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RESEARCH ARTICLE

Microplastics Isolated from Saltwater Clam *Paratapes undulatus* from Wet Market at Shah Alam, Selangor, Malaysia

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Abstract

Plastic waste has become a serious environmental threat because of large scale demand and poor disposal methods. Microplastics, defined defined as plastic waste with a diameter spanning from 5 mm to 1 µm, may easily enter the ocean and cumulatively ingested by marine life, which will eventually be consumed by humans. The goal of this study is to determine the types of microplastic that can be found in the saltwater clam *Paratapes undulatus* collected in Kuala Selangor, a fishery hub in Peninsular Malaysia. A total of 30 *P. undulatus* samples were collected and the soft tissue inside was dissected and digested using NaOH. The digestates were then vacuum-filtered to obtain the microplastics. Microplastics were identified based on their physical characteristics under microscopic examination (colour, shape and size). Fourier Transform Infrared Spectroscopy (FTIR) was used to determine the polymers based on the functional group of the plastics' molecular structure. A total of 2,072 microplastic particles were isolated from all clam samples. In terms of colour and shape, the majority of microplastics were black (64.48 %) and in the form of fibres (97.2 %). Most of the microplastic particles had sizes ranging from 0.5 to 1 µm and 1 to 2 µm. Polystyrene (PS) and polymethyl methacrylate (PMMA) were two common polymers. This study indicates that clams harvested off the coast Kuala Selangor may be contaminated with microplastics from their habitat. More research is needed to assess the toxicity and potential threat of microplastics to human health when consuming seafood.

Keywords: Microplastic, Seafood, *Paratapes undulatus*, FTIR analysis.

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Introduction

Plastic production has increased tremendously, peaking at 367 million tonnes worldwide in 2020 [44]. Plastics are cheap and designed to be versatile for many applications. With frequent use and inappropriate disposal, most of the waste will end up in oceans. Under normal conditions, they will take centuries or may never even decompose. After being exposed to physical, chemical and biological abrasions over a long period, they will break into small particles known as microplastics, which can be ingested by living organisms [1].

Clams are one of the most popular seafood in Malaysia. These bivalves are rich in nutrients, low in fat and a great source of lean protein for health-conscious individuals [35, 48]. *Paratapes undulatus*, also known as *siput lala* in Malay language, is a species of saltwater clam that lives in the sandy seabed of coastal areas along the Straits of Malacca. Due to limited mobility and feeding strategy, most shellfish are "filter feeders", where they trap and feed on organic matter found in seawater that is absorbed through their body. This feature makes them an ideal bioindicator because the amount of particles, heavy metals and toxic substances they accumulate will reflect the level of pollution in their surrounding environment. Currently, microplastics have been identified as the most common pollutant absorbed by shellfish [6, 26, 50].Clams are a direct route of microplastic ingestion by humans as they are a seafood that is highly consumed and usually harvested in polluted waters.

Microplastics are synthetic polymers with a diameter of less than 5 mm, and they come in a variety of shapes, such as fibres, fragments and beads, depending on the degradation process [5]. It may either be derived from commercial or household products, or degraded from larger plastics after being exposed to environmental abrasions, such as UV radiation (which causes plastics to become brittle), and wind and physical stress. The degradation of plastics will also release toxic substances like polycyclic aromatic hydrocarbons and polychlorinated biphenyls. Plastic waste and its chemical by-products can be easily washed into rivers and the ocean via stormwater runoff and atmospheric deposition [4, 11, 25].

Plastic additives are harmful to living organisms and they have been found in high concentrations in marine environments [15]. The consequences of ingesting these chemicals have been documented in various marine species. They have been observed to cause morphological changes in cells and tissues, besides reducing the species' reproductive capability and increasing population mortality [2, 17, 27]. Although the presence of plastic chemicals and additives do pose a threat to food safety and human health, the implications of ingesting microplastics itself are still not known [15, 46, 51].

This study aims to assess the level of microplastic contamination in *P. undulatus* harvested off the coast of Kuala Selangor in the state of Selangor, Peninsular Malaysia, Being the most populous state in the peninsula, the seafood harvested in Kuala Selangor (a fishing hub near the capital of Kuala Lumpur) will be consumed by a significant portion of the country's population. Being close to busy shipping lanes also exposes the clams to high pollution levels. Therefore, it is important to monitor the level of pollutants, especially microplastics, absorbed by the clams to ensure that they are safe to consume without leading to adverse health effects.

Materials and Methods

Sample Collection and Preparation

Thirty samples of *P. undulatus* were collected randomly from a wet market at Shah Alam, Selangor, Malaysia which is the standard sample size according to previous papers (Rangseethampanya *et al*., 2019; Ruairuen *et al*., 2022). All samples were stored in seawater and taken to the laboratory. Prior to tissue digestion, the *P. undulatus* flesh was cut out of their shells and weighed individually. The dissected samples were preserved in a glass bottle containing 95 % ethanol and kept in a freezer (-20°C) until further analysis [36].

Isolation of Microplastics

Tissue digestion was performed according to Karami *et al*. (2017). Briefly, the isolated tissues were thawed to room temperature and placed in 50 ml of 10 % sodium hydroxide (NaOH) before being incubated for 48 hours in a water bath at 40 °C. After incubation, the digestates were filtered through a 5-13 μm filter paper under vacuum. The sediment was resuspended in 10 to 15 ml of sodium iodide (NaI), followed by sonication and agitation. The micro-glass fibre process was repeated twice to ensure maximum recovery of microplastics, and the sheet was kept in a covered clean glass petri dish [10].

Microscopic Examination of Microplastics

The microplastics on the micro-glass filter papers were carefully sorted and examined under a dissecting microscope. Photos of sorted microplastics were then taken using a comparison microscope. Microplastics were classified by their size, shape and colour. When it was impossible to distinguish between polymers and organic components, the hot needle test method was applied. The recovered microplastics were placed in a new glass petri dish with filter paper to determine the chemical characteristics of the polymer [36].

Identification of Polymer using Fourier Transform Infrared Spectroscopy (FTIR)

Thermo Fisher Scientific NICOLET iS50 FTIR Spectrometer equipped with an XT-KBR beam splitter and a deuterated triglycine sulphate (DTGS)-potassium bromide (KBr) detector was used for FTIR study to characterise the molecular structure of polymers and identify plastics components. Attenuated total reflection (ATR) imaging was used to capture a background scan that was recorded as blank before each sample on a clean tape. This background scan was obtained utilising 32 co-added images in the mid-IR range of 4000-650 cm^{-1}. Using a pressure probe while collecting data, the sample was positioned so that it was in direct contact with the diamond surface and ATR imaging using the same parameters as for the background scan. To get the actual spectrum of microplastics, the blank spectrum was subtracted. The microplastic polymers were determined based on the FTIR spectrum ranges of previous studies [18, 38].

Quality Assurance and Control

To prevent airborne microplastic contamination throughout the experiment, non-plastic clothing such as cotton lab coats and nitrile/latex gloves were worn. Additionally, the samples were examined with metal tools and stored in glassware. Any potential contaminant was identified during filtering and sieving. To limit microplastic loss, air exposure was also kept to a minimum.

Results and Discussion

Characterisation of microplastics

A total of 2,072 microplastic particles were isolated from all 30 samples of *P. undulatus*, with each sample containing an average of 70 particles.

Based on colour, Figure 1 depicts the amount of microplastics recovered. The particles had eight colours: black, blue, brown, pink, red, yellow, orange and white. Black microplastics were the most prevalent, comprising 64.48 % of all the particles extracted. This was followed by blue (27.36 %), pink (2.80 %), brown (2.56 %), red (2.27 %), white/transparent (0.29 %) and yellow/orange (0.24 %) being the least. The primary source determines the colour of microplastics although they could have been affected by UV radiation, weathering and microbial deterioration [52]. The colour trend of microplastics identified in this study was similar to those seen in other studies, where black and blue were the most prominent. This might be attributed to the colours of fishing nets and ground ropes, which were the main contributors of microplastic pollution in coastal areas [33]. Marine life such as fish and crustaceans had been observed to have a preference for dark microplastics because they resembled natural prey and food, thus increasing the risk of ingestion [9, 12, 42].

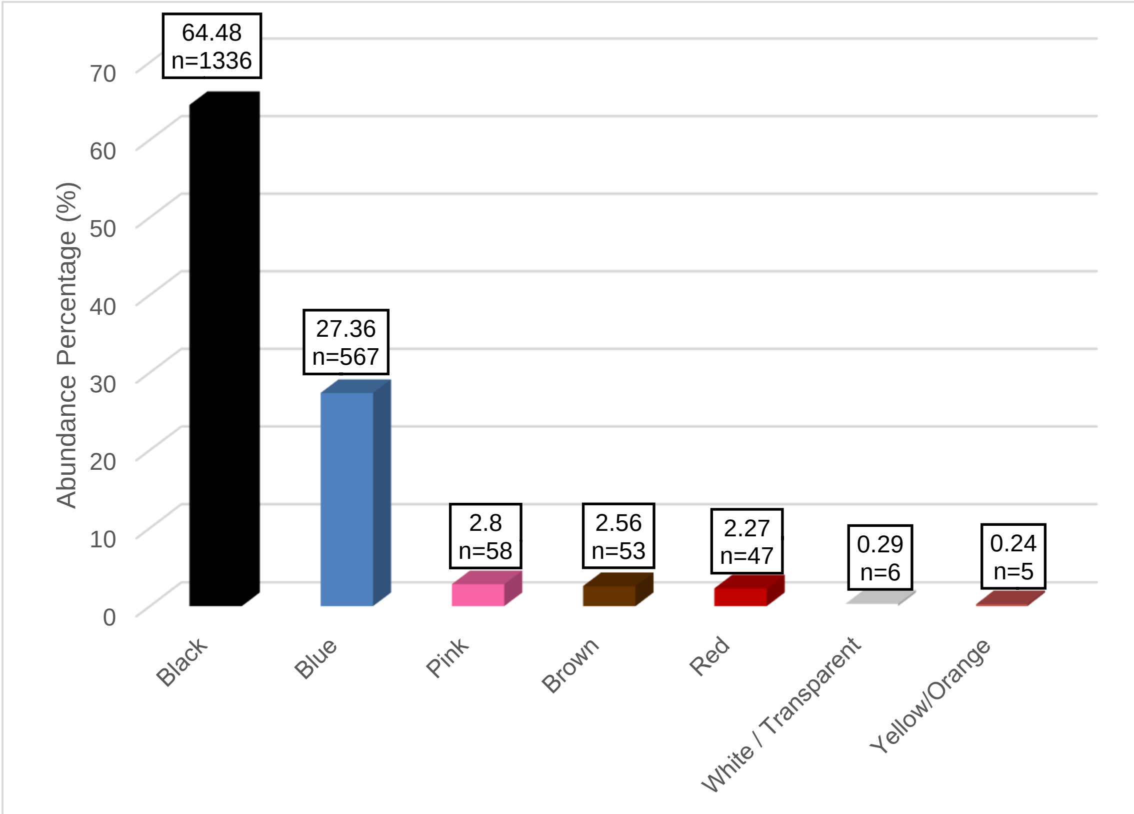

Figure 1. Percentage abundance (%) of microplastics isolated from soft tissue of *P. undulatus* based on colour

According to Figure 2, the size of the microplastic particles mostly ranged from 0.5 to 1 mm and 1 to 2 mm, accounting for 58.4 % and 21.57 % of the total particles, respectively. Microplastics that are larger than 5 mm were the least observed, comprising 0.68 % of all particles. The size of ingested microplastics depended on the clams' feeding mode. Small-sized microplastics were mostly ingested because these plastic particles were widely accessible in a diverse range of biota in both benthic and pelagic habitats. Smaller particles seemed to be more likely to be swallowed, causing stomach blockage, physiological consequences, chemical transfer and trophic transfer, while larger particles were prone to get entangled on marine life, which disrupted their movement and ability to hunt for food [5, 23, 28]. In certain cases, some feeding systems did not allow them to distinguish between prey and anthropogenic materials, while some might mistake plastics as prey and consume them directly [29].

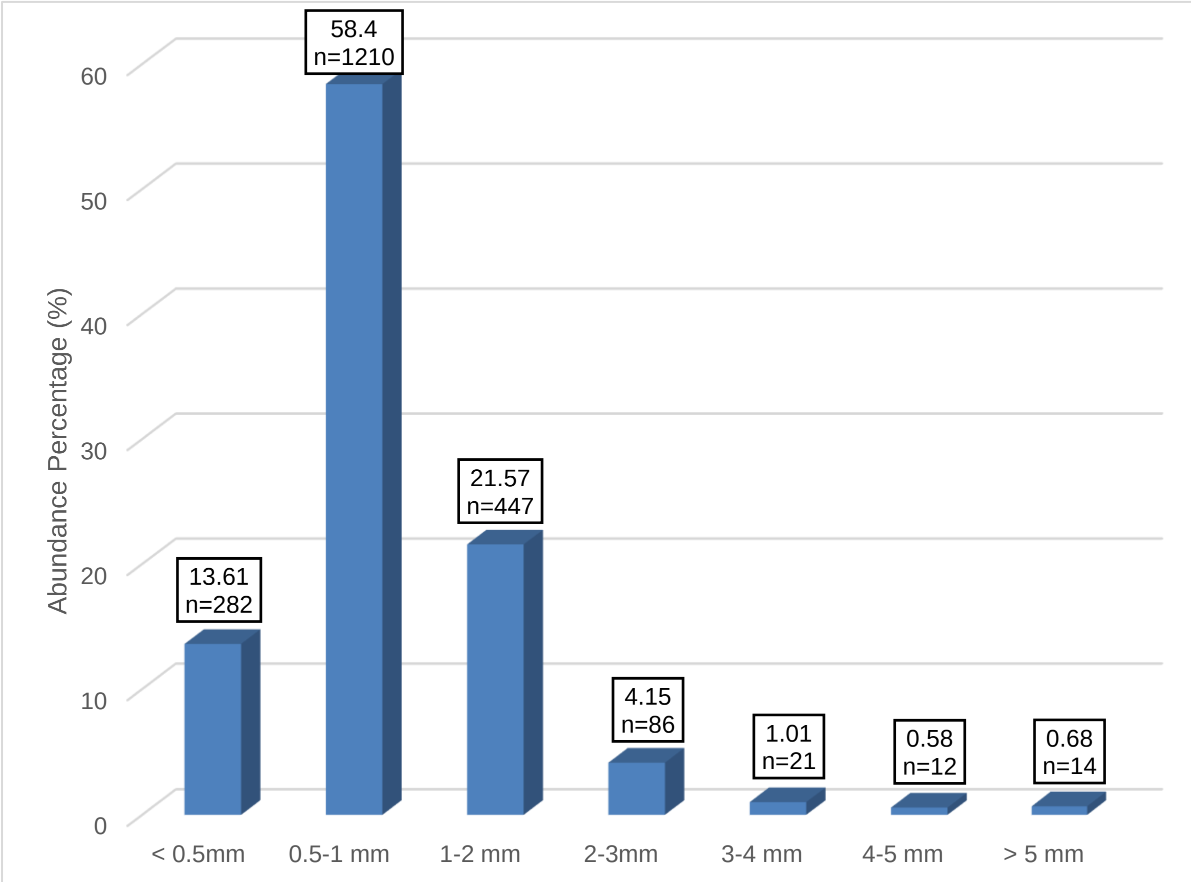

Figure 2. Percentage abundance (%) of microplastics isolated from soft tissue of *P. undulatus* based on size

As indicated in Figure 3, the most microplastics identified were fibres (97.2 %), followed by fragments. Fragmented microplastics accounted for 2.8 % only of the total. This result concurs with those observed in other studies [3, 10, 24, 40]. Asia is the world's biggest manufacturer of synthetic fabrics and largest contributor of microfibre pollutants [37]. Microfibre shedding from synthetic textiles in the clothing industry, cigarette butts, degradation of tyres, cosmetics, wastewater and fishing equipment were most likely the contributors of microfibres dispersion in the marine ecosystem [8, 30, 39, 49]. Ingestion of these fibrous microplastics has been reported in filter-feeding echinoderms [7, 13].

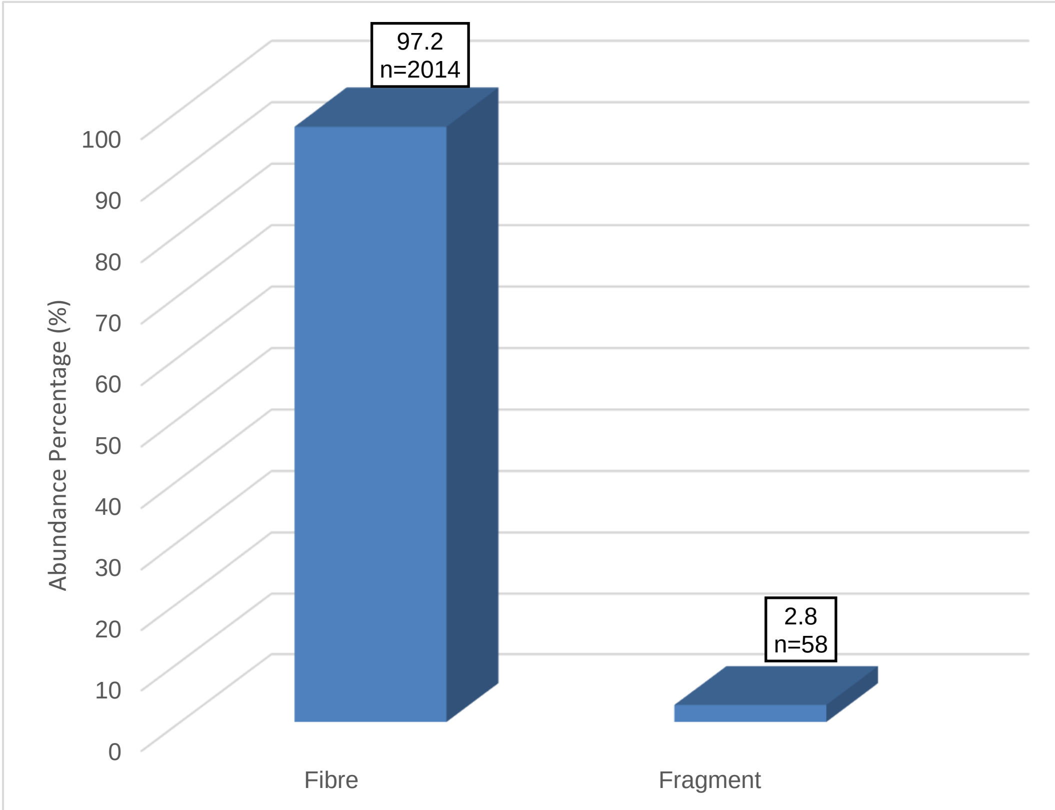

Figure 3. Percentage abundance (%) of microplastics isolated from soft tissue of *P. undulatus* based on shapes

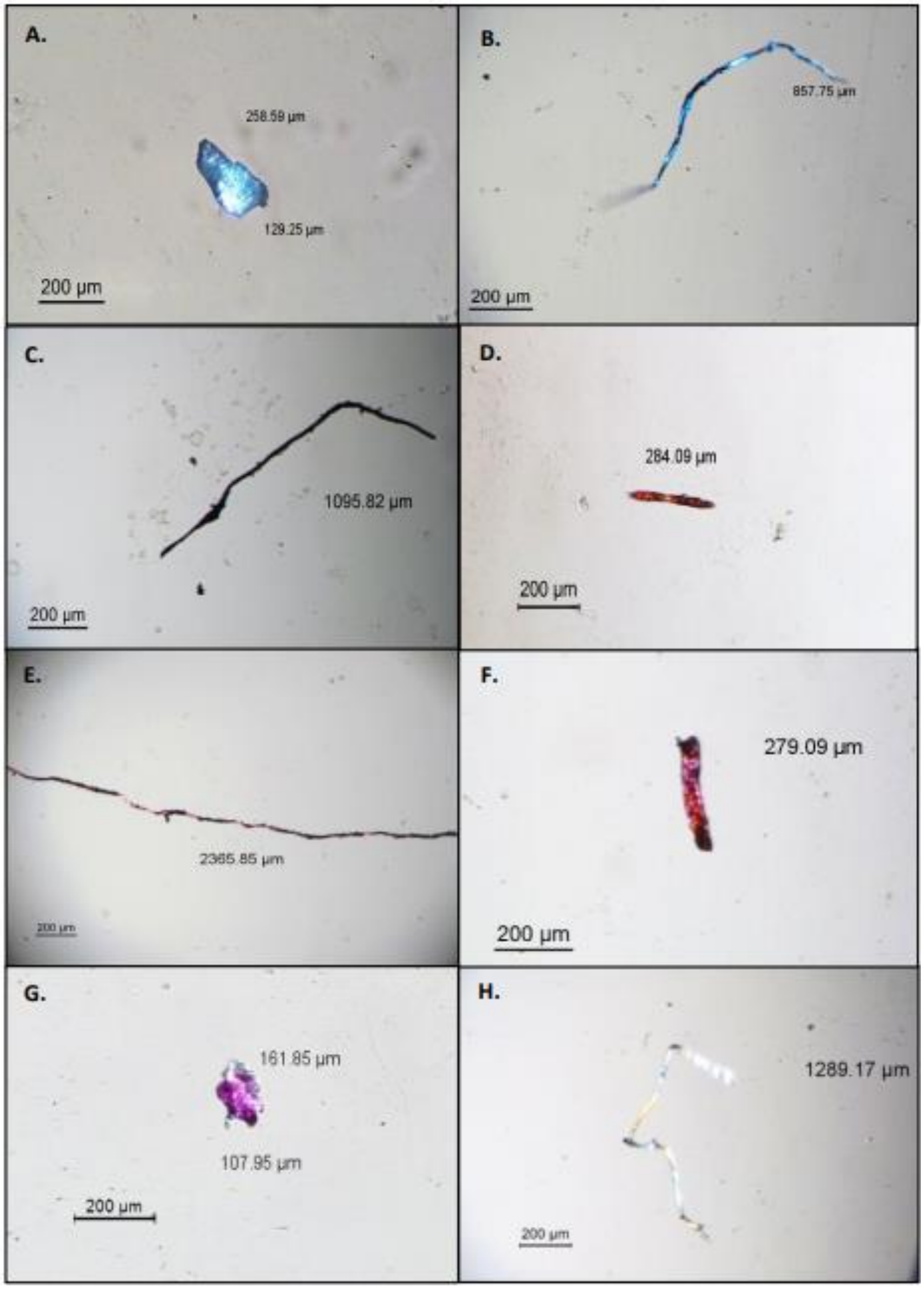

Figure 4. The shapes and colours of microplastic isolated from soft tissue of *P. undulatus.* **A)** Blue fragment, **B)** Blue fibre, **C)** Black fibre, **D)** Orange fibre, **E)** Pink fibre, **F)** Red fibre, **G)** Pink fragment, and **H)** White fibre [Scale bars: 200 µm]

Chemical Composition of Microplastics

The polymers found in microplastics extracted from *P. undulatus* samples in Kuala Selangor were mostly polystyrene and polymethyl methacrylate (PMMA). Figures 5 and 6 depict the FTIR spectra of the microplastic particles. In Figure 5, the FTIR spectrum shows the absorption band of aromatic C-H with the stretching vibration recorded around 2879.42 cm^{-1} to 2967.06 cm^{-1}. The absorption of the CH_2 bend was observed at 1465.05 cm^{-1}. Both FTIR polystyrene spectra carrying all major peaks were linked to pendant phenyls (C_6H_5) and were in close agreement with previous studies on the identification of commercial polymers of microplastics ingested by endangered marine animals and their environment [18, 34]. According to PlasticsEurope (2019), polystyrene is the fourth most plastic in demand in 2018 because of its application in food packaging and household items [16]. It was not surprising to find a high amount of this light-weight material as a microplastic waste in Kuala Selangor due to the town being a tourist attraction site due to improper littering practices and fishing hub, where polystyrene is used in most of the food and fish packaging. The high level of socio-economic activities may account for a significant part of pollution in this town [41]. The degradation of polystyrene waste into microparticles was likely to happen with direct exposure to sunlight, as well as abrasion from seawaves over a long period [5, 22].

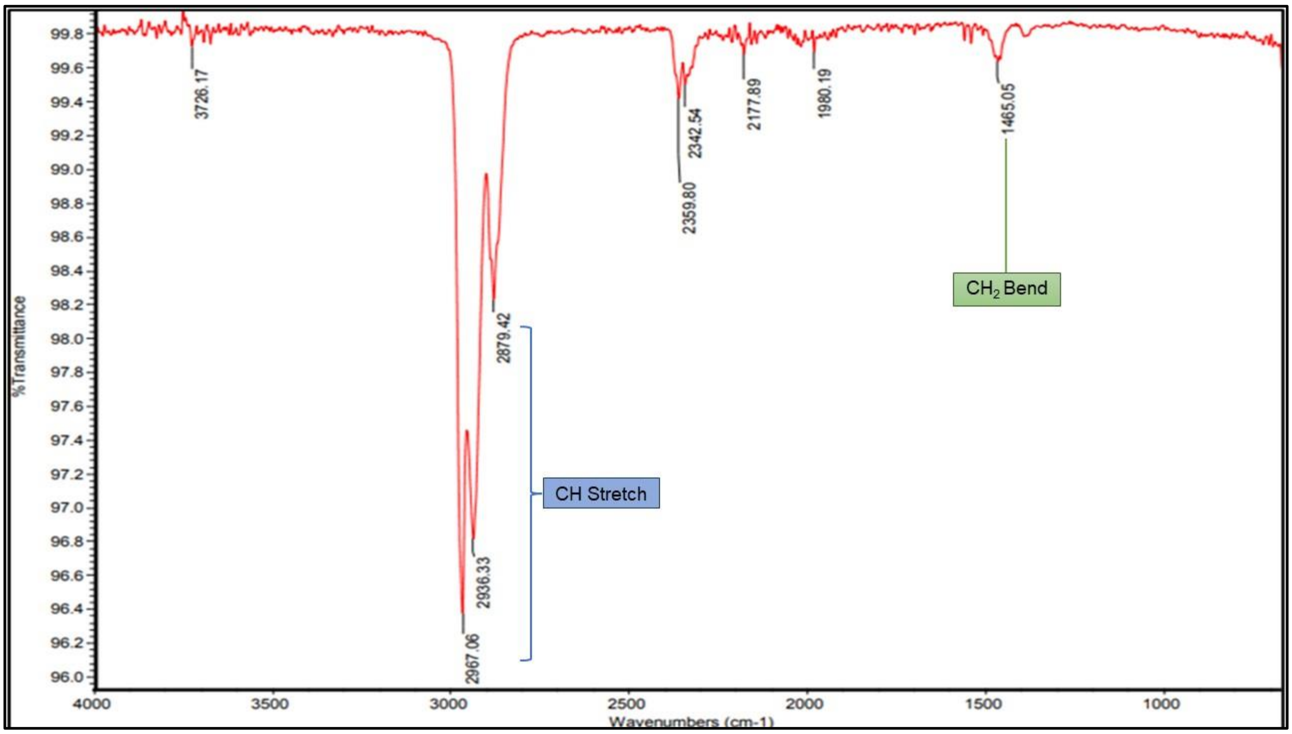

Figure 5. FTIR spectrum showing the functional groups of polystyrene composition obtained from microplastic particles extracted from *P. undulatus* samples

All significant peaks in Figure 6 for PMMA material were mainly composed of carboxylic esters and were identical to the spectrum reported by Jung *et al*. (2018) and Veerasingam *et al*. (2020). At peak range of 2821.84-2975.41 cm-1, the strong C-H stretch absorption spectrum could be noticed. The presence of the carbonyl compound stretching (C=O), which were related to PMMA polymers, exhibited a significant absorption bands at peak of 1731.20 cm^{-1}. The peaks of CH_2 bending deformation were found at 1456.87 cm^{-1}. The CH_3 bend was responsible for the high peaks at 1372.89 cm^{-1}. Medium bands at 1164.45 cm^{-1} were assigned to C-O bond stretching modes of PMMA at the fingerprint region, where the pattern of peaks was complex as each compound had its own distinct pattern. The C-H bend was shown at peak 978.18 cm^{-1}. PMMA, known as acrylic glass, is widely utilised in the automotive and maritime transport industries because of its excellent impact and ultraviolet resistance. Besides being a fishing area, Kuala Selangor is also located along one of the world's busiest shipping lanes, where anthropogenic activities would occur at a higher rate. Furthermore, rapid urbanization had also increased the level of microplastic pollution in Kuala Selangor. In the presence of oxygen, PMMA could undergo thermal oxidative degradation, which would result in it becoming brittle and breaking into small pieces [31, 41]. The denser PMMA particles would sink to the seabed and be ingested by filter feeders.

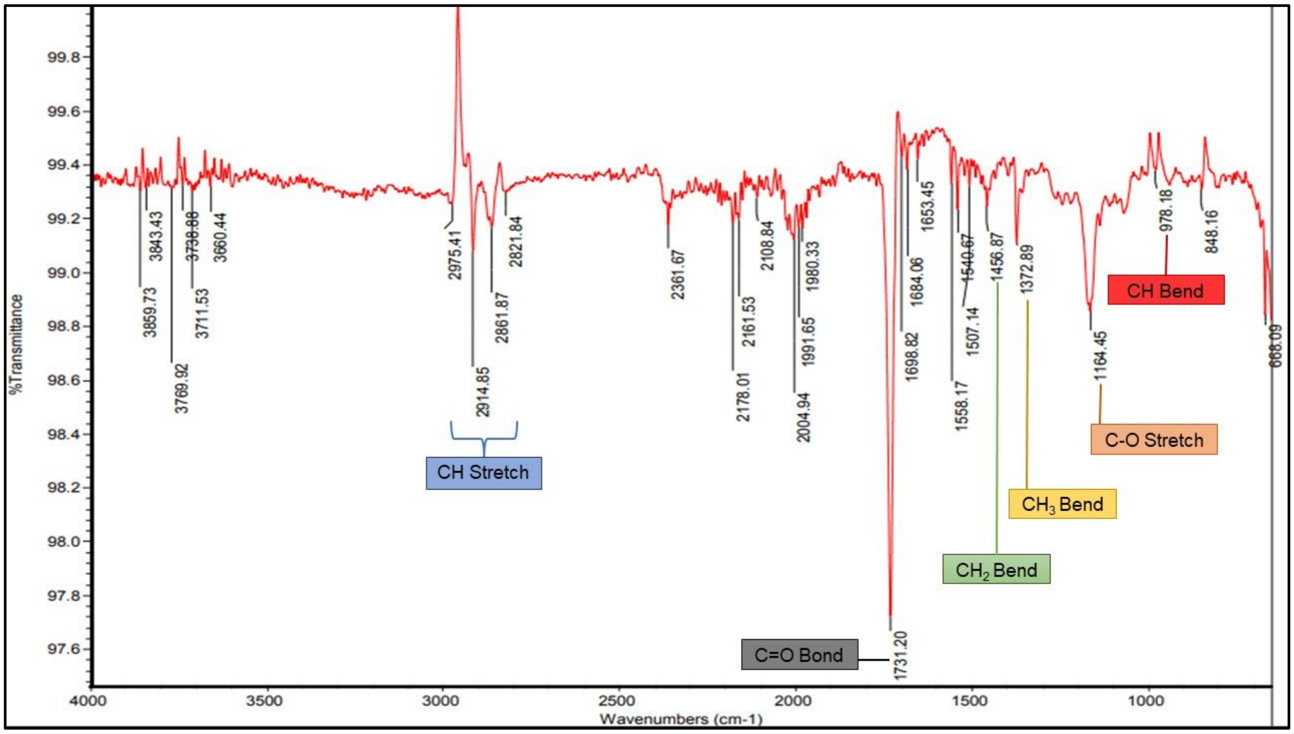

Figure 6. FTIR spectrum showing the functional groups of polymethyl methacrylate composition obtained from microplastic particles extracted from *P. undulatus* samples

Figure 7 depicts unidentified particles since the peaks of the functional groups were not significant. These microplastics could be mistaken for organic matter and other anthropogenic particles [32]. Algae and cellulosic fibres were some of the most frequent particles misidentified as plastics because they were easily breakable and had multiple cell patterns. In certain circumstances, chitinous materials could not be completely removed in the filtration process [29]. Other than that, the centre of spherical organic particles could appear similar to plastic films. Salt and sand fragments might also resemble plastic. Due to atmospheric microfibre pollution, natural polymers such as wool, linen and cotton could also contaminate the microscopic examination.

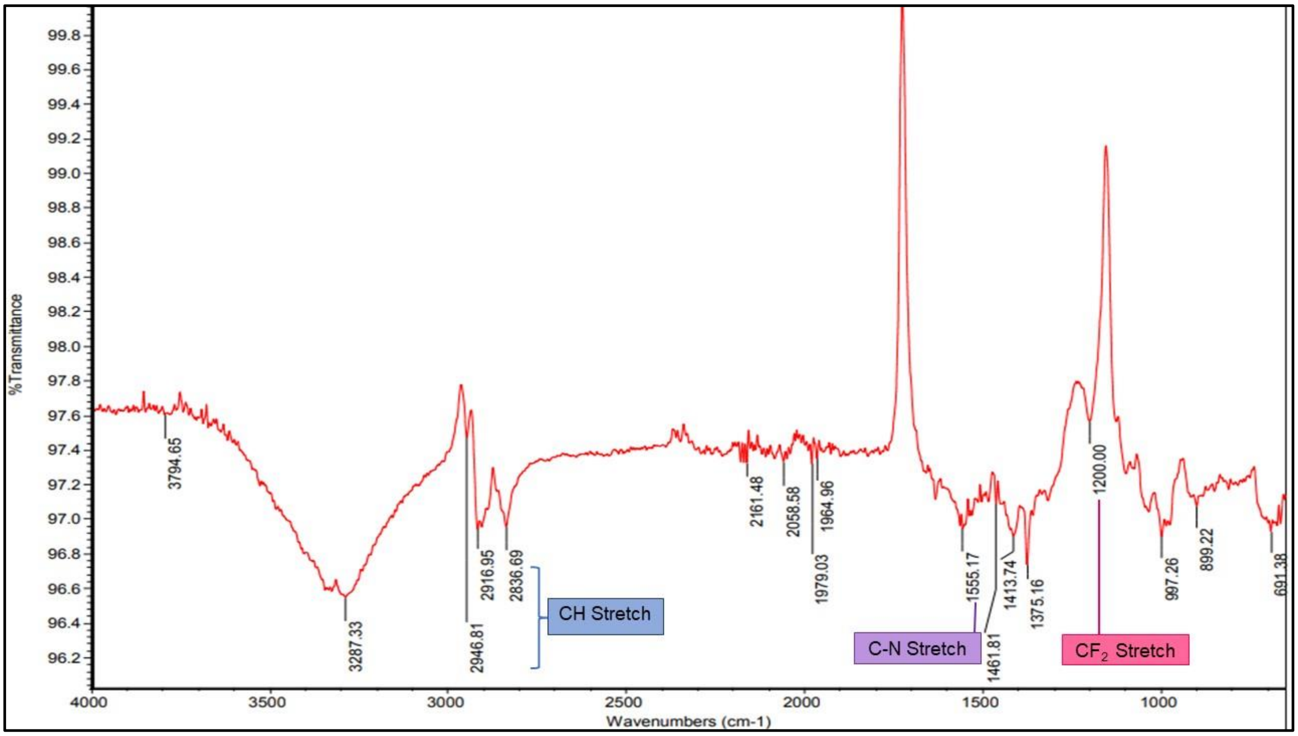

Figure 7. FTIR spectrum showing unidentified functional groups obtained from microplastic particles extracted from *P. undulatus* samples

Conclusions

Microplastics were isolated and identified from *P. undulatus*. Polystyrene and PMMA were the most common polymers found in *P. undulatus* harvested. It is undeniable that humans had been exposed to microplastics through their consumption of seafood. These findings could be utilised to conduct risk assessments to marine species and human health. Future research could also be conducted on various fishing sites off the coast of Kuala Selangor to monitor the distribution and severity of microplastic pollution.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

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