (g)	Dry weight (g)
S1	5.2±0.5	4.6±1.1	2.0885±0.6023	0.7305±0.2872
S2	3.9±0.7	3.7±0.8	1.3349±0.5996	0.5170±0.2408
S3	5.2±0.6	4.9±0.8	3.0500±0.8664	1.1133±0.5720
S4	5.4±0.7	4.8±0.7	2.2390±0.6359	0.8984±0.4844

S5	4.9±0.7	4.1±0.6	1.1942±0.4000	0.3658±0.0845
S6	5.0±0.6	4.2±0.8	1.5890±0.4782	0.6317±0.3122
S7	4.5±0.9	3.6±0.7	0.9929±0.4516	0.4452±0.2496
S8	4.4±0.7	3.7±0.5	1.0947±0.2798	0.3478±0.0959
S9	4.6±0.4	3.9±0.4	1.3707±0.2418	0.4717±0.1586

3.2 Microplastic abundance

The negative blank and positive blank utilized in the experiment underwent filtration using filter paper. The negative blank showed no presence of microplastics on the filter paper, while the positive blank demonstrated that the microplastics remained intact throughout the experiment. These observations confirm the absence of cross-contamination and deterioration during the experimental procedures [35]. In terms of microplastic abundance in S. cucullata, Penarak (S7) exhibited the highest concentration with approximately 0.40 item per wet weight of soft tissues, and Kok Beach (S9) recorded the highest abundance with 0.43 item per individual (see Figure 2). This result aligns with previous studies where microplastic contamination in bivalves ranged from 0.1 to 20 microplastics per gram of wet tissue [12]. The selected sampling sites displayed varying levels of microplastic pollution, with Penarak and Kok Beach experiencing heavy pollution due to fisheries equipment and boating activities, respectively. Our findings indicated that the microplastic concentrations in both sites exceeded those found in bivalves from Vietnam [35] and Europe [37]. However, differences in results could be attributed to the distinct protocols used by Phuong et al. [36]. The identification technique played a crucial role, as visual observation alone was insufficient for characterizing microplastics. Advanced methods such as Raman spectroscopy, capable of detecting samples as small as 1 μm, and μFTIR, capable of identifying microplastics ranging from 3 to 800 μm depending on the working mode, are recommended [35]. Conversely, Pasir Panjang (S2) and Pasir Hitam (S8) exhibited the lowest microplastic abundance, aligning with the biometric data, where both locations had low yields of wet weight tissues.
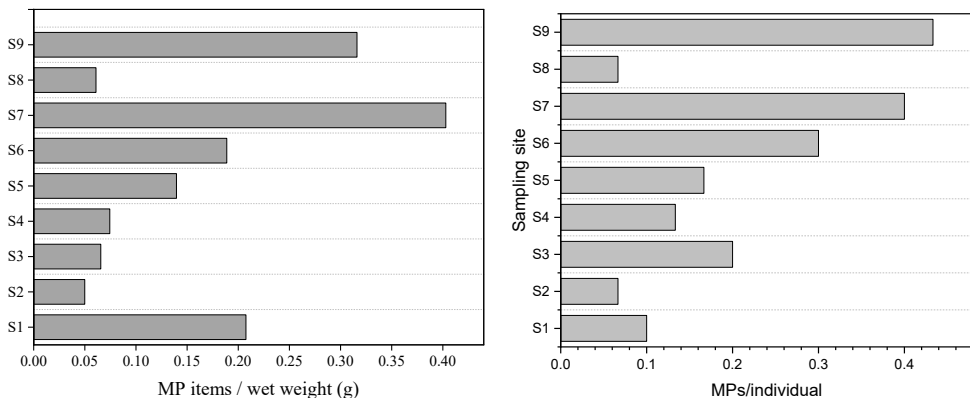

Fig. 2. Abundance of microplastics per wet weight according to sites (left) and microplastics per individual of *S. cucullata* according to sites (right).

3.3 Physical characteristics of microplastics

The predominant shape of microplastics found was filaments with a black colour, followed by fragments exhibiting a red colour (see Figure 3). However, the sources of microplastics

based on colour have not been determined, as there were no additional sediment and seawater samples collected for establishing a correlation between the colour of microplastics in soft tissues and the sources of pollution. In a previous microplastic study in the coastal waters of Penang and Langkawi, fragments, film, granules, filaments, and foam were commonly identified in surface water using visual sorting identification [15,32]. Microplastic beads were detected in the soft tissues at sites in Langkawi, a finding not consistent with other studies that did not detect microplastic beads except in fish samples from Skudai, Johor [43], and Northwest Peninsular Malaysia [15]. The distribution of microplastic shapes and colours is depicted in Figure 3.
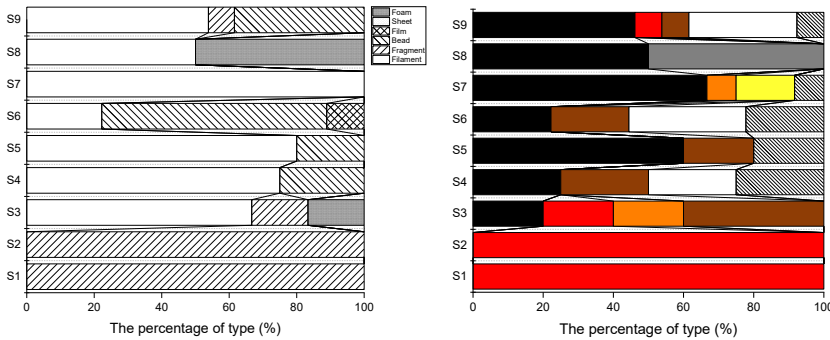

Fig. 3. Distribution of shapes of microplastics (left) and colour of microplastics (right) found according to sites.

The sizes of the identified microplastics ranged from 167.93 µm to 11,268 µm, with most samples falling around 5,000 µm (see Figure 4). Microplastic size is crucial, as plastics of different sizes interact with various levels in the food pyramid [11]. Microplastics can interact at the foundational level of the food pyramid, potentially leading to bioaccumulation or biomagnification [25]. Plastics, irrespective of size, can interact with toxic pollutants such as plasticizers, polybrominated diphenyl ether (PBDE), heavy metals, and polychlorinated biphenyls (PCBs), inducing toxicity, especially at the cellular level [19,21,57]. Moreover, plastics with a size less than 1000 µm can penetrate cell barriers and be transported to other organs through a process known as adherence, ultimately causing oxidative stress and an inflammatory response by cells [12,27].
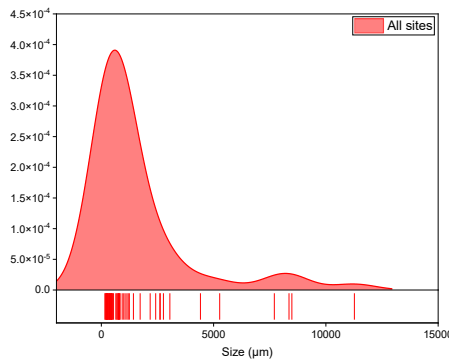

Fig. 4. Distribution of microplastic counts versus size and its kernel density estimation for microplastic in *S. cucullata* for all sampling sites.

3.4 Polymer distribution and chemical composition

A total of 36 microplastic items were successfully identified based on their chemical composition, revealing the presence of six distinct polymers. The most prevalent microplastics were cellulose triacetate (CTA) and polycyclohexanedimethylene terephthalate (PCT) (see Figure 5). The composition of these polymers can serve as a valuable tool for tracing the origin of the plastics. For instance, CTA can originate from natural sources or may be used in the production of cigarette filters, textile fibers, photographic film, surface coatings, and membranes for various separation processes [13]. Polyester polymers like PCT indicate diverse activities in the area, potentially linked to single-use plastic items [49]. Sites with elevated microplastic abundance in both individual and wet tissues, specifically Penarak (S7) and Kok Beach (S9), exhibited the highest polymer identification. The heavy pollution from boat activities in these sites justifies the diverse array of polymers found in *S. cucullata*.
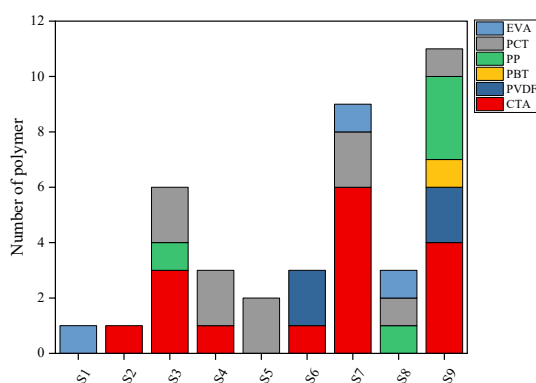

Fig. 5. Distribution of the number of polymers according to sites for microplastic found in *S. cucullata*.

The FTIR spectra of the polymers identified in this study are illustrated in Figure 6. For PVDF, distinctive peaks appear at wavenumbers of 2924 cm-1 (CH2 stretching), 1447 cm-1 (CH2 deformation), 1040 cm-1 (strong C-F stretching), and 855 cm-1 (strong CH bending) [33]. CTA displays peaks at wavenumbers of 3337 cm-1 (-OH stretching of unacetylated cellulose), 2972 cm-1 (C-H stretching of CH2 or CH3), 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 cellulose backbone) [13]. PBT exhibits peaks at wavenumbers 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) [5]. PP presents peaks at wavenumbers of 2918 cm-1 (C-H stretch), 1457 cm-1 (CH2 bend), 1375 cm-1 (CH3 bend), 1169 cm-1 (CH bend, CH3 rock, C-C stretch), 998 cm-1 (CH3 rock, CH3 bend, CH bend), 975 cm-1 (CH3 rock, C-C stretch), and 843 cm-1 (CH2 rock, C-CH3 stretch) [22]. PCT demonstrates peaks at 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 (strong C-H bending) [5]. EVA shows peaks at wavenumbers of 2918 cm-1 (C-H stretching), 1736 cm-1 (C=O stretching), 1468 cm-1 (CH2 bending, CH3 bending), 1238 cm-1 (C(=O)-O stretching), and 1018 cm-1 (C-O stretching) [5].
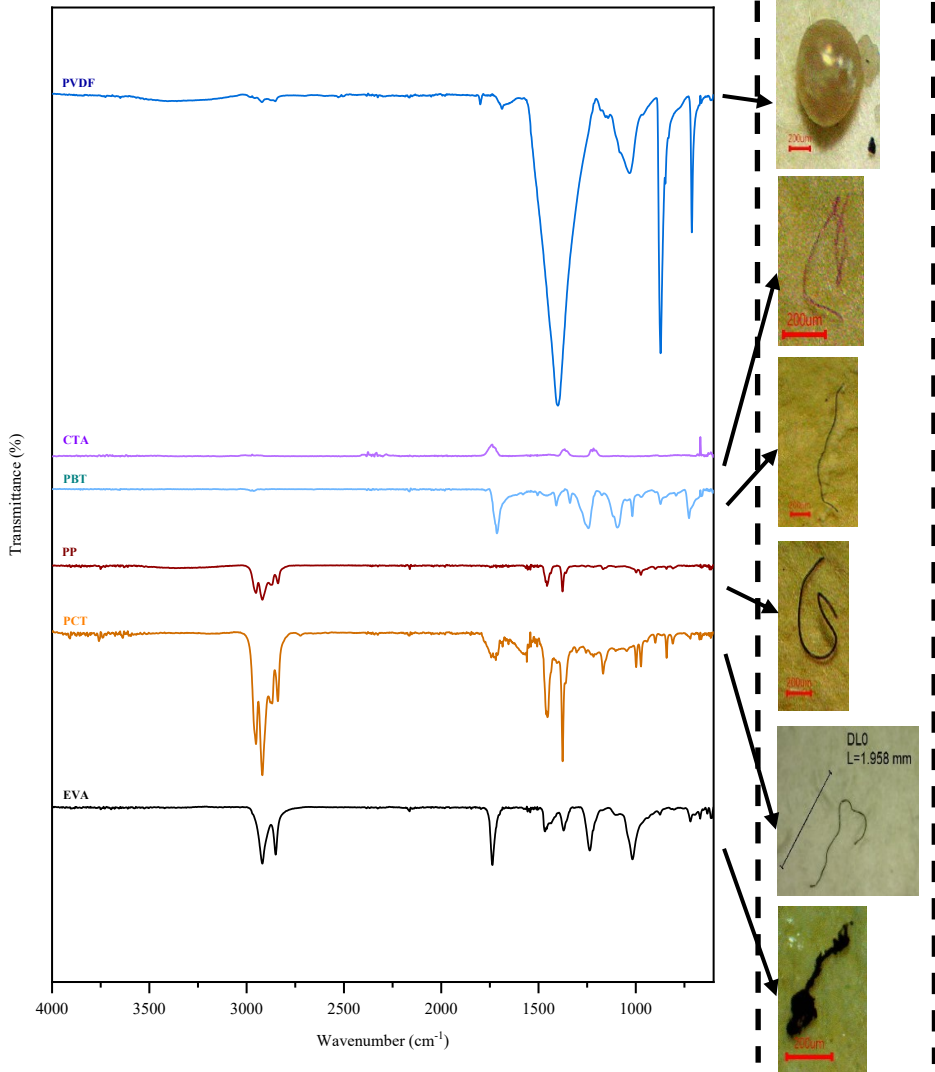
Fig. 6. Compilation of FTIR spectra for microplastic polymers found in Langkawi and Penang.

3.5 Health risk analysis

Exposure to microplastics through shellfish consumption raises potential concerns for human health. Given that the Reference Dose (RfD) value for the polymer remains undetermined, we employed the RfD value of the monomer for the most abundant microplastic found at each site. The methodology, including the calculation for Estimated Daily Intake (EDI) and Hazard Quotient (HQ), has been detailed in the methodology section. Continuous consumption of contaminated shellfish could potentially lead to hepatic and urinary diseases [52,63]. It's crucial to note that the study solely focused on the impact of microplastics and did not consider potential toxicity from other sources. All evaluated sites displayed a Hazard Quotient exceeding 1 (refer to Table 3), indicating a potential risk in consuming oysters from these sites. However, sites without an RfD value for the monomer were labeled as "Not Evaluated" (NE). Table 3 provides information on the dominant pollutant found at each site

based on polymer identification in subsection 3.4, along with its Estimated Daily Intake (EDI) projection and Reference Dose (RfD). The Hazard Quotient was calculated for sites with an available RfD monomer value, and any value exceeding 1 predicts potential health effects such as hepatic and urinary issues if consumption continues according to the estimated daily intake (EDI) projection.

Table 3. Hazard quotient (HQ) calculations for all sampling sites.

Site	Pollutant	EDI	RfD	HQ	Health Effects
S1	EVA	0.023	NE	NE	NE
S2	CTA	0.318	0.03	10.611	Hepatic, urinary [50]
S3	CTA	0.116	0.03	3.857	Hepatic, urinary [50]
S4	PCT	0.261	0.10	2.608	Urinary [51]
S5	PCT	1.367	0.10	13.667	Urinary [51]
S6	PVDF	1.679	NE	NE	NE
S7	CTA	4.545	0.03	151.503	Hepatic, urinary [50]
S8	CTA	4.633	0.03	154.437	Hepatic, urinary [50]
S9	CTA	1.307	0.03	43.552	Hepatic, urinary [50]
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					

4 Conclusion

This study offers a comprehensive overview of the physical and polymer characteristics of microplastics, along with potential health effects that may arise from the consumption of *S. cucullata* contaminated with microplastics (see Table 4). The gathered data establishes a baseline for the investigation of microplastic contamination in bivalves. This information holds valuable implications for informing public policy and can serve as a predictive tool for the Malaysian fisheries industry in the future.

Table 4. Summary of microplastic data for all sampling sites

Site	Item / w.w.	Item / individual	Shape	Colour	Size range (µm)	Polymer found	HRA
S1	0.2075	0.1000	Fragment	Red	167-1057	EVA	>1
S2	0.0499	0.0667	Fragment	Red	198-423	CTA	>1
S3	0.0656	0.2000	Filament, fragment, foam	Black, orange, brown	270-8485	CTA, PCT, PP	>1
S4	0.0744	0.1333	Filament, bead	Black, brown, white, colorless	164-2608	PCT, CTA	>1
S5	0.1396	0.1667	Filament, bead	Black, brown, colorless	295-1727	PCT	>1
S6	0.1888	0.3000	Filament, bead, film	Black, brown, white, colorless	226-1428	CTA/PVDF, PVDF	>1

S7	0.4029	0.4000	Filament	Black, orange, yellow, colorless	227-2765	CTA, EVA, PCT	>1
S8	0.0609	0.0667	Filament, foam	Black, grey	747-11268	EVA, PP, PCT	>1
S9	0.3161	0.4333	Filament. fragment, bead	Black, red, brown, white, colorless	291-7702	PBT, CTA/PVDF, CTA/PVT, CTA, PP	>1

Acknowledgements

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