

## ASSESSING MICROPLASTIC CONTAMINATION IN SHELLFISH: INSIGHTS FROM PANTAI REMIS KUALA SELANGOR, STRAIT OF MALACCA, MALAYSIA

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### Abstract

**Introduction:** Microplastic (MP) contamination poses a global environmental threat, affecting terrestrial and marine ecosystems, and human health. This study investigates the presence, density, and composition of MPs in three commercially important shellfish species, oriental angel wing clam (*Pholas orientalis*), bamboo clam (*Ensis leei*), and blood cockles (*Tegillarca granosa*) at Pantai Remis Jeram, Kuala Selangor. **Methods:** Microplastics in shellfish were quantitatively analyzed for their abundance, colour, size, shape, and composition using microscopic techniques and micro-Fourier Transform Infrared (FTIR). Standard experimental protocols were followed. Statistical analysis of the data was performed using SPSS to identify correlations between these parameters. **Results and Discussion:** Our findings reveal a significant presence of MP particles in shellfish with *T. granosa* exhibiting the highest density (2.417 particles/cm<sup>3</sup>) compared to *E. leei* (0.721 particles/cm<sup>3</sup>) and *P. orientalis* (1.449 particles/cm<sup>3</sup>). Fibers and fragments were the dominant MP morphotypes, primarily in black color. *P. orientalis* and *T. granosa* contained a majority of MPs within the 1 - 5 mm size range, totalling 41 and 56 particles, respectively. Shellfish samples contain polymers of cellulose acetate and polyethylene terephthalate, indicating possible origins from plastic bottles and textile fibres. A statistically significant difference in the mean MP densities in the different species of shellfish was found by one-way ANOVA analysis ( $p = 0.042$ ,  $p < 0.05$ ). **Conclusion:** This study provides relevant data on MP pollution in commercially significant shellfish species. To effectively mitigate this environmental concern and comprehend the long-term ecological ramifications of MP intake by shellfish, more research is required.

## INTRODUCTION

Microplastic (MP) contamination has become a major environmental in recent years because of its extensive distribution and possible hazards to ecosystems and human health. Consuming shellfish introduces people to MPs in a particularly concerning way since MPs can infiltrate the food chain through a variety of pathways (1). This raises concerns about the possible adverse effects of eating crustaceans contaminated with MPs (2). Filter-feeder organisms, oysters, mussels and clams, incorporate MPs and various other particles from their environment as well.

While consuming shellfish as part as commonly chosen food daily, despite their nutrition rich food status, these organisms basically also serve as the bioindicators for the bioaccumulation of MP in water bodies (3). Therefore it is important to assess the level of MP concentration in such aforementioned marine species and its potential impacts on human health.

There were several studies previously highlights the high amount of MPs exist in the bivalves species and potential health risks related to its consumption. For instance, a holistic studies carried out in United Kingdom between year of 2016 to 2017 (4) has suggested that

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those bivalves collected in such estuaries and coastline were heavily polluted with MPs. On top of that, another group of researcher has also found out that MPs already proven to be in the human wastes, henceforth strongly suggest that microplastic eventually may build up and absorbed respectively in human body (5). All these findings fundamentally suggest a clearer picture to which magnitude does the MPs may incorporated in our daily life.

Even though the evidence of MPs ingestion through shellfish and mollusc consumption is arbitrary, factors such as size, shape and chemical composition of MPs have to be take into consideration, since all of these criteria's may enhance or affecting the bioaccumulation and biomagnification of MPs in both shellfish and mollusc, thus pose different hazards rate to human health. Mixture of conceptual knowledge in studying the impact of MPs towards human health will eventually require interdisciplinary studies including the involvement of environmental science and toxicology area with tagging on public health. This will help a better understanding on aforementioned dynamics and interaction thereafter.

This study addresses the growing concern over MP contamination in Malaysian waters, where different types of plastic polymers have been found. The extensive use and inappropriate disposal of plastics have led to MP pollution, which has a serious negative impact on marine ecosystems. Shellfish, an essential component of Malaysia's seafood economy, have been identified as potential carriers of MPs, raising worries about associated health hazards for human consumers. Due to the widespread consumption of shellfish in Asian cultures, particularly those in the West, and the location of the regional shelf where land-based pollutants are most likely to be found due to concentrated industrial, shipping, trading, and infrastructure activities. This study aims to investigate the presence of MPs in shellfish from Pantai Remis Jeram, Kuala Selangor. The results of this study address MP contamination and are consistent with Sustainable Development Goal 14.1 of the United Nations, which aims to prevent and minimize marine pollution, including MPs. Understanding the concentration of MPs in shellfish is crucial for creating effective pollution control measures.

## METHODS

### Study Location and Sample Collection

The study was conducted at Pantai Remis Jeram, Kuala Selangor, Selangor (3.2009°N, 101.3057°E). Pantai Remis is a mangrove area with a muddy recreational beach suitable for shellfish habitat. The area maintains a traditional fishing village, with fishing boats

lining the beach and local fishermen engaged in their daily activities. Nets, ropes, and hooks are commonly used in traditional fishing methods by the local community from small boats or directly from the beach. Tourist and fishing activities may contribute to plastic pollution in the area. Furthermore, effluent from nearby industrial and agricultural operations could transport plastic particles into the coastal waters. The currents and tides of Pantai Remis can exacerbate the accumulation of MPs along the shoreline, making it a high risk for pollution. The region's importance as a breeding and harvesting ground for various seafood species, including clams and cockles, monitoring MP concentrations, is crucial to assess potential risks to these organisms.

Three commercially important shellfish species, bamboo clam (*Ensis leei*), mentarang / oriental angel wing clam (*Pholas orientalis*), and blood cockles (*Tegillarca granosa*) were selected for this study due to their high demand in the local markets of Kuala Selangor and surrounding areas, which suggests that they are commonly consumed and traded in this area. To minimize sampling error, 30 samples of each shellfish species were collected from the study site. The collected shellfish samples were promptly packed in an icebox and transported to the laboratory at the Faculty of Health Sciences, UiTM, where they were stored at -20 °C for subsequent laboratory analysis.

### Microplastic Extraction in Shellfish

The fresh shellfish was thawed at ambient room temperature (27°C) on a clean metal tray. The mussels were then carefully dissected to extract the entire soft tissue, which was weighed to determine wet mass. To eliminate any external MPs, the soft tissues were rinsed with filtered deionized water and filtered through a Whatman 0.7GF/G filter. Tissues from ten individuals were pooled to create three replicates for each shellfish species. The pooled samples were then transferred to 1000 mL glass bottles and treated with 200 mL of a 10% potassium hydroxide (KOH) solution to facilitate tissue digestion.

These bottles were sealed with aluminum foil and incubated in a humidifier at 65°C for 24 hours, followed by an additional 24 hours at ambient temperature. To facilitate MP flotation, 800 mL of saturated sodium chloride solution was added to each container. After 24 hours of floating at ambient temperature, the overlying water was filtered under vacuum through a 47 mm diameter filter with a 20 µm pore size. The filtered samples were then transferred to clean Petri dishes, covered, and examined using a Leica DM2500 stereoscopic microscope and a Dino-Lite digital microscope for microplastic components.

The sampling and MP extraction procedures followed those described in a previous study (6).

### Characterization and Quantification of Microplastics

In this research, microplastic characteristics in samples were observed in three categories: morphotype, size, and color. According to research investigations, microplastics can be divided into distinct morphotypes based on their features. A previous study revealed there are five main categories of microplastics: (i) fragments (hard and jagged pieces of plastic debris); (ii) fibers (thin and straight strands of plastics); (iii) pellets (rounded and solid plastic particles); (iv) films (thin and flimsy plastic sheets); and (v) foams (lightweight and spongy plastic materials) (7). Meanwhile, microplastics were discovered to be categorized based on their size. Two size groups were identified: large microplastics ranging from 1 millimeter to 5 millimeters, and tiny microplastics ranging from 1 micrometer to 1000 micrometers (8). Another study found another layer of detail in microplastic research by highlighting color variation. They identified several colors of microplastics such as black, blue, brown, and ash. This finding helps a better understand and characterize the microplastics in different environments (9).

### FTIR Analysis

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) was used to identify, measure, and analyze microplastics in the shellfish. The microscope's detector used was 128 × 128 pixel Focal Plane Array (FPA). It was able to obtain 16,384 spatially resolved spectra on an area of 700 × 700 micrometers per tile (10). The selected microplastics represent some of the most common particle-type samples in visual inspection. The microplastics finally spread all over the KBr crystals. Spectral comparisons are done comprehensively by comparing the observed spectrum with the reference spectrum of Perkin Elmer particles.

### Quality Control

All procedures are consistently followed throughout the experimental work in order to prevent any contamination of the sample. All laboratory equipment was rinsed with distilled water and tightly wrapped in aluminum foil until needed. Glassware, metal spoons, and metal trays were used for all sampling and analysis procedures to avoid the potential for plastic contamination. Personal protective equipment, such as cotton lab coats, masks, and nitrile gloves, was worn throughout the experiment to safeguard the researchers. To protect samples and glassware from

exposure to air, both were covered with aluminum foil during analysis and transportation. Shellfish dissection and digestive tract separation were performed as quickly as possible. To reduce the risk of infection, the digestive system was immediately placed in a Petri dish and covered. A control Petri dish containing several milliliters of distilled water was placed near the microscope to monitor for any ambient contamination.

### Data Analysis

The SPSS Statistics 27.0 software was used for statistical analysis. Microplastics in shellfish were tested using a one-way analysis of variance (ANOVA). Pearson correlation analysis examined the relationships between microplastics in different shellfish species. In all cases,  $p < 0.05$  was considered statistically significant.

## RESULTS

### Microplastic Density in Shellfish

Microplastic particles (MP) are prevalent across various shellfish species, with notable variations in density. The blood cockles (*T. granosa*) displayed a significantly high concentration, averaging 2.417 particles per cubic centimeter ( $\text{cm}^3$ ), surpassing other samples. In contrast, bamboo clam (*E. leei*) exhibited the lowest average MP density at 0.721 particles per  $\text{cm}^3$ , while oriental angel wing clam (*P. orientalis*) recorded 1.449 particles per  $\text{cm}^3$ . Figure 2 illustrates these differences, with *E. leei* consistently maintaining a lower average MP density than *P. orientalis* (31%) and *T. granosa* (53%) samples at 16%.

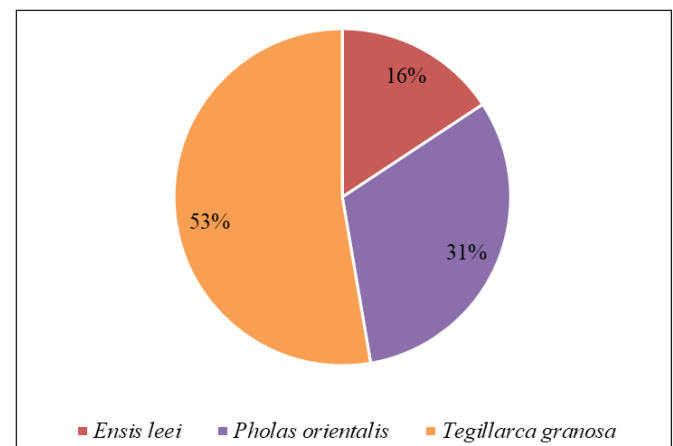


Figure 2. Percentage of Shellfish with an Average MP Density (particles/  $\text{cm}^3$ )

### Morphotype of Microplastic in Shellfish Shapes of Microplastics

This study illuminates the composition of microplastics (MPs) in diverse shellfish species, offering insights into potential ecological repercussions in



aquatic ecosystems. MPs contamination was evident in oriental angel wing clam (*P. orientalis*), totaling 261 MPs, averaging around 87 particles per replicate. Bamboo clam (*E. leei*) and blood cockles (*T. granosa*) showed 119 and 435 MP particles, averaging approximately 40 and 145 particles per replicate, respectively. Morphotypes, such as pellets, films, fibers, and fragments, vary among shellfish species (Figure 3 (a)(b)(c)). Different shellfish species exhibited variations in feeding habits and habitats, influencing MP concentrations (11).

Figure 4 illustrates the presence of MP in shellfish soft tissue, categorized into pellets, films, fibers, and fragments. Fiber morphology dominated, constituting 82.0% in *T. granosa* and 4.9% in *E. leei*. Fragments were highest in *P. orientalis* (67.9%), while film constituted 41.5% in *E. leei* and 4.9% in *P. orientalis*. Pellets, the most unusual form, were highest in *T. granosa* at 2.7%. Fibers and fragments emerged as predominant MP types, likely due to their abundance and widespread distribution in inshore environments, aligning with the previous findings in oysters (12).



Figure 3. The Different Shapes of MP Obtained From a) *P. orientalis*; b) *E. leei* ; c) *T. granosa*

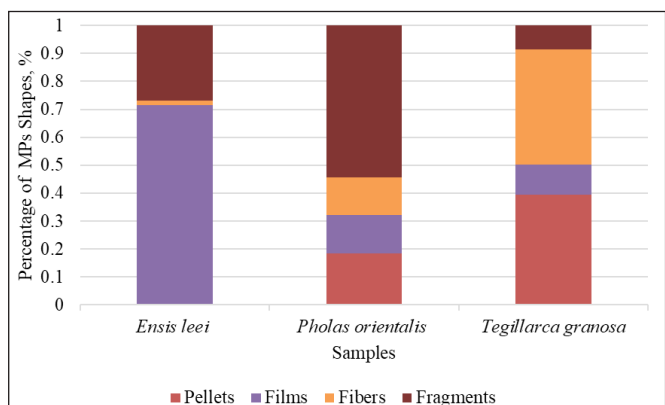


Figure 4. The Percentage of MP Shapes in the Sample of Shellfish

### Colors of Microplastics

The diverse array of microplastic (MP) colors identified in shellfish, including white, transparent, red, yellow, purple, and black (Figure 5), underscores the intricate nature of marine microplastic pollution. Black microplastics dominate, constituting 46.34% in *E. leei*, 53.09% in *P. orientalis*, and 40.82% in *T. granosa* samples. Transparent microplastics rank second in abundance, representing 31.71% and 13.58% in *E. leei* and *P. orientalis*, respectively. White microplastics make up 25.17% in *T. granosa*, while yellow in *E. leei*

at 4.88% is the least prevalent color. Red microplastics are relatively high in *T. granosa* at 8.84%, and purple microplastics are the least common, constituting only 8.64% in *P. orientalis* and absent in *E. leei*.

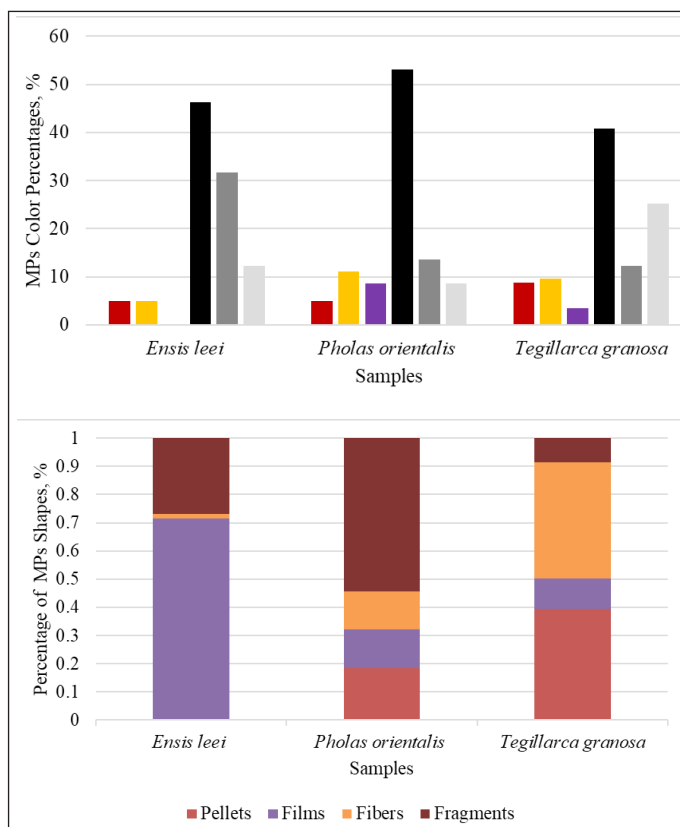


Figure 5. The Percentage of MPS Colors in Shellfish

**Sizes of Microplastics**

Plastic particles in this study were categorized into three size classes: <0.5 mm, 0.5 mm–1 mm, and 1 mm–5 mm (Table 1, Figure 6), aligning with the approach used in a prior study in Bangladesh (13). Analysis of *P. orientalis* and *T. granosa* samples revealed the prevalent size range of MPs to be 1 to 5 mm, with 41 and 56 particles, respectively. Conversely, *E. leei*'s predominant size was 0.5 to 1 mm, constituting 18 particles. The distribution of MPs in shellfish exhibited a significant portion within the 1.0 to 5.0 mm range (41.0% of the total), followed closely by the 0.5 to 1.0 mm category (37.0%). Notably, 22.0% were below 0.5 mm. This distribution underscores

the frequency of microplastics in shellfish samples and provides insight into possible ecological consequences.

**Table 1. Quantity of MP Particle Count in Shellfish Across Various Length Ranges (mm)**

Samples	Quantity of MPs Particle Count		
	<0.5	0.5 – 1.0	1.0 – 5.0
<i>P. orientalis</i>	18	29	41
<i>E. leei</i>	6	18	16
<i>T. granosa</i>	35	55	56

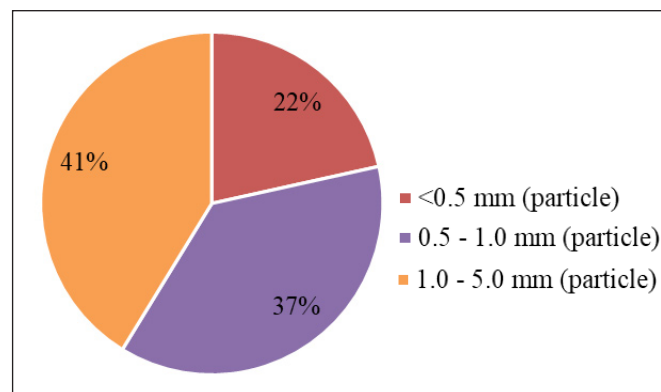


Figure 6. The Percentage of Overall MP's Size

**Chemical Composition of Microplastics**

Three samples from each of the *P. orientalis*, *E. leei*, and *T. granosa* species were chosen for a comprehensive investigation, and the results were confirmed using FTIR analysis. A thorough observation of spectra revealed that all three samples have a common polymer type—polyethylene terephthalate (PET) and cellulose acetate (CA). In the analyzed samples, the distinctive peaks associated with CA and PET were identified, with Figure 7 (a) emphasizing notable peaks at key wavenumber regions: 3299.65 cm<sup>-1</sup> (O-H Stretch), 2887.61 cm<sup>-1</sup> (C-H Stretch), 1318.44 cm<sup>-1</sup> (O-H Bend), and 1020.56 cm<sup>-1</sup> (C-O Stretch). While Figure 7 (b) also shows PET produces prominent peaks around wavenumber regions 3419.02 cm<sup>-1</sup> (O-H Stretch), 1644.96 cm<sup>-1</sup> (C=C Stretch), 1281.84 cm<sup>-1</sup> (C-O Stretch), 856.48 cm<sup>-1</sup> (C-H Bend) and 676.66 cm<sup>-1</sup> (=C-H Bend).

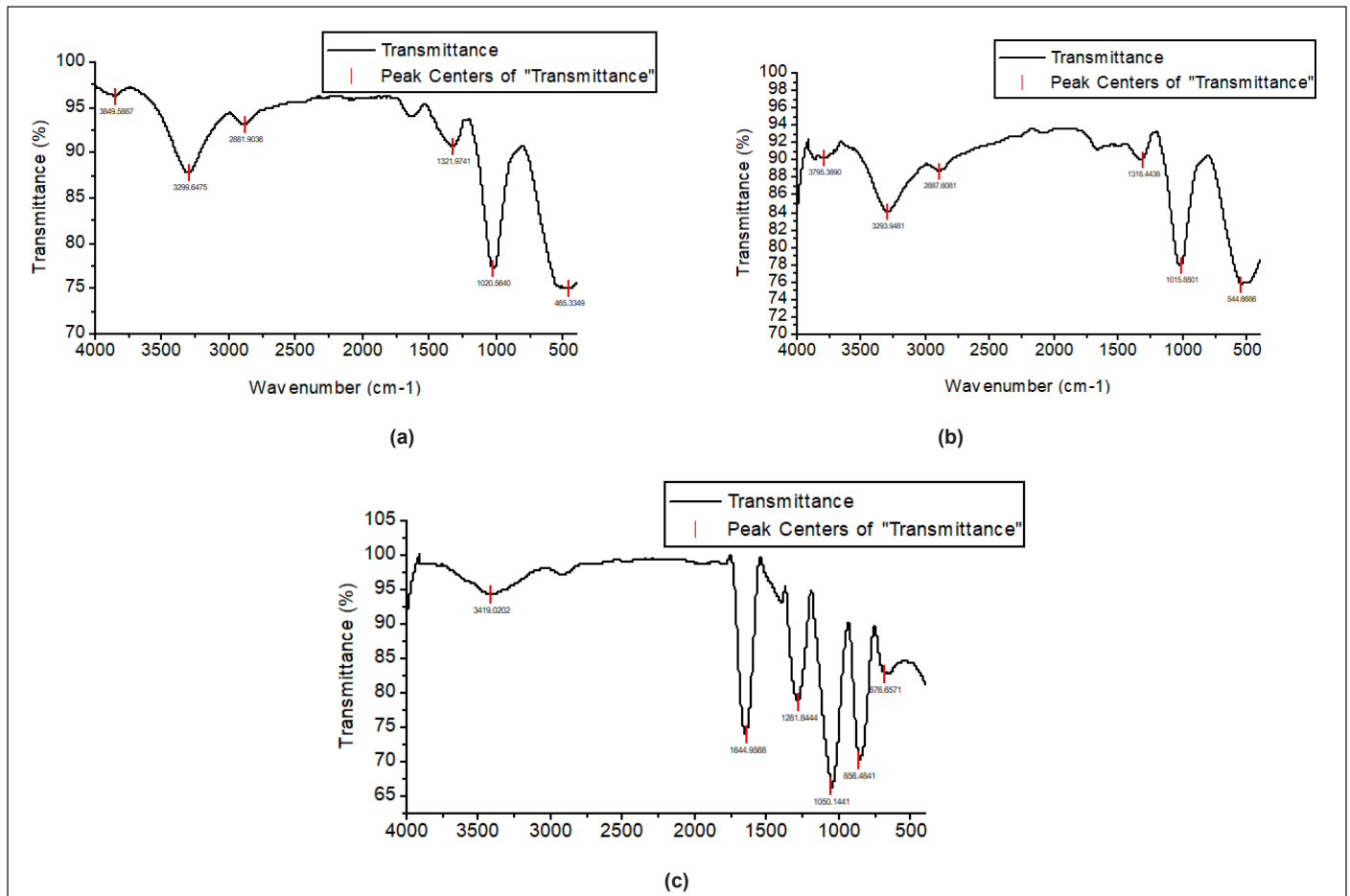


Figure 7. FTIR spectra of typical microplastic particles found in shellfish, (a) CA in *P. orientalis*; (b) CA in *E. leei*; (c) PET in *T. granosa*

**Assessing the Relationship Between the Microplastic in Shellfish**

Based on Table 2, applying a one-way ANOVA test yielded compelling results, indicating a statistically significant difference among the mean densities of MPs across various types of shellfish samples ( $p= 0.042$ ,  $p < 0.05$ ). A statistically significant disparity in MPs density was identified, with *T. granosa* samples exhibiting a notably higher level than *E. leei* samples ( $p < 0.05$ ) as shown in Table 3. This finding underscores the significance of the observed difference and suggests that *T. granosa* may be more susceptible to MPs contamination or are situated in environments with elevated MPs concentrations.

Table 2. One-way ANOVA Test Between the Sample of Shellfish

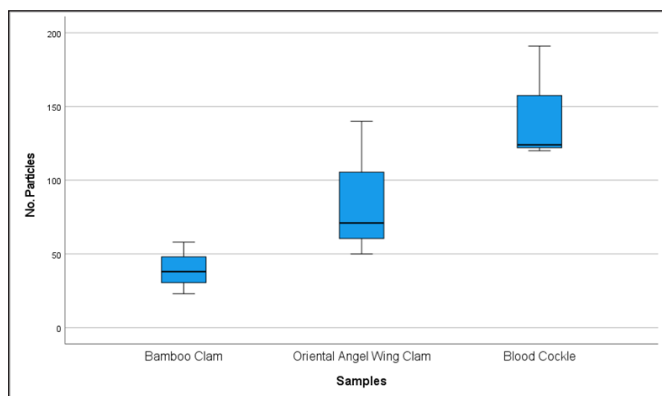
ANOVA					
MPs Density (particles/cm <sup>3</sup> )					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.340	2	2.170	5.614	.042
Within Groups	2.319	6	.387		
Total	6.660	8			

Table 3. Multiple Comparisons Post Hoc Test Between the Sample of Shellfish

Multiple Comparisons					
Dependent Variable: MPs Density (Particles/cm <sup>3</sup> )					
Tukey HSD					
(I) Samples	(J) Samples	Mean Difference (I-J)	Std. Error	Sig.	
<i>E. leei</i>	<i>P. orientalis</i>	-.728	.507	.383	
	<i>T. granosa</i>	-1.695*	.507	.036	
<i>P. orientalis</i>	<i>E. leei</i>	.728	.507	.383	
	<i>T. granosa</i>	-.966	.507	.218	
<i>T. granosa</i>	<i>E. leei</i>	1.695*	.507	.036	
	<i>P. orientalis</i>	.966	.507	.218	

The findings, illustrated in Figure 8, underscore notable differences in the box plot representing microplastic levels among the studied groups. Notably, the data for Oriental Angel Wing Clam (*P. orientalis*) stands out with its exceptional dispersion, revealing unique characteristics in microplastic distribution within this species compared to others. Furthermore, the concentration of microplastics in blood cockle (*T. granosa*) is noteworthy, ranking highest among the

sampled shellfish, followed by Oriental Angel Wing Clam (*P. orientalis*) as the second-highest, and bamboo clam (*E. leei*) with the lowest concentration.



**Figure 8. Comparison of No. Particles of MPs in Different Species of Shellfish**

## DISCUSSION

MP density in shellfish is influenced by factors such as fishing activity, the use of fishing gear, human activities, and industrial materials (14). This result aligns with previous studies, emphasizing the diverse nature of factors influencing MP prevalence in coastal areas (15-16). Microplastics in shellfish can be attributed to both coastal pollution from human activities and household sources. Shellfish collected from areas near residential and commercial zones are particularly vulnerable to contamination from these sources (17).

The prevalence of MP fibers is closely related to coastal fishing activities, with materials such as fiberglass (18) and plastic used in fishing gear contributing significantly to their release into the marine environment (19). Abandoned or improperly disposed of fishing gear further adds to this release (20). The problem of MP pollution is worsened by abandoned fishing gear and plastic bags from coastal markets. These items can break down into smaller pieces and contaminate the marine environment (21). Over time, these bags degrade into fragment-type MPs (22).

The presence of colourful particles heightens the risk of marine biota mistaking them for food, as reported by previous studies (23). The visual similarity of these particles to lower trophic level species increases the likelihood of marine animals mistaking them as viable food, potentially ingesting hazardous materials (24). A study found that black microplastics can be mistaken for tiny insects by other organisms because of how light reflects off them. This similarity could lead to confusion and potentially harm these organisms (25). A study on microbeads in facial scrubs aligns with this research, showing a connection between wastewater and the presence of white and clear microplastics in shellfish

(26). The variety of microplastic colour found in shellfish likely comes from broken-down plastic waste, especially colourful plastic bags, that ends up in the ocean.

Consistent with previous research in Southeast Asia and other countries, this study reveals a predominant size range of 1.0 to 5.0 mm for MPs. The convergence of data across studies highlights the resilience and repeatability of this observed pattern, emphasizing the prevalence of mid-sized microplastics in shellfish samples (27-28). The presence of cellulose acetate (CA) in shellfish suggests a likely source from textile fibers, given its versatility and wide application in industries producing fibers, cigarette filters, fabrics, films, membranes, and molded objects (29). Although considered environmentally beneficial, CA's chemical modification affects its (bio)degradability in natural environments (29). The identified polymers hint at a potential origin from clothing fibers, entering seawater through laundry and wastewater inefficiencies, or improper disposal practices, including cigarette filter discarding by beach visitors.

Similar to the previous one on *Saccostrea ucellate* oysters, this research highlights polyethylene terephthalate (PET) as a major type of microplastic contaminant (6). PET, the third most extensively used polymer in packaging, especially in beverage bottles, constitutes a significant portion of plastic consumption in the European packaging sector (30). The study identifies various sources of PET microplastics in shellfish, emphasizing the impact of market activities relying on PET for drinking water packaging (31). Understanding the life cycle of plastic bottles becomes crucial in comprehending the entry and accumulation of microplastics in marine habitats and shellfish.

The diverse eating behaviors of *P. orientalis*, *T. granosa*, and *E. leei*, three filter-feeding species, result in different degrees of exposure to MPs. As observed in clams and oysters (32), the non-selective feeding characteristics of filter feeders make them more susceptible to ingesting MPs. These results enhance our knowledge of the variations in MP ingestion (33). *P. orientalis* and *T. granosa* interact with greater amounts of water through filter-feeding behaviours, which may expose them to more MPs in the water column. Through utilising burrowing and efficient filter feedings, *P. orientalis* has the potential to unintentionally pick up MPs from sediment or water (33). *T. granosa* may consume MPs, plankton, and debris because it lives in shallow coastal environments and filters close to the sediment surface (34-35). By using long syphons and an effective foot to draw in water, *E. leei* use a filter-feeding method that exposes it to tiny particles on muddy or sandy



substrates, including MPs (36). Inequality in the retention or removal of MPs are partly caused by variations in the digestive processes. Furthermore, *T. granosa*'s selective feeding behavior might result in preferences based on the properties of MPs (37-39). The types and quantities of MPs that these species come into contact with are greatly influenced by their capacity to adapt to particular settings, which is demonstrated in their eating habits (40). The complex dynamics of MPs interaction in these ecosystems are further influenced by biological factors and local environmental variations.

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### AUTHORS' CONTRIBUTION

AAA: Writing-Original draft preparation. MSO: Methodology, Data Analysis. NWR: Data Analysis, Data Curation. FAS: Data validation, Writing-Reviewing and Editing. NASF: Writing-Reviewing and Editing. MAZ: Writing-Reviewing and Editing. SRMY: Conceptualization, Methodology, Original draft preparation.

### CONCLUSION

Taking everything into account, we can conclude that shellfish sampled from Pantai Remis Jeram in Kuala Selangor District show a positive presence of different types of MPs in terms of its particle size, shapes and colours. On top of that, commonly found types of MPs are in the form of fibres and fragments. All of this findings were consolidated from commercially valuable species of shellfish hence increase the concern on potential health impact might be posed by the ingestion of these contaminated shellfish. It is noteworthy to report that, through this study, Polyethylene terephthalate (PET) and Cellulose Acetate (CA) were the most abundant polymers found in the samples. Both of these polymers indicating that the MPs resources are highly contributed from textile fibres and plastic bottles. Different densities of the MPs was statistically proven using One – Way ANOVA analysis by considering three different species of shellfish including blood cockles. It was found out that the highest amount of MPs is in the *T. granosa* species. This finding generally suggested that high consumption of blood cockles (*T.granosa*) may pose potential health impact to human being and as well as to the marine

ecosystem too. Hence, extensive strategies are needed to overcome or to reduce pronounced impacts of MPs contamination in our ecosystem.

For instance, concerted effort need to be exercise by various stakeholders in preventing and reducing the plastic materials into aquatic and marine ecosystem, which therefore may prevent the infiltration of MPs fragment into aquamarine medium which later translated to biomagnification and bioaccumulation in the major food chain pathways. Other than that awareness on the mishandle of plastic material which may lead to normalization of plastic material presence in dynamic ecosystem without properly handled must be inculcated and refreshed from time to time in multilayer generations and community attributes. Rather than focusing on awareness alone, public must also feeds up by information on potential MPs contamination in various food and selection of food source hence may prevent or reduce the future complex interaction between the needs of the food source especially protein, with possible negative impact due to the intake of it. Holistic advance study henceforth become fundamental and much needed in near future in exploring better understanding of health impact due to intake of contaminated shellfish. Expectation on threshold and exposure period must be identified and published in future research therefore the health impact indices could be forecasted and gauge rationally nationwide.

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