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RESEARCH ARTICLE

Occurrence and confirmation of microplastics in cockles and mussels from aelected sites on the West Coast of Peninsular Malaysia using FTIR analysis

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ABSTRACT

The global use of plastics continues to increase annually, leading to widespread accumulation of microplastics (<5 mm) in marine ecosystems. These degraded particles often settle in sediments and may be ingested by filter-feeding organisms, enabling microplastics to enter the aquatic food web and potentially pose toxicity risks to consumers. This study aimed to determine the occurrence, physical characteristics, and polymer types of microplastics present in the tissues of two commercially important shellfish species, cockles (*Anadara granosa*) and mussels (*Corbicula leana*). Three samples each of cockles and mussels (three replicates per site) were collected from Sebatu, Melaka, and Tanjong Karang, Selangor, representing selected sites along the west coast of Peninsular Malaysia. Possible microplastics were isolated after Nitric Acid (HNO_3) digestion and observed using light and fluorescence microscopy at 40× magnification. Physical characterisation revealed several distinct morphotypes, including red subangular jagged fragments, grey irregular fragments with surface grooves, black elongated fibres with broken edges, red subangular fragments, red spheruloid-shaped pellets, and crystalline clear thin elongated filaments. These characteristics are consistent with the commonly reported forms of microplastics in bivalves worldwide. Polymer identification using Fourier Transform Infrared (FTIR) spectroscopy (4 cm^{-1} resolution) confirmed the presence of Polypropylene (PP), Polystyrene (PS), and Polyethene (PE) as the dominant polymer types. Principal Component Analysis (PCA) demonstrated clear spatial separation between sampling sites and species, accounting for 96% of the total variance, with significant differences observed between groups ($p < 0.05$). Overall, this study provides important baseline data on the physical and chemical characteristics of microplastics in Malaysian shellfish and supports future efforts in environmental monitoring, seafood safety assessment, and marine pollution management.

1. INTRODUCTION

Plastics are materials made up of large organic molecules that can be transformed into a variety of products. Plastics are synthetic materials composed primarily of long-chain polymers derived from petrochemical sources such as oil, natural gas, and coal. Their durability, low cost, light weight, and versatility have led to extensive applications across numerous sectors, including packaging, construction, and consumer goods (D'ambrières, 2019). However, the rapid escalation in plastic production and consumption has resulted in widespread environmental contamination, particularly in aquatic ecosystems. It has been projected that the volume of plastic waste entering the oceans may increase fourfold between 2010 and 2050 if current trends continue. As larger plastic items undergo environmental weathering, they fragment into smaller particles known as microplastics through physical abrasion (e.g., wave and tidal action), chemical processes (e.g., oxidation and pollutant adsorption), and biological interactions such as biofilm formation (Ahmad et al., 2025). Additionally, microplastics are commonly defined as plastic particles smaller than 5 mm in size and are characterised by diverse shapes, sizes, and colours (Aparna, 2019).

In recent years, microplastics have been increasingly recognised as emerging food contaminants with potential implications for food safety and human health, owing to their persistence in the environment and their capacity to act as vectors for chemical toxicants (Karim et al., 2025). Moreover, the growing reliance on single-use polymeric products, including food packaging, containers, and plastic bottles—particularly associated with online delivery services—has further exacerbated microplastic pollution (Osman et al., 2022). Global plastic production now exceeds 320 million tonnes annually, posing substantial risks to ecosystems and potentially disrupting food chains through the accumulation and transfer of plastic-derived contaminants (Waring et al., 2018). Common sources of microplastics include degraded consumer products, such as bottles, wrappers, cups, and Polystyrene (PS)-based packaging. These materials may further fragment via photolysis, thermal oxidation, hydrolysis, and mechanical abrasion, generating secondary microplastics in the environment (Pfohl et al., 2022). In contrast, primary microplastics originate from intentionally manufactured small particles, such as microbeads in personal care products and industrial plastic pellets (Sarijan et al., 2019).

Furthermore, microplastics are of particular concern due to their ability to interact with and transport chemical pollutants within aquatic systems. When ingested by lower-trophic-level organisms, microplastics may cause false satiation, physical damage, or facilitate the transfer of hazardous compounds through the food web, thereby posing risks to both marine organisms and human consumers (Karami et al., 2018). Specifically, the Association of Southeast Asian Nations (ASEAN) region accounts for approximately 20% of global plastic production, with Malaysia as a notable contributor (AIT RRC.AP, 2018). Since the introduction of plastics in the 1950s, global plastic consumption has increased dramatically—from approximately 2 million tonnes to over 400 million tonnes by 2015—reflecting the scale of the environmental challenge posed by plastic waste.

Notably, Malaysia is among the largest consumers of plastic packaging, accounting for a substantial share of global marine litter (Zhang et al., 2023). Daily municipal solid waste generation in Malaysia ranges from 0.5 to 1.9 kg per capita, with plastics accounting for approximately 24% of total waste, making them the dominant component (Md Amin et al., 2020). Nonetheless, despite these statistics, comprehensive environmental data on microplastic contamination in Malaysian marine ecosystems remain limited. This lack of data is concerning, as unrealistic or poorly constrained exposure estimates may lead to uncertainty when assessing the ecological and health impacts of microplastics.

Additionally, recent estimates suggest that Malaysians consume an average of 16.78 kg of plastic packaging annually, with more than half of the resulting plastic waste eventually entering marine environments. Approximately 90% of marine plastic debris consists of microplastics, which can enter the human body via inhalation or ingestion, particularly through seafood consumption (Smith et al., 2018). Seafood, such as shrimp, bivalves, oysters, cockles, mussels, and fish, represents important dietary exposure pathways. Malaysia is among the world's top seafood-consuming nations, with an estimated per capita fish consumption of 56.5 kg per year—well above the global average of less than 20 kg (The Star, 2014; Izzah et al., 2016). This high consumption rate amplifies concerns regarding dietary exposure to microplastics.

On the other hand, shellfish aquaculture plays a significant role in Malaysia's fisheries sector, with the country accounting for approximately 93% of certain shellfish production in Asia, particularly adult cockles, which contribute more than 50% of national aquaculture output (Saffian et al., 2020). Filter-feeding bivalves such as cockles (*Anadara granosa*) and mussels (*Corbicula leana*) are especially vulnerable to microplastic ingestion due to their non-selective feeding behaviour (Yahya et al., 2023). Several Malaysian studies have reported significant microplastic contamination in these species. For example, Ratnam and Mohd Zanuri (2022) revealed an average of 5.9 ± 0.62 microplastic particles per blood cockle in Penang, while Aziz et al. (2024) documented high microplastic densities dominated by fibres and fragments in shellfish from Kuala Selangor. Similar findings have been identified for green mussels (*Perna viridis*) in Johor, where Polyethene (PE) and Polypropylene (PP) were commonly detected (Mat Zin et al., 2022). Likewise, saltwater clams (*Paratapes undulatus*) in Selangor exhibited predominantly black fibres and polymers, including PS and Polymethyl Methacrylate (PMMA) (Shukhairi et al., 2024).

Conversely, studies from the west coast of Peninsular Malaysia have further highlighted the potential for human exposure, reporting microplastic concentrations of 0.26 ± 0.15 particles g^{-1} in edible cockle tissues and estimated annual intakes of up to 93.5 particles per person (Foo et al., 2022). Regional reviews reinforce the widespread occurrence of microplastics in bivalves and emphasise their value as bioindicators of marine pollution (Tong et al., 2025). Nevertheless, spatial data remain scarce for strategic coastal locations such as Tanjong Karang (Selangor) and Sebatu (Melaka), which are situated near major industrial zones and busy shipping routes. These areas supply seafood to local communities and are therefore critical for assessing potential food safety risks. In addition, organic contaminants such as hexachlorinated hexanes and Polycyclic Aromatic Hydrocarbons (PAHs) may adsorb onto plastic debris and subsequently transfer to animal tissues upon ingestion, further facilitating contaminant entry into the food web (Conesa, 2022).

Although cockles and mussels can survive in polluted environments by accumulating contaminants in their tissues, this adaptive mechanism may inadvertently increase consumers' health risks. Persistent pollutants such as

Polychlorinated Biphenyls (PCBs) and mercury can enter aquatic ecosystems through industrial discharges, urban runoff, e-waste recycling, and atmospheric deposition (United States Environmental Protection Agency, 2019; Mohd Ali et al., 2025). Notably, these substances can adsorb onto sediments and microplastics, enhancing their transport within riverine and coastal systems. In Malaysia, rivers such as the Selangor River traverse highly industrialised regions before discharging into the Straits of Malacca, increasing the likelihood of plastic and chemical pollution (Othman et al., 2018). Similarly, Melaka lies along one of the world's busiest maritime trade routes, connecting the South China Sea and the Indian Ocean, where intensive shipping and industrial activities contribute to environmental contamination (Ahmed, 2025).

Therefore, given these considerations, the present study aims to assess the occurrence, physical characteristics, and polymer types of microplastics in the tissues of cockles, mussels, and shrimps collected from selected sites along the west coast of Peninsular Malaysia, specifically Tanjong Karang, Selangor, and Sebatu, Melaka. Microscopic examination combined with Fourier Transform Infrared (FTIR) spectroscopy was employed to confirm the presence and composition of microplastics. The findings are expected to provide valuable baseline data on microplastic contamination in Malaysian coastal seafood, supporting future research on marine pollution, seafood safety, and sustainable fisheries management. Furthermore, the results may inform policymakers, environmental agencies, and aquaculture stakeholders in developing effective monitoring strategies and mitigation measures to protect marine ecosystems and public health.

2. MATERIALS AND METHODS

2.1. Sample collection

Samples of cockles (*Anadara granosa*), mussels (C*orbicula leana*), and coastal water were collected at the sea located at Tanjung Karang, Selangor, and Sebatu, Melaka. Coastal water consists of seawater where the samples of cockles and mussels were cultivated. Approximately 2 kg of each shellfish species and 3 L of seawater were collected to screen and identify the presence of microplastics. The cockles and mussels from Tanjong Karang, Kuala Selangor, were harvested in an area around a 5 km radius from the seashore in March, July, and October 2021. The coordinates of the sampling sites for each sample were recorded. Table 1 and Figure 1 depict the coordinates and the location of the cockles and mussels collected at the cultivation site.

Note that all samples were stored in a PS box with a dry ice pack, and the temperature was maintained below 0°C during transportation. Upon arrival at the laboratory of the Faculty of Science and Technology, USIM, Nilai, Negeri Sembilan, Malaysia, about half of the cockle and mussel samples were shucked and immediately processed prior to physical analysis. In contrast, the remaining samples were packed in an aluminium pouch and stored at -20°C for further analysis. Coastal water, on the other hand, was stored in the glass container at -20°C. All analyses were done in triplicate.

Table 1: Coordinates of the cockles and mussels sampling site

Samples	Sampling site			
	Tanjong Karang, Selangor		Sebatu, Melaka	
	Latitude	Longitude	Latitude	Longitude
Cockles	3°13'29.8"N	101°17'19.4"E	2°05'59.7"N	102°27'16.4"E
Mussels	3°14'41.3"N	101°17'34.3"E	2°06'43.4"N	102°27'16.4"E

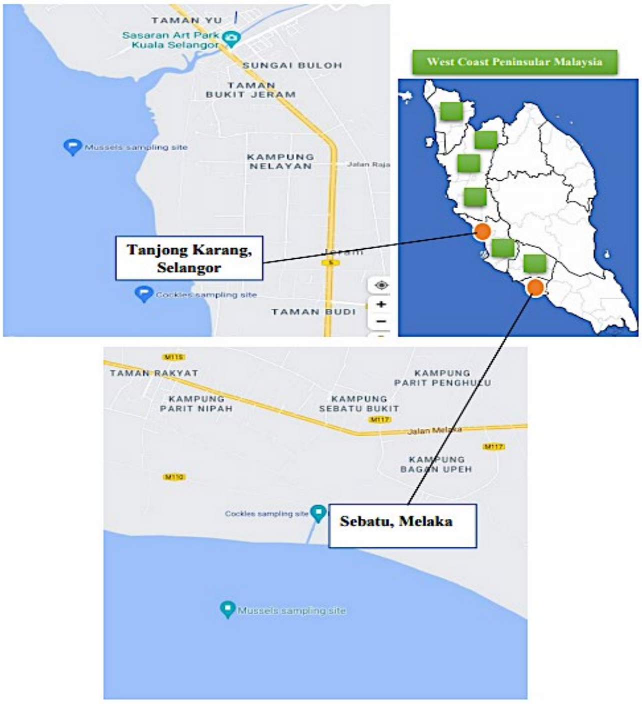


Figure 1: Sampling sites of cockles and mussels from two strategic sites on the West Coast of Peninsular Malaysia

2.2. Microplastic screening on cockles, mussels, and seawater samples

Microplastic extraction from mussel and cockle tissues was conducted based on the method reported by Espiritu et al. (2019), with minor procedural adjustments. Prior to tissue processing, the shell length and wet weight of the organisms were recorded. Samples were prepared in triplicate, with each replicate consisting of 30 g of shucked cockle or mussel tissue. In particular, the tissues were transferred separately into clean conical flasks and dried in a laboratory oven at 60°C for at least 2 hours to remove residual moisture.

Following dehydration, 20 mL of concentrated Nitric Acid (HNO_3; Merck, Germany) was added to each flask to facilitate chemical digestion of organic material. The digestion process was allowed to proceed for approximately two days until complete breakdown of soft tissues was achieved. Additionally, to enhance digestion efficiency, the samples were subsequently heated on a hot plate at 80–90°C for 20 minutes. After cooling, each digest was diluted with 20 mL of pre-heated distilled water (80°C).

Subsequently, the resulting solutions were filtered under vacuum using glass fibre grade C (Whatman GF/C glass-fibre) filters. The filters were transferred to glass Petri dishes and dried at 50°C for 1–2 hours until constant dryness was attained. Potential microplastic particles retained on the filters were examined using both light and fluorescence microscopy at 40× magnification. Suspected microplastics were documented and categorised by sampling location and organism type, and their physical characteristics were described according to the classification criteria presented in Table 2.

Following this, microplastic assessment in mussels and cockles was conducted according to Espiritu et al. (2019) with slight modifications. The weight and length of the remaining mussels and cockles were measured before shucking in triplicate, each consisting of 30 g of shucked cockles and mussels. The cockles and mussels were placed in separate conical flasks, then oven-dried at 60°C for at least 2 hours until all the water had dried out. After oven-drying, 20 mL of concentrated HNO_3 (Merck, Germany) was added to each sample, which were then left to dry for at least 2 days until no more organic material remained and the digestion had reached its optimum. Moreover, each sample was heated on a hot plate at 80–90°C for 20 minutes. Consequently, 20 mL of warm distilled water (80°C) was added to each conical flask prior to dilution. The samples were then subjected to vacuum filtration (Whatman Grade GF/C). The filters were eventually placed on glass Petri dishes and oven-dried at 50°C for 1-2 hours until the samples were dry. The dried samples on the filter papers were then observed under a light and fluorescence microscope (magnification: 40x) for the detection of potential microplastic particles. Identified microplastics were documented and characterised using descriptions based on location and sample type, as demonstrated in Table 2.

2.2.1 Assessment of microplastics in seawater samples

The extraction of microplastics from coastal water samples was performed following the approach described by Espiritu et al. (2019), with minor methodological modifications. Water samples were first vacuum-filtered through a filter paper saturated with distilled water to concentrate suspended particulate matter. The retained solids were transferred into pre-weighed glass beakers and dried in an oven at 90°C until a constant mass was obtained (± 0.0001 g).

To further remove residual organic matter and enhance microplastic recovery, Wet Peroxide Oxidation (WPO) was applied. Briefly, 20 mL of 0.05 M aqueous ferrous sulphate ($FeSO_4$; R&M, Malaysia) was added to the dried material, followed by the gradual addition of 20 mL of 30% Hydrogen Peroxide (H_2O_2). The reaction mixture was allowed to stand at room temperature for 5 minutes to ensure homogeneity, after which it was heated on a hot plate with continuous stirring at 75°C until effervescence was observed. The mixture was subsequently removed from the heat and allowed to cool until bubbling subsided. Notably, this heating–cooling cycle was repeated for an additional 30 min. Where necessary, supplementary aliquots of 30% H_2O_2 (5 mL) were added incrementally until complete digestion of organic material was achieved.

Furthermore, density separation was then conducted by dissolving Sodium Chloride (NaCl; Merck, Germany) into the oxidised solution at a ratio of 6 g per 20 mL. The mixture was agitated in a shaking incubator at 50°C and 100 rpm for 15 minutes to ensure complete dissolution of the salt. The solution was transferred into a clean beaker and allowed to stand undisturbed overnight to facilitate phase separation (Masura et al., 2015). Floating particles were thereafter recovered by filtration and examined under a light microscope at 40x magnification. Suspected microplastics were visually categorised according to particle morphology (e.g., fragments, fibres, and pellets) and colour characteristics commonly reported in environmental samples (Table 2). The isolated particles were transferred into glass vials and oven-dried at 40°C to minimise spectral interference prior to FTIR analysis. Additionally, physical characterisation of microplastics was conducted following the classification criteria outlined by Hidalgo-Ruz et al. (2012).

2.2.2 Characterisation of the microplastics isolated from the samples

The potential microplastic particles detected under light microscopy were subsequently classified and described based on multiple criteria. This includes particle size (estimated using a 5 mm scale reference), polymer composition (confirmed by FTIR analysis), particle type and morphology, surface erosion features, and colour, in accordance with the classification framework presented in Table 2. Information on the likely origin of the particles, together with their shape and degree of surface degradation, was also documented.

Table 2: Characterisation of microplastics according to Hidalgo-Ruz et al. (2012).

Characteristics	Description
Source	Consumer product degradation and raw industrial fragment
Type	Plastic pellet, fragment, plastic film, filament, granule, foamed plastic, and Styrofoam
Shape	For fragment: Rounded, subrounded, subangular, angular For pellet: cylindrical, flat, disk, ovoid, spherical General: irregular, degraded, elongated, rough, and broken edges
Erosion	Fresh, linear fractures, irregular surface, unweathered, and level of crazing (conchoidal fractures), weathered, incipient alteration, grooves, jagged fragments, subparallel ridges, and degraded
Colour	White, crystalline, clear-white, orange, cream, blue, red, black, opaque, brown, pink, green, grey, yellow, tan, and pigmentation

Source: (Espiritu et al., 2019)

2.2.3 Identification of microplastics by FTIR analysis

Individual microplastic particles obtained from the physical analysis were isolated and stored in a small glass vial prior to chemical analysis. The transmission spectra were generated using an IRTracer-100 FTIR spectrometer (PerkinElmer, USA) with 8 scans averaged at a resolution of 4 cm^{-1}. The spectral range was set to 4000-600 cm^{-1}. The spectra obtained were compared with the microplastic standards of PE, PP, and PS obtained by FTIR spectroscopy.

2.3 Statistical analysis

Statistical data analysis was performed using Minitab Version 17 (Stat Inc., USA). A two-way Analysis of Variance (ANOVA) was conducted to analyse the distribution of microplastics across locations and sample types. Differences of p-value ($p < 0.05$) were considered statistically significant. Principal Component Analysis (PCA) was performed on a data matrix of FTIR spectra of functional group presence for each sample from two strategic sampling sites, compared with polymer types of microplastic standards, to evaluate the relationships among microplastic presence, sample type, and sampling site. Specifically, PCA analysis was performed using Unscrambler X Version 10.4 (CAMO, USA).

2.4 Quality control measures

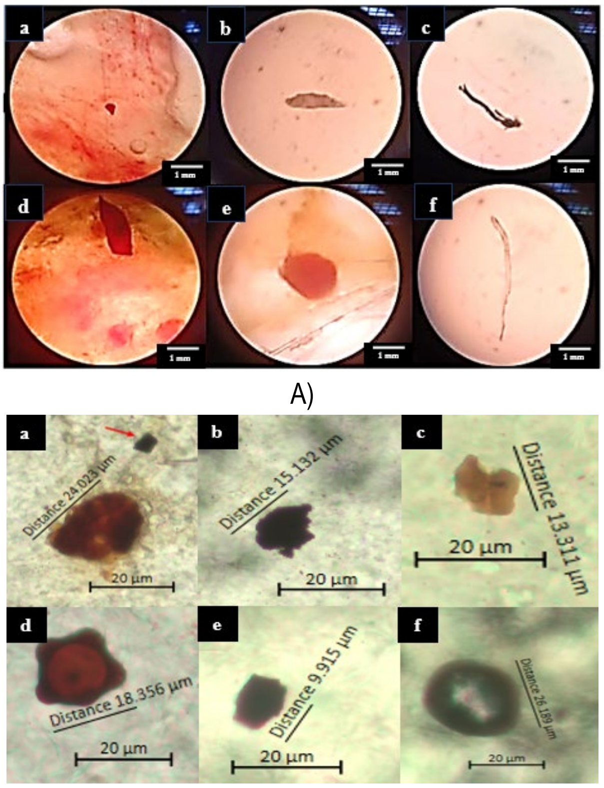
To minimise errors and airborne contamination throughout all steps of the experiment, including sampling, handling, processing, and analysis, safety measures need to be implemented (Hermsen et al., 2018). Furthermore, to prevent contamination, all liquids, including distilled water, ethanol, and HNO_3, were filtered through cellulose nitrate membrane filters with a 0.45 µm mesh size (Zaki et al., 2021). All glassware was rinsed with filtered distilled water and 70% ethanol prior to use. Note that all apparatus, including Petri dishes, was made from non-plastic polymer to avoid contamination from plastic debris coming into contact with the samples. The entire preparation and extraction procedure was performed under a fume hood, except for microscope analysis, to minimise foot traffic and reduce the possibility of airborne microplastics. All the glassware utilised in the preparation and extraction protocol was covered with aluminium foil to prevent airborne contamination.

3. RESULT AND DISCUSSION

3.1 Physical analysis of microplastic presence in cockles and mussels collected from Tanjong Karang, Kuala Selangor

In this study, microplastics in shellfish tissue were screened using light and fluorescence microscopy after digestion of organic material. The digestion protocol described by Huang et al. (2023) initially employed HNO_3 at high concentration, enabling extensive removal of biological tissue but raising concerns about polymer damage. Notably, recent method evaluations indicate that while HNO_3 remains effective, acid-based digestion may degrade certain polymers and alter spectral signatures during FTIR/Raman identification (Zeng et al., 2025). As such, this approach followed a modified protocol that balanced digestion efficiency with polymer preservation as recommended in recent reviews of microplastic pre-treatment (Murugan et al., 2023). The microscope was coupled with a camera to capture the possible presence of microplastics. Moreover, the observation and characterisation of possible microplastics were performed by microscopy (light and fluorescence) and confirmed via FTIR spectroscopy, following established protocols (Bitencourt et al., 2020) and more recent method reviews (Murugan et al., 2023). Initial identification of suspected microplastics was performed through visual screening, a widely applied approach for preliminary differentiation prior to spectroscopic confirmation, according to its physical attributes such as shape, size, colour, and appearance (Kundu et al., 2021).

Consequently, the result demonstrates that the shape and colour identification of the microplastics presence in cockles` tissues collected from Tanjong Karang, Selangor, under a light microscope were categorised into red subangular jagged fragment, grey irregular shape with groove, black elongated fibre with broken edges, red subangular fragment, red spheruloid-shaped pellet, and crystalline clear thin elongated filament (Figure 2A). On the other hand, under a fluorescence microscope, the shape and colour identification of the microplastics present in similar samples were categorised into orange degraded fragment, black subangular jagged fragment, yellow irregular shape with groove, red degraded fragment with broken edges, grey subangular fragment, and grey spheruloid-shaped pellet (Figure 2B). The size of microplastics is approximately 10 µm to 50 µm (< 1 mm).



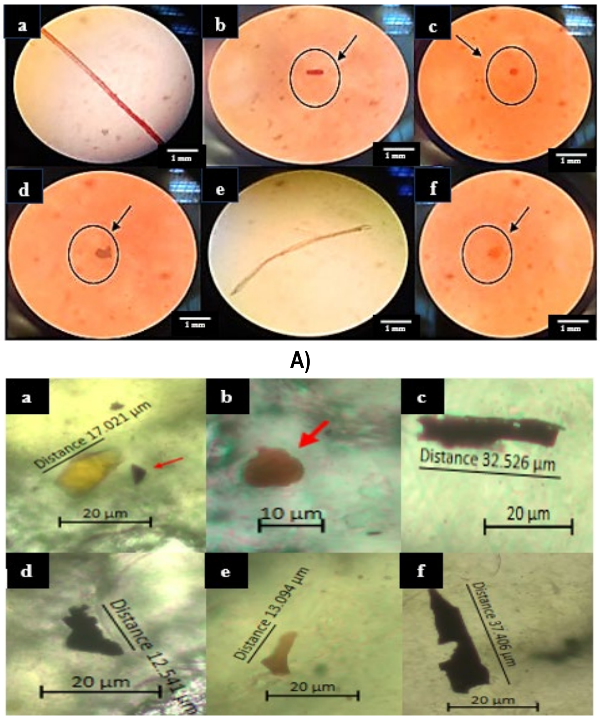
A)

B)

Figure 2. Microplastic identification on cockles' tissues from Tanjong Karang, Selangor. Microplastic shaped and color identification from light microscope (A) are categorised into red subangular jagged fragment (a), grey irregular shape with groove (b), black elongated fibre with broken edges (c), red subangular fragment (d) red spheruloid-shaped pellet (e) and crystalline clear thin elongated filament (f) (scale bars: 1 mm) and fluorescent microscope (B) are categorised into orange degraded fragment (a), black subangular jagged fragment (b), yellow irregular shape with groove (c), red degraded fragment with broken edges (d), grey subangular fragment (e) and grey spheruloid-shaped pellet (f) (scale bars: 20 µm).

Generally, the most abundant characteristic identified in the cockles' tissues from Tanjong Karang was a degraded fragment with an irregular shape. Based on the observation, degraded fragments, filaments, and pellets are the dominant shapes of microplastics in cockle and mussel tissues. This finding aligns with broader regional and global patterns. For instance, a 2025 systematic review of microplastic contamination in Thailand revealed that fibres (especially PE, PP, and polyester) were the overwhelmingly dominant form in filter-feeding organisms such as mussels and shrimp, highlighting that these polymers are highly bioavailable in benthic (bottom) habitats (Sawangproh & Paejaroen, 2025). Thus, this supports the hypothesis that the types of microplastics detected in the shellfish reflect pervasive regional pollution sources (e.g., consumer plastics, clothing, fishing gear).

Additionally, the possible appearance of microplastics in mussel tissue collected from the exact location under a light microscope was categorised into red thin elongated filament, red degraded fragment, orange spheruloid-shaped pellet, brown jagged fragment with irregular surface, brown long linear fracture, and orange irregular spheruloid-shaped pellet (Figure 3A). Subsequently, with similar samples under a fluorescence microscope, the shape and colour of microplastics were categorised into yellow degraded fragment, red irregular spheruloid-shaped pellet, brown thick-elongated filament, black jagged fragment with irregular surface, yellow degraded fragment, and black degraded fragment with linear fracture (Figure 3B).



A)

B)

Figure. 3: Microplastic identification on mussels' tissues from Tanjong Karang, Selangor. Microplastic shaped and color identification from light microscope (A) are categorised into red thin elongated filament (a), red degraded fragment (b), orange spheruloid-shaped pellet (c), brown jagged fragment with irregular surface (d), brown long linear fracture (e) and orange irregular spheruloid shaped pellet (f) (scale bars: 1 mm) and fluorescent microscope (B) are categorised into yellow degraded fragment (a), red irregular spheruloid shaped pellet (b), brown thick-elongated filament (c), black jagged fragment with irregular surface (d), yellow degraded fragment (e) and black degraded fragment with linear fracture (f) (scale bars: 20 µm).

Since microplastics may be embedded within tissues and are therefore difficult to isolate, tissue and gut contents are often digested using oxidative, acid, alkaline, or enzymatic methods prior to visual separation (Dey et al., 2021). However, in this experiment, digestion with HNO_3 and moderate temperature exposure (85°C for 20 minutes) were sufficient compared to the thermal degradation process, which requires temperatures exceeding 200°C to cause polymer degradation of plastics (Ibrahim et al. 2018). Furthermore, optimum tissue digestion of cockles and mussels using the following method is sufficient, where it has been reported that applying strong organic acids such as HNO_3 is associated with outstanding results for tissue digestion regarding biota tissue, mainly consisting of carbohydrates, proteins, and fats (Jaafar et al., 2020).

Notably, one of the microplastics discovered in cockles' tissues collected from Tanjong Karang, Selangor, demonstrates a clear crystalline colour under a light microscope. Hence, it is believed that microplastics may originate from plastic packaging (Emenike et al., 2023). In a study by Sarijan et al. (2019), microplastics identified in the gastrointestinal tracts of various species in the Skudai River, Malaysia, were characterised as fibrous, small foam, thin filaments, and a variety of colourful mixtures of film and degraded fragments, such as red, blue, pink, purple, and white. Nevertheless, the colour of microplastics can sometimes be misidentified under light microscopy due to uncertainty about the exposure period and environmental conditions. Essentially, microplastics exposed to environmental conditions over uncertain periods, such as physical abrasion, solar radiation, and biological processes, may undergo physical changes and colour variants (Binda et al., 2023).

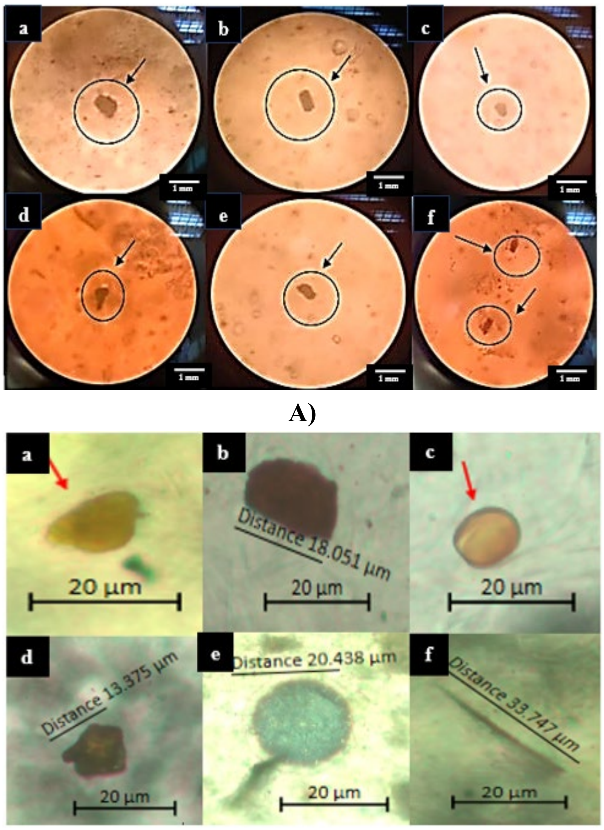
Notably, the most common microplastics' colour detected on both cockles and mussels' tissues was grey and red. Three common shapes of microplastics observed in the tissues of cockles and mussels from Tanjong Karang, Kuala Selangor were filament or fibre, fragment, and pellet. These three prevalent types of microplastics identified on the shellfish, with a variety of colours, were associated with particles originating from the degradation of intact plastic debris. Correspondingly, it was ingested by the species, dominating and accumulating in the tissues due to their filter-feeding properties. Conversely, the presence of intact plastic debris may be linked to intensive offshore human activities, including commercial fishing operations, waste and sewage discharge, maritime traffic, and shellfish aquaculture. It represents major marine-based sources of plastic inputs that can ultimately accumulate within the tissues of filter-feeding organisms (Thushari & Senevirathna, 2020). This intact debris may be associated with the abundance of offshore activities, such as commercial fisheries, waste or sewage disposal, navigation, and shellfish culture sites, as key marine-based sources that contribute to the accumulation of plastic debris, which ends up in the tissues of filter feeders (Thushari & Senevirathna, 2020). On the other hand, environmental factors, such as pH, temperature, salinity, and physical abrasion from waves, tides, and currents, may lead to variation in the characteristics and distribution of microplastics in aquatic environments (Zhang et al., 2019). Other microplastics may also come from manufacturing and packaging processes (Chaplin et al., 2018).

As the Selangor River flows south-eastward, traversing about 110 km before passing into the Straits of Malacca (Othman et al., 2018), it is an industrialised area with mining activities that contribute to plastic waste entering the sea. Notably, PE was reported as the dominant polymer detected in filter-feeding organisms, accounting for 88.4% of identified microplastics, followed by PP (9.3%) and PE terephthalate (2.3%) (Karbalaei et al., 2019). The prevalence of these polymers in cockle and mussel tissues is likely influenced by nearby anthropogenic activities, including residential settlements, port operations, industrial zones, and fishing-related infrastructure. In particular, the occurrence of microplastics at Tanjong Karang may reflect inputs from surrounding port facilities, coastal communities, industrial activities, and jetty-based fishing operations. According to Lusher et al. (2015), fishing and shipping activities may contribute to the presence of microplastics in less densely populated and non-urbanised areas.

On the contrary, high use of plastic materials, such as face masks, gloves, personal protective equipment, and food packaging, also increases the likelihood of microplastic contamination in shellfish. Disposable face masks made from PS and PP (Aragaw, 2020) can leach into the sea, where they are unintentionally taken up by filter feeders such as cockles and mussels. These products may enter aquatic and terrestrial environments in various ways, including flooding, leaching, littering, and blowing, introducing environmental chaos caused by microplastic contaminants (Chaplin et al., 2018). Simultaneously, plastic food packaging also received significant attention due to delivery issues. A study reported that the most prevalent characteristics of microplastics detected in Malaysian marine water were fragment or fibre type and high-density polymers (>1.02 g cm-3), such as polyester, PS, polyamide, polyvinyl chloride, PE, and PP (Karbalaei et al., 2019). Food plastic packaging widely consumed during pandemics also originated from polymers such as PE terephthalate, PE, PP, PS, and even polyvinyl chloride (Binda et al., 2022). The accumulation of these plastic polymers in the human food chain can pose hazards and affect human health. Therefore, the identity of microplastic polymer on cockles and mussels' landings from Tanjong Karang, Kuala Selangor, was further analysed and confirmed using FTIR spectroscopy.

3.2 Physical analysis of microplastic presence in cockles and mussels collected from Sebatu, Melaka

The results demonstrate that the shape and colour identification of microplastic presence in cockles' tissues collected from Sebatu, Melaka, under a light microscope were categorised into grey or black degraded fragments with irregular surfaces, degraded fragments, spheruloid-shaped pellets, and jagged fragments with broken edges (Figure 4A). On the other hand, under a fluorescence microscope, the shape and colour identification of the microplastics in similar samples were categorised as yellow degraded fragment with irregular surface, red degraded fragment, orange and green spheruloid-shaped pellets, brown jagged fragment with broken edges, and greyish-black thin elongated filament (Figure 4B). In general, almost all the microplastics revealed in the cockles' tissues were degraded fragments with irregular surfaces and shared a grey colour.

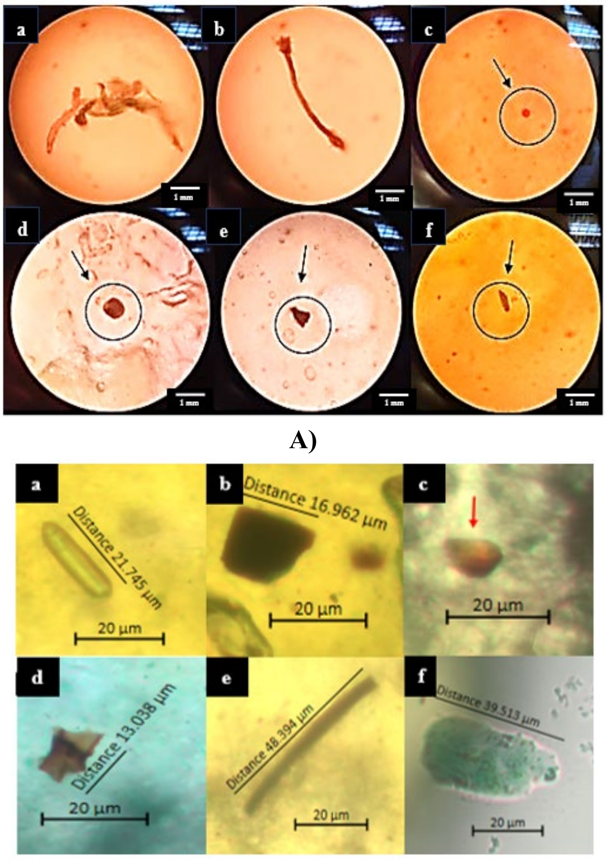


A)

B)

Figure 4. Microplastic identification on cockles' tissues from Sebatu, Melaka. Microplastic shaped and color identification from light microscope (A) are categorised into grey or black of degraded fragment with irregular surface (a), degraded fragment (b, e, f), spheruloid-shaped pellet (c), and jagged fragment with broken edges (d) and fluorescent microscope (B) are categorised into yellow degraded fragment with irregular surface (a), red degraded fragment (b), orange and green spheruloid-shaped pellet (c, e), brown jagged fragment with broken edges (d), and greyish black thin elongated filament (f) (scale bars: 20 µm).

Furthermore, as for mussels' tissues from Sebatu, Melaka, the result illustrates that the shape and colour identification under the light microscope were categorised into red degraded fragment with conchoidal fractures, red thin elongated filament with broken edges, red spheruloid-shaped pellet, black subangular jagged fragment, and grey degraded fragment (Figure 5A). In contrast, under a fluorescence microscope, the shape and colour of the microplastic in the same samples were categorised into clear cylindrical fragment, black, red, and green degraded fragment, orange subangular jagged fragment, and black thin elongated filament (Figure 5B). However, the spheruloid shape pellet was dominantly observed in mussels' tissues, mostly grey and red in colour. This suggests that dark-coloured microplastics, such as black, grey, and red, were ingested by filter feeders because they appeared like food to them, unlike light-coloured microplastics (Zhao et al., 2015).



A)

B)

Figure 5. Microplastic identification on mussels' tissues from Sebatu, Melaka. Microplastic shaped and color identification from light microscope (A) are categorised into red degraded fragment with conchoidal fractures (a), red thin elongated filament with broken edges (b), red spheruloid-shaped pellet (c, d), black subangular jagged fragment (e) and grey degraded fragment (f) and fluorescent microscope (B) are categorised into clear cylindrical fragment (a), black, red and green degraded fragment (b, c, f), orange subangular jagged fragment (d) and black thin elongated filament (e) (scale bars: 20 µm).

Melaka, which is strategically located at the Straits of Malacca, is the sea passage connecting the South China Sea and the Indian Ocean. The Strait of Malacca is rich in aquatic resources and ranks among the busiest shipping lanes in the world, with upwards of 90,000 vessel transits annually and handling about 30% of global trade flows (WEF, 2024; Ahmed, 2025). Therefore, this strategic site is highly likely to be contaminated with microplastics due to the world trade shipping route and rapid industrial development. Additionally, from a food-safety and ecological-risk perspective, the prevalence of PE, PP, and PS in edible shellfish tissues is concerning. These polymers are among the most used globally and are common in packaging, textiles, and fishing gear. The Thai systematic review (Sawangproh & Paejaroen, 2025) underscored that these polymers are among the most frequently detected in filter feeders, making the findings directly relevant to regional risk assessments. Given that filter-feeding bivalves non-selectively filter particles from their environment, the ingestion of these microplastic polymers may represent a direct route of exposure to humans, particularly in regions with high shellfish consumption.

Zhao et al. (2015) also reported that coloured microplastics predominated in the subsurface of estuaries in urban areas. Different colours may provide clues about the origin of plastic materials (Khalik et al., 2018). Notably, black or dark grey-coloured microplastics were detected, which may be associated with the degradation of synthetic fibres, tyres, and fishing gear at strategic sites in Melaka, which are at the centre of the Straits of Malacca (Zaki et al., 2021). The presence of microplastics indicates direct sources from multiple commercial activities and the primary route for ships, boats, and yachts (Acarer Arat S. 2025). As Melaka is a strategic site for high economic growth, industrialisation, and urbanisation, it generates higher waste volumes. Its inappropriate waste management system can contribute to microplastic contamination of marine environments. Filter feeders like cockles and mussels would unintentionally ingest microplastics that sank to the bottom of the sea, or prey already contaminated with microplastics. Small plastic particles attract these filter feeders, which mistakenly ingest them as food. This indicates the potential for biomagnification, bioaccumulation, and trophic transfer of microplastics within the food web, which may eventually be transferred to humans through shellfish consumption (Zaki et al., 2021). Notably, colour separation may not allow for the confirmation of the debris. Thus, to confirm the presence of microplastics in the tissues of cockles and mussels from Sebatu, Melaka, FTIR analysis was performed and compared with the spectra of PS, PE, and PP standards.

3.3 Physical characterisation of microplastics on seawater samples

As illustrated in Figure S1, microplastics were absent or could not be detected in the seawater samples collected within the coordinates of the cockles and mussels sampling site (Table 1). Seawater samples were collected at the sampling site to assess the water quality in the habitat of live filter feeders. Since shellfish actively filter particles and organic matter from the surrounding water using specialised feeding structures, they serve as important natural water-clarifiers. Additionally, recent studies have confirmed that these filter-feeding bivalves also enhance water quality by reducing suspended debris and excess nutrients in aquatic environments (McAfee et al., 2023; Filippini et al., 2023). The zone for collecting seawater samples may be associated with shellfish habitat, where shellfish live, thereby contributing to the clarification of water free of microplastics, as filter feeders involuntarily filter out tiny particles. Filter feeders like cockles, mussels, and oysters can filter out small particles and even toxins from the water, enhancing water clarity (Ribeiro et al., 2023).

Moreover, images obtained under a light microscope illustrated the accumulation of salt crystals resulting from density separation with NaCl. Other than that, microplastics' buoyancy may also be associated with these unidentified results, as the plastic particles sink in the ocean due to biofouling (Alfaro-Núñez et al., 2021). Specifically, biofouling and other interactions with aquatic biota, such as fragmentation, degradation, or additive leaching, may enhance the sinking of plastic particles (van Sebille et al., 2019). Hence, the sunk microplastic particles on the ocean's floor may be ingested and filtered by shellfish bivalves to enhance the water clarity. Therefore, the presence of microplastics in the seawater samples at the cultivation site of shellfish samples cannot be detected. However, the salt particles obtained from the physical analysis were further analysed using FTIR spectroscopy to rule out the presence of microplastic particles in the seawater samples.

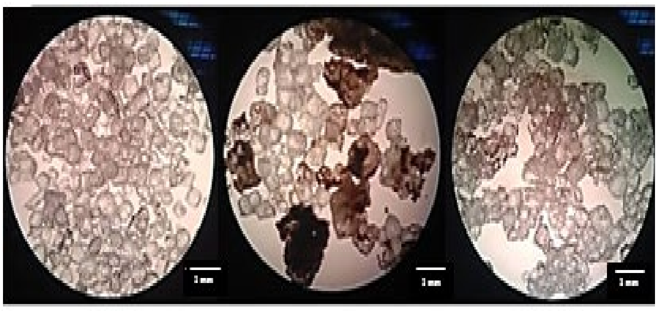


Figure S1. Observation of seawater samples under a light microscope. No traces of microplastics were identified in the seawater samples. Images illustrate salt crystals prior to density separation using sodium chloride (NaCl). (Supplement Figure).

3.4 FTIR analysis of microplastics on cockles and mussels' tissues from Tanjong Karang, Selangor

Since these three types of plastic polymers (PE, PP, and PS) are commonly used by the industries, they are selected as standards in this experiment for comparison with the spectrum obtained by the samples of cockles and mussels from two different sites in West Coast Peninsular Malaysia, Tanjong Karang, Selangor, and Sebatu, Melaka. Figure S2 depicts the overlapping spectrum of microplastics polymer standards, which are PE, PP, and PS, as a reference to the presence of microplastics on cockles and mussels' landings. From Figure S2, the presence of functional groups in PS was detected at wavenumbers between 2800 and 3000 cm^{-1} and between 1300 and 1500 cm^{-1}. The other two standards, PE and PP, also exhibited spectra similar to PS, with the peaks falling within the PS standard range. Essentially, mid-Infrared (mid-IR) spectroscopy is commonly applied for sample characterisation. In particular, it is typically segmented into four spectral regions based on vibrational features: the single-bond region (2500–4000 cm^{-1}), the triple-bond region (2000–2500 cm^{-1}), the double-bond region (1500–2000 cm^{-1}), and the fingerprint region (600–1500 cm^{-1}) (Kassem et al., 2023). The spectrum demonstrated below 1500 cm^{-1} is the fingerprint region (Nandiyanto et al., 2019). Although PE and PP are both polyolefins, they can be readily distinguished by FTIR (Cunsolo et al., 2021). When compared across all microplastics standards, PS has the highest transmittance for the functional group at a wavenumber between 2800 cm^{-1} and 3000 cm^{-1}. This may be due to the density of PS, which is the highest among PE, PP, and PS at 1.04 g/cm^3, 0.86 g/cm^3, and 0.85 g/cm^3, respectively. Among all, PS has the highest density (1.02 g/cm^3), which is higher than that of seawater. Hence, it sinks deeper into the sea. In contrast, the other two standards have densities lower than seawater. Thus, they float at the surface of the water (Das & Ali, 2025).

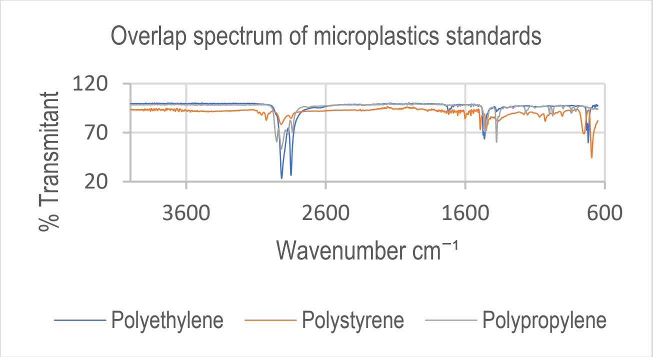
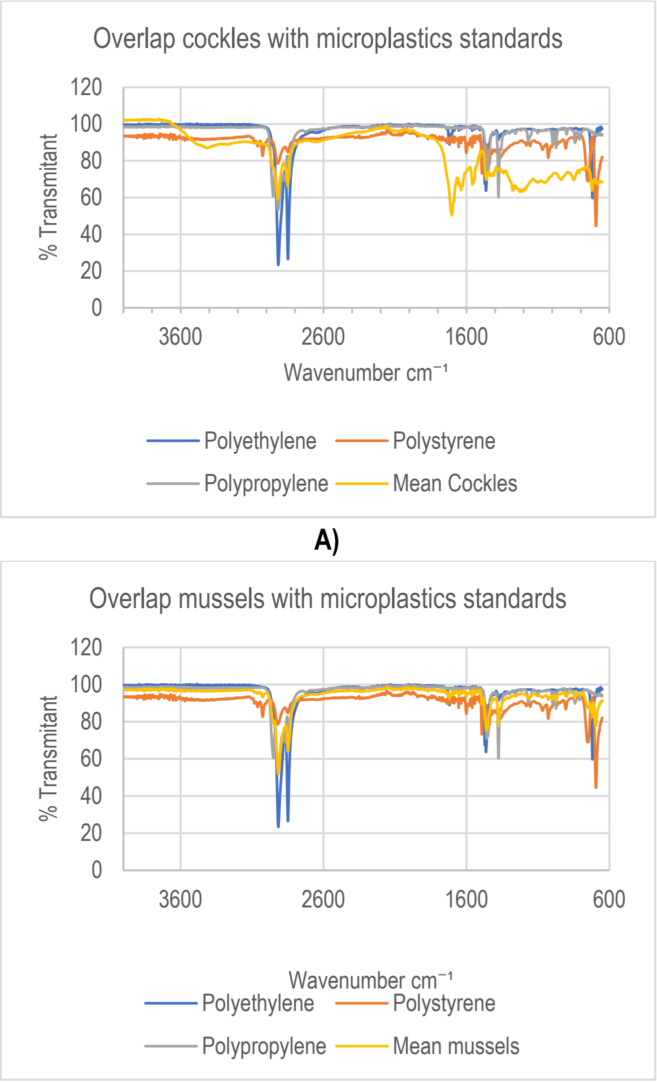


Figure S2. The overlapping spectrum of microplastics standards: a) Polyethene (blue), b) Polystyrene (orange), and c) Polypropylene (grey), respectively. The images on the right illustrate the chemical structures of the microplastic standards, which consist of polyethene, polystyrene, and polypropylene (Supplement Figure).

Based on the FTIR analysis, the most dominant microplastic identified in the tissues of cockles and mussels from Tanjong Karang, Selangor, exhibits chemical properties of PE, PP, and PS (Figure 6). The mean spectrum peaks obtained by cockles from Tanjong Karang are similar to the spectrum of the microplastics standards, suggesting that the presence of microplastics is confirmed by physical analysis using a light microscope (Figure 6A). From the graph, the peaks appear at wavenumbers between 2800 cm^{-1} and 3000 cm^{-1}, which are similar to the PS standard peaks (2918.5 cm^{-1}), indicating the presence of functional groups CH$_2$ (symmetrical stretching) and CH2 (asymmetrical stretching) (Razak et al., 2018). Furthermore, an absorption band observed near 1700 cm^{-1} corresponds to carbonyl (C=O) stretching vibrations (Zarshenas et al., 2015).

A prominent signal at approximately 1300 cm^{-1} corresponds to nitro (NO$_2$) stretching, characterised by vigorous absorption intensity. Additional bands detected around 800 and 900 cm^{-1} are associated with vinylidene C–H out-of-plane bending and trans C–H out-of-plane bending, respectively (Jung et al., 2018). Spectral features in this fingerprint region arise from overlapping and interacting vibrational modes, resulting in complex absorption patterns. Although interpreting this region can be challenging due to spectral complexity, each organic compound exhibits a distinctive combination of absorption bands. Consequently, compound identification is typically achieved by comparing the acquired infrared spectra with reference spectra of known materials (Nandiyanto et al., 2019). Figure 6B presents the mean of mussels' tissues, which also closely matched the mean spectrum of cockles' tissues. When compared to the spectrum of the standards, the overlapping spectrum at the wavenumber 2800 cm^{-1} and 3000 cm^{-1} was assigned to the =C-H stretch, similar to the spectrum of cockles' tissues mentioned above. Hence, this confirmed the presence of PE, PP, and PS in mussels' tissues as well as cockles' tissues that may be associated with the locations of Tanjong Karang, Kuala Selangor, in which it is heavily influenced by wastes from fishing and maritime activities, as well as industrial and residential areas. Since PE, PP, and PS continue to be widely used in packaging, particularly in food and beverage films, rigid containers, and textile applications, their prevalence in the packaging sector remains significant despite growing sustainability concerns (González-López et al., 2023; Thangamuniyandi et al., 2025).

A study conducted by Erni-Cassola et al. (2019) stated that the most abundant manufactured plastic polymers identified in seawater were PP, PE, and some forms of PS, which are less dense than water and can sink to the bottom of the sea when biofouled due to increased density. Several types of plastic polymers that are denser than seawater (density > 1.02 g cm^{-3}) tend to sink and accumulate on the ocean floor. Recent findings confirm that heavier polymers, such as polyvinyl chloride (PVC) and polystyrene (PS), as well as biofouled plastics, substantially contribute to deep-sea deposition of microplastics (Zhang et al., 2023). Filter feeders like cockles and mussels that live at the bottom of the sea may unintentionally ingest microplastics, thereby circulating them through the food chain. The potential presence of microplastics in the tissues of shellfish bivalves is assessed by physical analysis using a microscope. However, to prevent misidentification and overestimation of microplastics during physical observation, chemical analysis using infrared spectroscopy is needed to substantiate chemical and polymer identification (Huang et al., 2023).



A)

B)

Figure 6. A) The mean spectrum of cockles (yellow) from Tanjong Karang overlapping with microplastics standards; a) Polyethene (blue), b) Polystyrene (orange), and c) Polypropylene (grey), respectively. B) The mean spectrum of mussels (yellow) from Tanjong Karang overlapping with microplastics standards; a) Polyethene (blue), b) Polystyrene (orange), and c) Polypropylene (grey), respectively.

Conversely, regarding polymer composition, the FTIR-confirmed presence of PE, PP, and PS is consistent with findings from other studies of coastal and estuarine bivalves. In a 1-year temporal study conducted in the Aveiro Lagoon, Portugal, concentrations of microplastics in mussels (*Mytilus galloprovincialis*) and cockles (*Cerastoderma edule*) varied seasonally. Nonetheless, PE was consistently abundant, especially in the warmer months (Botelho et al., 2023). This temporal study suggests that microplastic exposure at the sampling sites might also fluctuate with environmental conditions (e.g., water temperature, filtration rates), and that the single-time-point measurements may capture only part of the contamination picture.

Overall, the results from both samples, cockles and mussels, depict spectra that overlap with the standards but differ slightly at specific wavelengths, as the microplastics detected in the samples have undergone chemical changes due to their high processing and have been mixed with other substances during processing. It is crucial to note that polymers in plastics have undergone various processing and are rarely used in their pure form, as most plastics produced prior to commercial use utilise colourants, additives, plasticisers, and other substances to enhance polymer properties (Vinay et al., 2021). Therefore, these substances tend to alter the polymer's spectrum in the samples, resulting in slight differences from the spectra of the microplastics standards obtained. Secondly, the prolonged presence of microplastics exposure to the environment is associated with various factors such as physical decomposition (heating or cooling, wetting or drying, or abrasive forces), biofouling by the action of microorganisms, and ageing of the polymer, leading to changes in polymer properties, thereby altering their spectra in FTIR analysis (John et al., 2024).

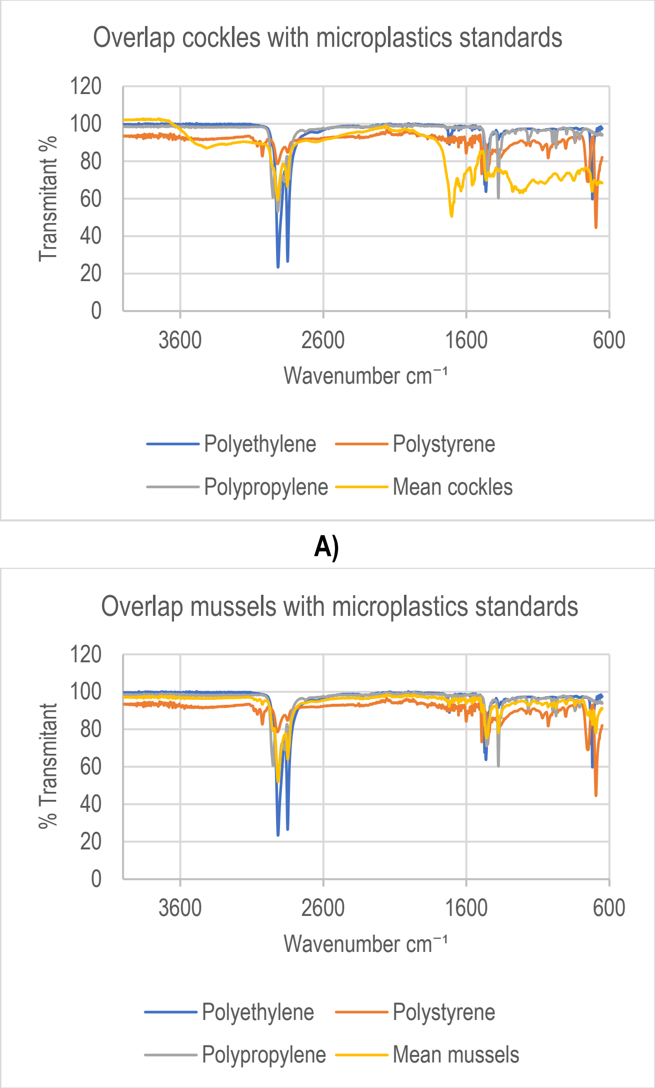
PE is divided into two groups: high-density PE and low-density PE. Both types are heat-resistant, made from petroleum, and considered safe for food packaging. However, they can potentially jeopardise human health if harmful substances, such as Bisphenol A (BPA) or phthalates, are present (Proshad et al., 2017). Moreover, a previous study also reported that long-term exposure to high-density PE to sunlight's UV rays may be harmful to human health (Okunola et al., 2019). PP, on the other hand, a semi-transparent plastic and stronger than PE, is considered safe for food and drink packaging (Proshad et al., 2017) and is also the safest among all plastics due to the absence of BPA. PP is unlikely to leach into food unless it is exposed to sunlight for an extended period. PS, whose monomer is styrene, exhibits potential health hazards, where styrene exposure, even at low levels over time, has been associated with neurotoxicity and is "reasonably anticipated to be a human carcinogen" according to the US government's 15th Report on Carcinogens (Collins, 2021; National Toxicology Program, 2021). Nevertheless, the Plastics Foodservice Packaging group identified that the styrene exposure from the use of food intact packaging made from PS is exceptionally low, with the calculated daily intake estimation at 6.6 micrograms per person daily, which is 10,000 times below the safety limit set by the US Food and Drug Administration (Cao et al., 2018).

3.5 FTIR analysis of microplastics on cockles and mussels' tissues from Sebatu, Melaka

Sebatu, Melaka, has been associated with contamination by various pollutants, including microplastics, due to its strategic location along the busiest shipping routes, shipping activities, and transportation (Looi et al., 2015). Therefore, it is expected to detect microplastic contamination in the tissues of cockles and mussels. The results (Figure 7) illustrate that the mean spectrum peaks of cockles are similar to those of the standards (PE, PP, and PS). As Melaka is also known as an eco-tourism region (Shariff & Rahman, 2022), it is well known for its historical sites and buildings, as well as its strategic location as a transportation and trade hub. Therefore, these features attract more tourists and increase the population in the Melaka area, thereby increasing the amount of plastic introduced into the environment through food packaging, textiles, and domestic waste.

The spectral peaks of the microplastics isolated from the mussel samples were similar to those of cockles from Tanjong Karang, Kuala Selangor, due to the same species and the presence of organic compounds in the tissues. In addition, when compared with the spectra of microplastic polymer standards, the prominent peaks in cockles' tissues are also similar in wavenumber to those of the standards, occurring between 2800 cm^{-1} and 3000 cm^{-1}, 1700 cm^{-1}, and in some fingerprint regions. This demonstrated the presence of microplastics, including PE, PP, and PS, in cockles' tissues from Sebatu, Melaka. The spectrum obtained by the cockles' tissues was closely similar to that of the mussels' tissues, as presented in Figure 7.

Furthermore, when compared, the results of cockles and mussels from both Sebatu, Melaka, and Tanjong Karang, Kuala Selangor, revealed similarities in the presence of functional groups and an overlapping spectrum with the standards. Moreover, the seasonal variation study in the Aveiro Lagoon (Botelho et al., 2023) documented that the number, size, shape, colour, and polymer type of microplastics changed over a year, with winter being dominated by fibres and a mix of polymer types, and summer indicating more PE fragments and mixed shapes. This dynamic pattern suggests that microplastic contamination is not static and that filter feeders' contamination could respond strongly to environmental flux. For the study sites in Tanjong Karang and Sebatu, it would therefore be valuable to conduct longitudinal sampling (e.g., across seasons) to understand how microplastic accumulation in shellfish varies over time.



A)

B)

Figure 7. A) The mean spectrum of cockles (yellow) from Sebatu, Melaka, overlapping with microplastics standards; a) Polyethene (blue), b) Polystyrene (orange), and c) Polypropylene (grey), respectively. B) The mean spectrum of mussels (yellow) from Sebatu, Melaka, overlapping with microplastics standards; a) Polyethene (blue), b) Polystyrene (orange), and c) Polypropylene (grey), respectively.

Based on the results, filter feeders like cockles and mussels can readily ingest microplastics that accumulate in seawater when mistaken for food. In particular, filter-feeding organisms ingest both target food particles and unintended particulates from the surrounding water due to their non-selective feeding mechanisms (Joshi et al., 2024). Although some organisms have developed adaptations and modifications to avoid the consumption of unwanted materials—such as preventing the ingestion of particles larger than a specific size by adjusting the mesh size of gill rakers and other anatomical components (Rahman, 2019) - microplastics can still be consumed. Notably, these adaptations occurred over a thousand years ago, whereas the current issue of microplastics has raised global concern for less than a century (Lusher et al., 2015).

3.6 FTIR analysis on seawater samples

As expected, the results of the FTIR analysis confirm the previous physical analysis, which revealed no microplastics in the seawater samples from both sampling sites, Tanjong Karang, Kuala Selangor, and Sebatu, Melaka. From Figure 8, the spectrum between 3000 cm⁻¹ and 2800 cm⁻¹ presented no overlap between the seawater samples and the microplastic standards. This region is a very significant functional group for plastic polymers, indicating the presence of functional groups from aliphatic and aldehyde compounds (Nandiyanto et al., 2019). Therefore, the absence of these functional groups means the absence of the polymeric substances crucial to the microplastics configuration. Furthermore, the absence of microplastics in seawater samples may be due to a filter-feeding community at the sampling site, which also plays a significant role in water clarification. From the graph (Figure 8), a prominent peak in the mean seawater spectrum was observed at 3400 cm⁻¹, which corresponds to the O-H stretching vibrations (van der Post et al., 2015), as the presence of the hydroxyl group (O-H) originates from the H_2O compound in seawater. The sample pre-treatment that was subjected to WPO using H_2O_2 also contributes to the O-H stretching vibrations of hydrogen-bonded hydroxyl groups in polymeric association. In microplastics standards, there was no O-H configuration in the functional groups of PE, PP, and PS. Thus, the presence of an O-H group denied the presence of microplastics in the seawater samples.

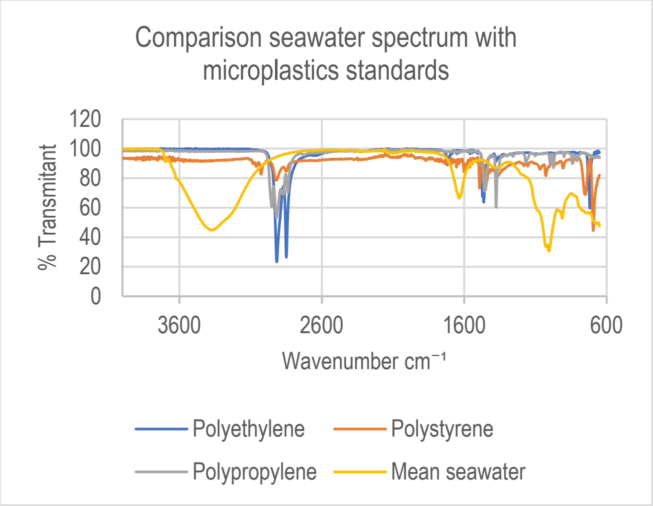


Figure 8. Comparison of seawater spectrum (yellow) with microplastics standard; a) Polyethene (blue), b) Polystyrene (orange), and c) Polypropylene (grey), respectively.

Other studies reported the presence and emergence of microplastics in Malaysian marine waters, such as in Kuala Nerus and Kuantan port (Khalik et al., 2018), two selected beaches from Kuching, namely Santubong and Trombol (Noik et al., 2015), and selected beaches in Kuala Terengganu, such as Teluk Kemang, Batu Burok, Tanjung Aru, Seberang Takir, and Teluk Likas (Fauziah et al., 2015). The occurrence of microplastics in these areas was associated with anthropogenic activities, industrialisation, and recreational sites. Other than that, the different methods for accessing microplastics in the water samples and the different sampling locations also contributed to the contradictory results regarding microplastic occurrence in these areas. However, in this study, filter feeders such as cockles and mussels can filter out small particles and even toxins from the water, thereby enhancing water clarity (Ribeiro et al., 2023). As for seawater at Tanjong Karang, Selangor and Sebatu, Melaka, the areas on collecting the water samples were the cultivation sites for cockles and mussels sampling where these filter feeders contribute for filtering the water column of fragments or debris by actively pumping water across the filtering organs, as most of the cultured marine-suspension feeders can trap small fragments and particles up to >4 µm with 100% efficiency under optimal conditions (Hamann et al., 2022). Methodologically, this approach, which combines light and fluorescence microscopy with FTIR spectroscopy, is well supported by current best practices. Studies such as Kovačić et al. (2024) utilised Scanning Electron Microscope (SEM) plus Energy Dispersive X-ray Spectroscopy (EDS) as a confirmatory method, whereas the Aveiro Lagoon research employed mid-infrared FTIR to chemically identify microplastics (Botelho et al., 2023). This suggests that this methodological framework is robust and appropriate for detecting and characterising microplastics in bivalve tissues.

3.7 Principal Component Analysis (PCA) on cockles and mussels' tissues from Tanjong Karang, Selangor, and Sebatu, Melaka

The shellfish samples from the FTIR analysis were analysed using PCA to compare the presence of microplastics between two samples, cockles and mussels, at two sampling sites on the West Coast of Peninsular Malaysia: Tanjong Karang, Selangor, and Sebatu, Melaka. PCA results from Figure 9 suggest that two variables (sample type and sampling sites) describe the overall trend or spatial distribution of cockles and mussels across the two sampling sites in two-dimensional coordinates. Figure 9 illustrates Principal Component 2 (PC2) plotted against Principal Component 1 (PC1) in a PCA of the data set obtained from FTIR analysis of two samples, cockles and mussels, from two sampling sites, Tanjong Karang and Sebatu, and Melaka. In particular, PC1 and PC2 together account for more than 95% of the total variance. Most of the samples depicted strong discrimination between samples from two different sites, with a total variation of 96%, clearly distinguishing cockles and mussels from Tanjong Karang and Melaka. Consequently, this data supports the variation in the distribution of microplastics on cockles and mussels from Tanjong Karang and Melaka, as the locations were associated with offshore and anthropogenic activities that contribute to the high total variance (Looi et al., 2015). PC1, explaining 72% of the total variance, had high loadings for cockles and mussels from Melaka, which are positioned on the left side of the plot (negative direction). This component represents the correlation between cockles and mussels from Melaka, which are mainly associated with the global shipping route through the Straits of Malacca (Zaki et al., 2021).

Conversely, PC2, explaining 24% of the total variance, was dominated by cockles from Tanjong Karang and some mussels from Tanjong Karang, as these samples were positioned in the positive direction (right-hand side of the score plot). The PC2 reflects another cluster of cockles and mussels from Tanjong Karang, which exhibits a correlation with samples from Tanjong Karang, with less trading activity than in Melaka but focused on fishing and shipping (Lusher et al., 2015). Based on the PCA distributions, the likelihood of detecting microplastics is higher in mussels harvested in Tanjong Karang and cockles harvested in Melaka in a microplastic-contaminated environment than in cockles harvested in Tanjong Karang and mussels harvested in Melaka. However, the results remain inconclusive. More studies on microplastics, with larger sample sizes or broader scales at these two sampling sites, are required to analyse their distribution patterns.

In terms of spatial distribution, cockles and mussels were distributed within the outlier, with some samples indicating similar fingerprinting, originating from two sampling sites, Tanjong Karang and Sebatu, Melaka, and exhibiting high microplastic abundance, leading to similarities in the same component. A few samples of cockles and mussels from Tanjong Karang and Melaka, respectively, were discriminated far apart from each other, which may be due to replication purposes. Additionally, seasonal changes, environmental conditions, and human error may lead to variations in the occurrence of microplastics (Zhang et al., 2019). Overall, results reveal significant differences ($\rho < 0.05$) between cockles and mussels in Tanjong Karang, Selangor, and between the two types of shellfish from Sebatu, Melaka, using one-way ANOVA. The differences in location contribute to distinct microplastic distributions between the two sampling sites, as PCA results present that all the data discriminate from each other and are positioned in both positive and negative directions along the first and second principal components.

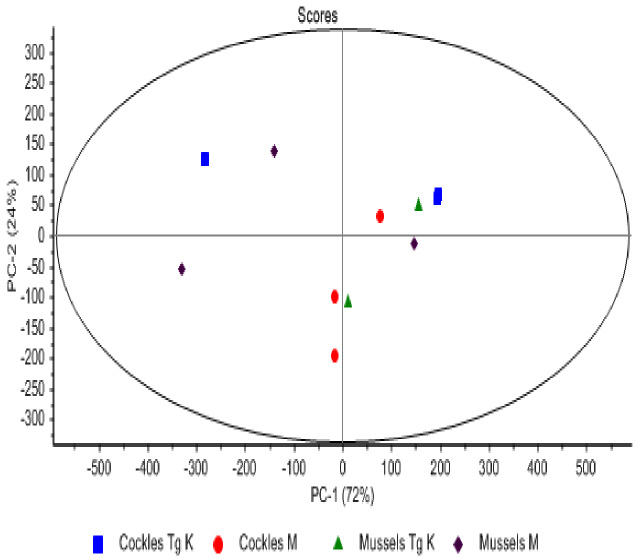


Figure 9. Score plot between the selected principal components obtained from applying the FTIR data set to PCA analysis. Samples labelled with different colours and symbols indicate different shellfish samples from two sampling sites.

4. CONCLUSION

4.1 Conclusion

Shellfish, such as cockles and mussels, are filter feeders that potentially obtain food by filtering organic matter and small organisms from the surrounding water. In this experiment, the presence of PE, PP, and PS has been confirmed on cockles and mussels' samples from Tanjong Karang, Kuala Selangor, and Sebatu, Melaka, through microscopy and FTIR analysis. The microplastics, detected in 30 g of cockles and mussels, exhibited characteristics of degraded fragments, thin, elongated filaments, and spheruloid-shaped pellets in a variety of colours, including black, red, grey, and yellow. Essentially, this preliminary study on the occurrence of microplastics in urbanised and rural areas can be used to monitor and implement food safety measures, as well as to support future comparative studies. If plastic waste from current pandemics, industrialisation, shipping, or trade activities is not managed correctly, it may lead to severe microplastic pollution in the environment. In brief, the presence of microplastics in each sample was significantly different ($\rho < 0.05$) from that in the others, and the PCA results were inconclusive due to differences in geographic social activities at both sampling sites.

4.2 Future recommendations

For further studies on the presence of microplastics in cockles and mussels, a quantification method is needed to assess the abundance and distribution of microplastics in shellfish bivalve tissues. Moreover, additional studies on the quantification of microplastic occurrence in biota, sediments, water, and the atmosphere (airborne) from other sampling dimensions in Malaysia are significant for determining and evaluating the sources, pathways, and fate of microplastics. Therefore, to expand understanding of cockles' ability to trap particles from surrounding water, research on their structure and capacity to absorb impurities is needed, which is crucial for microplastics studies.

4.3 Acknowledgments

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