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MICROPLASTICS CONTENTS IN NATURAL AND MARICULTURED SHELLFISH FROM PASIR PUTIH ESTUARY IN JOHOR, MALAYSIA

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Abstract: Microplastics (MPs) are plastic debris diameters ranging from 1 μm to 5 mm. The prevalence of MPs in green mussels and their environment is now being questioned as to whether aquaculture practices or human environmental activities cause them. Hence, this study focuses on looking into the prevalence of microplastics (MPs) in natural environments and mariculture mussels *(P.veridis*) which comprise physical characteristics to compare microplastics (levels, type, size and colour) uptake based on the mood of living from Pasir Putih estuary in Johor, Malaysia. Specimens *of P.veridis* were obtained from two sampling stations in the Pasir Putih estuary. Soft tissues were removed and digested with a 10% KOH solution and the density of microplastics was isolated using 1.2 g/cm^3 NaCl solution respectively. Microplastics were visually inspected using a stereo microscope and my-solution premium (IMT Cam^3) software at magnifications ranging from 40x to 45x. The ATR-FTIR spectroscopy was then utilised to verify the presence of microplastics. In cultured *P.veridis* samples, microplastic abundance ranged from 0 to 6.67 Mp particles/individual with average values of 2.23 ± 1.04 MPs particles/individual and 0.4 ± 0.24 for each gram of soft tissue. Meanwhile, the microplastic found in wild *P.veridis* averages 1.29 ± 1.19 Mp particles/individual and 0.44± 0.34 Mp particles/gram. The shapes of microplastics found in the tissue of the green mussel are high in fragments. Most polymers in fragments found in the natural and wild mussels were standard plastic used in plastic products such as polyethylene (PE) and polypropylene (PP). In comparing microplastic abundance in wild and farmed mussels, cultured mussel was the most contaminated with microplastics. However, a statistical test revealed no significant differences in the microplastic concentration between farmed and wild green mussels (T-test, confidence level of 5%, P value <0.05). The presence of MPs in mussels has an impact on health and provides a pathway for human exposure.

Keywords: Microplastics, green mussel, mariculture, seafood.

Introduction

The production of plastic products manufacturing has grown enormously with global production was 1.5 million tons in the 1950s and reaching 348 million tons in 2017 (Liu *et al.,* 2020). The constantly increasing production trends, usage patterns and poor waste management have resulted in a vast quantity of plastic. They have sparked widespread public concerns worldwide due to their discovery in living organisms and their persistence in the aquatic ecosystem. Due to environmental factors such as exposure to weather, wave action, UV light and microbial action, plastics in aquatic environment systems undergo physical and environmental degradation, ultimately breaking down and producing massive quantities of small microplastic particles creating change in the polymer chain. Microplastics (MPs) are small plastics particle with a diameter range between 1 μm to 5 mm and are ubiquitous in an aquatic environment (Andrady, 2017). In fact, because of their small size and high specific surface area, microplastics have the potential to be ingested by a wide range of aquatic animals at various trophic levels (Xu *et al.,* 2020).

Moreover, recent research has revealed that microplastics are extensively prevalent in daily diets, particularly in seafood ingested by humans and consequently provide a pathway to reach the food chain and affect human health (Phuong *et al.,* 2018; Teng *et al.,* 2019). For a long time, seafood items have long been considered an important source of protein and nutrients and they are growing increasingly popular among consumers. The increased demand for seafood has led the government sector to look for various alternatives to meet customers' needs. So far, mariculture plays an essential role as a resource-saving way to supply seafood worldwide. In addition, mariculture production accounts for more than half of worldwide seafood consumption, with a projected increase to 62% by 2030 (FAO, 2018; World Bank, 2013).

The practice of mariculture in seafood supply has become trending in Asia countries (Chen *et al.,* 2018). As aquaculture uses plastic base material in the operation system, there are questions and concerns about the possibility of microplastic pollution from marine culture activities. As found by Anderson *et al.* (2016), Waite *et al.* (2018) and Sparks *et al.* (2021) reported that plastic debris found in farmed is higher than wild mussels which may be a result of farming practices and facilities that use plastic-base materials. The study found by Li *et al.* (2019) reveals that the plastic debris found in fields is intimately connected to human anthropogenic activity. There is evidence of a significant positive and quantitative linear relationship between microplastics in farmed mussels and nearby waterways. Conflicting results also exist in the literature. For instance, Digka *et al.* (2018) and Birnstiel *et al.* (2019) claim that no significant difference was observed in the microplastic content in farmed and natural mussels. However, there is still a pertinent information gap in current studies on the presence of microplastics in mariculture ecosystems whether human activities in the surrounding environment are the key contributors or aquaculture systems being used in cultivating the organisms. The mariculture gear used in infrastructure is a potential source of microplastics in the marine environment. However, significant research on the effects of mariculture on cultivated species is currently insufficient (Chen *et al.,* 2018). Despite these worries concerning microplastic in seafood, the interplay of microplastics in organisms and their environments remains unknown; therefore, more comprehensive research should be carried out. In short, the literature offers that the aquaculture industry is a significant source of microplastics in aquatic environments but the impact of aquaculture on microplastics, especially in farmed organisms remains unclear.

The geographical influence is based on environmental factors that promote the survival of *P.veridis* such as water temperature and salinity of water, causing green mussels aquaculture activity to be recommended and become popular seafood for coastal communities in Pasir Putih estuary in Johor, Malaysia. On the other hand, the industrial and residential area surrounding the Pasir Putih estuary is predicted to result in a significant concentration of microplastics. The selection of green mussels in this study is a great bioindicator because of their ability to consume environmental pollutants, their value to seafood protection and their popularity in seafood dishes; they may also increase public health concerns. Consequently, it is a suitable and typical location for investigating microplastic contamination in a small-scale mariculture context. In order to clarify the uncertainty about the impact of the aquaculture system on the accumulation of microplastic levels in farmed green mussels, we aim to investigate the microplastic contents (levels, type, size and colour) in mariculture and natural mussels from two sampling stations in Pasir Putih estuary. In addition, we sampled *P.veridis* based on a different mood of life in the Pasir Putih estuary and analysed the characteristics and possible sources of microplastics accumulated in the samples. At the same time, the substrate used in aquaculture activities was collected and analysed to determine the type of polymer type of microplastics. Our research can provide thorough data on the contamination level of microplastics in green mussels and provide a meaningful perspective for a better understanding of the probable source of microplastics from aquaculture activities.

Materials and Methods

Sample Collection

The sampling events were held in the Pasir Putih estuary from June 2021 to December 2021. The location of sample sites is shown in Figure 1 and the detailed coordinate and overview of the location are shown in Table 1. A total of 378 individuals of *P. veridis* were collected. These locations were chosen based on population density, industrial activities and residential zones. Because of its geographical and hydrological characteristics, the Pasir Putih estuary is an ideal location for mariculture operations. The collection of green mussels was conducted between two stations namely, Station 1 (ST 1) (1 km to 1.2 km) from station 2, which represent farmed green mussel with a longline system, meanwhile station 2 (ST 2) represent the natural green mussel. The problem created by aquaculture activity used plastic-based materials such as rope and large blue plastic containers, which were suspected to be one of the sources of microplastic in cultivated mussels. The mussels were obtained from each sampling site, with 27 individuals pooled with three replicates of adults (80 mm to 120 mm), juveniles (50 mm to 79 mm) and babies (20 mm to 49 mm). The *P.veridis* were individually wrapped in aluminium foil, placed in plastic zip lock bags, transferred to the laboratory and maintained at 20°C until further testing.

Quality Control of Analysis

To address and analyse the potential procedural contamination, procedural blanks without tissue were put up concurrently. Before usage, all the liquids used in the experiment (potassium hydroxide, sodium chloride) were filtered using 0.45 µm filter paper. All samples were promptly wrapped in aluminium foil to eliminate potential airborne microplastics. To minimise airborne contaminants, precautions were taken during the experiments by wearing a cotton laboratory coat and gloves throughout the sample preparation and analysis steps. Finally, all glassware was washed and rinsed with distilled water before use.

Extraction of Soft Tissue with Potassium Hydroxide (KOH)

P.veridis extraction technique and microplastic analyses were modified based on (Ding *et al.,* 2018). The mussels were cleaned with tap water before recording their weight. A vernier calliper was used to measure the length, height and width of the shell. Each green mussel's soft tissue was dissected and documented. Three individual mussels of the same age were put in a 250 mL conical flask and treated as replicates. Three replicates were prepared for each location monthly. Approximately 150 mL of 10% KOH was added to each conical flask containing green mussel soft tissue, sealed with aluminium foil and placed in an oscillation incubator at 40ºC with 90 rpm for 24 hours, followed by 24 hours at ambient temperature, depending on soft tissue digesting impact. The digestion processes were stopped and regarded as entirely digested when the solution was clear and there were no visible particles.

Density Separation Using Sodium Chloride (NaCl)

The separation methods are based on density separation, employing a concentrated saline solution (1.2 g/cm^3, NaCl) to float microplastics and other anthropogenic waste from a dissolved liquid. Each flask containing *P.veridis* soft tissue received 150 mL of filtered saturated NaCl solution and was capped with aluminium foil. The mixture was then combined and left to float the microplastics overnight. Using a vacuum system, the overlying water was gently extracted and filtered through a GF/F (1.2 m pore size; 47 mm in diameter) glass microfibre filter (Whatman). The filter paper was placed in clean Petri dishes with a labelled cover and secured with tape for further analysis.

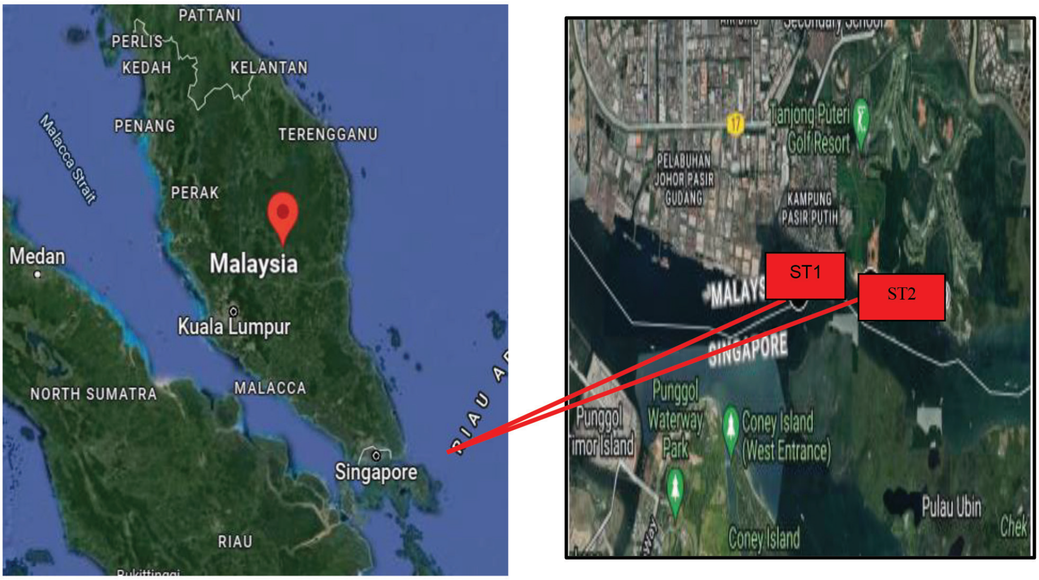


Figure 1: Sampling stations

Table 1: Coordinate of the sampling stations

Station No.	Location Overview	Coordinate	Mode of Live
ST 1		1.430237,103.942019	Farmed (Longline)
ST 2		1.431094,103.928223	Natural

Observation of Microplastics

After drying, the filter papers were inspected, photographed and labelled using a microscope (HSZ-600) at 40x - 45x magnification. First, a visual assessment was used to identify probable microplastics based on the physical characteristics of the particles. Based on the technique stated by Ding *et al.* (2018), several common and undeterminable particles were chosen and validated by ATR-FTIR Spectroscopy.

Verification of Microplastics Using ATR-FTIR Spectroscopy

All marked items were verified using Attenuated total reflectance fourier transform infrared (ATR-FTIR) Spectroscopy, Bruker brand, based on the approach described by (Ding *et al.,* 2018). The spectrum range was set from 650 to 4,000 cm^{-1}. The polymers were identified by comparing the functional group of the resulting reported in the literature with known polymers and infrared spectra references. Higher than 70% spectral similarity was considered reliable and microplastics (K. Zhang *et al*., 2016; Jung *et al*., 2018; Jian *et al*., 2020; Su *et al*., 2020).

Results and Discussion

Microscopic photographs found in *P.veridis* under a microscope with 40x-45x magnification with four shapes of microplastic particles are shown in Figure 2. The microplastics collected in the natural and wild green mussel in Pasir Putih estuary were classified based on their appearances, namely fibres, fragments, films, foam and others (including pellets, some irregularly complex shapes). The study found that different shapes and colours of microplastics variability, suggesting that different locations may provide different results due to environmental conditions, land use and other sources.

ATR-FTIR spectroscopy was used to identify MPs particles. Most polymers in fragments found in the natural and wild mussels were standard plastic used in plastic products such as polyethylene (PE) and polypropylene (PP). As pointed out by Ding *et al.* (2018) and Mathalon and Hill (2014), the prevalence of microplastics in farmed mussels was typically attributed to infrastructure used in mariculture as the longline system often employed plastic polypropylene plastic lines to anchor the mussels, which may present as a source of microplastics in their environments. In addition, polyamide was also discovered. The source of polyamide derived from the cultivation system used where a suspended rope was intensely employed in the aquaculture operation. Furthermore, nylons were favourable common plastic substances used in fishing gears.

The Abundance of Microplastics in P.veridis

Microplastic particles were found in the edible tissues of both cultivated and wild *P.veridis.* In cultured *P.veridis* samples, microplastic abundance ranged from 0 to 6.67 Mp particles/individual, with average values of 2.23 ± 1.04 MPs particles/individual and 0.4 ± 0.24 for each gram of soft tissue. Meanwhile, the microplastic found in wild *P.veridis* averages 1.29 ± 1.19 Mp particles/individual and 0.44± 0.34 Mp particles/gram. The result reveals that farmed green mussels contained more microplastics than wild green mussels. As shown in Figure 3, the amounts of accumulated microplastics observed slightly differed between farmed and wild mussels. These results correspond to finding by Mathalon and Hill (2014), Anderson *et al.* (2016), Ding *et al.* (2018) and Phuong *et al.* (2018) found that a higher amount of microplastic was identified in the farmed mussel compared to wild that may result of farming practice. However, the statistical test showed no significant differences in the microplastic concentration between farmed and wild green mussels (T-test, confidence level of 5%, P value <0.05). The use of polypropylene (PP) carrier lines as suspended ropes in green mussels aquaculture practice may present a source of microplastic to green mussels as the line degrades and is considered a key explanation for the greater MP levels in cultured green mussels (Hantoro *et al.,* 2019; Lyu *et al.*, 2020). Some short and old PP plastics may be found in green

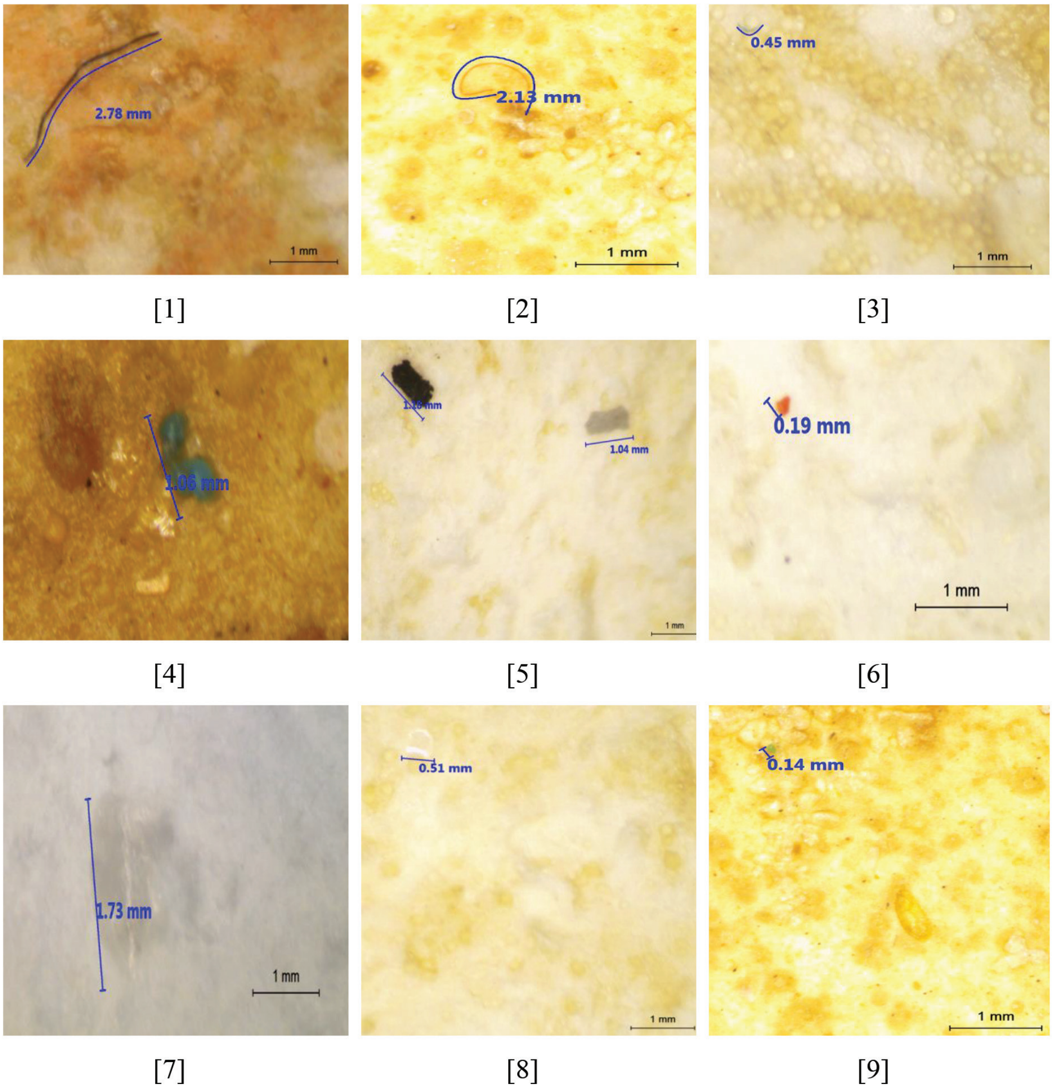
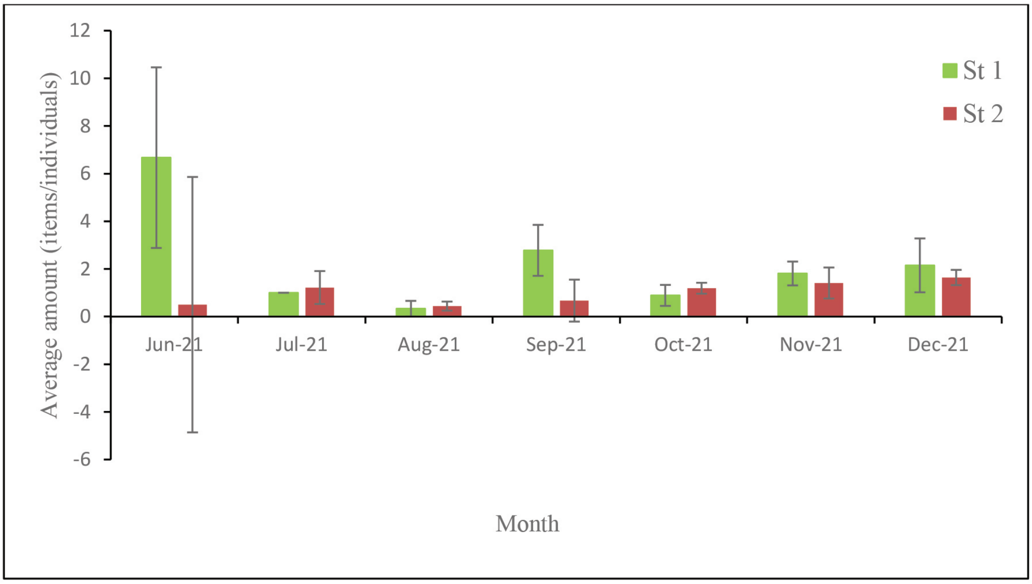


Figure 2: Images of different types of microplastics obtained under 40-45x magnification microscope in wild and farmed *P.veridis* (1-3) fibre, (4-6) fragment and (7-9) film/bead

mussel aquaculture environments, increasing the chances of plastics being consumed and inadvertently mistaken as food.

In contrast, Li *et al.* (2016) found the contrary, highlighting that the commercial mussel samples had considerably more MPs than aquaculture farm samples. Meanwhile, Davidson and Dudas (2016) and Mercogliano *et al.* (2020) reported no significant differences in the microplastic contents between cultured and natural mussels. Hence, the different concentrations of microplastics in green mussels might be significantly different in different locations considerably because of the level of microplastic contamination in the aquatic environment and should be closely related to the plastic pollution in their living areas.



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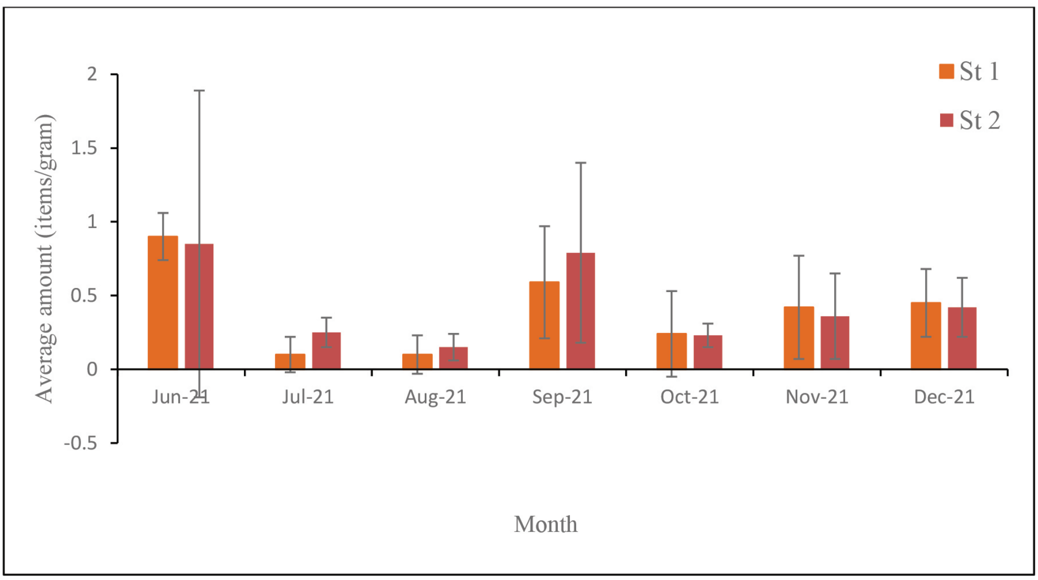
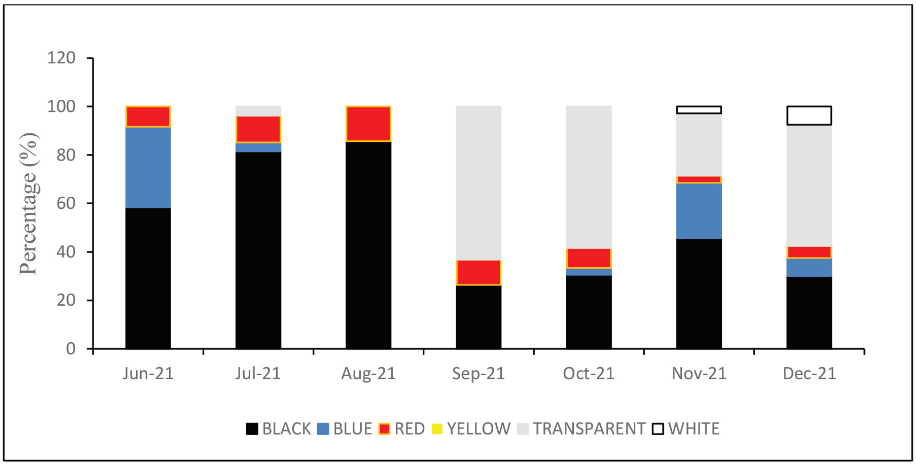


[B]

Figure 3: Average amount of microplastics items/individuals [A] and items/gram uptake by longline cultivated and wild *P.veridis* [B]

Microplastic Colour, Sizes and Shape

All six physical characters, namely blue, red, black, yellow, white and transparent were recorded throughout the sampling period (Figure 4). Microplastics with colours such as red, blue and yellow have a higher probability of being synthetic compared to transparent, black or white ones (Dehaut *et al.*, 2019). The dominant colour of microplastics extracted from *P.veridis* found in both stations was black



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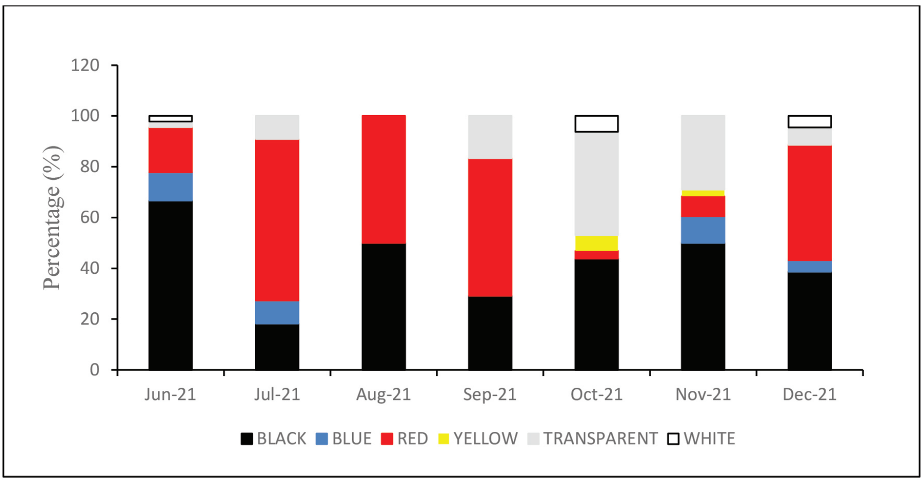


[B]

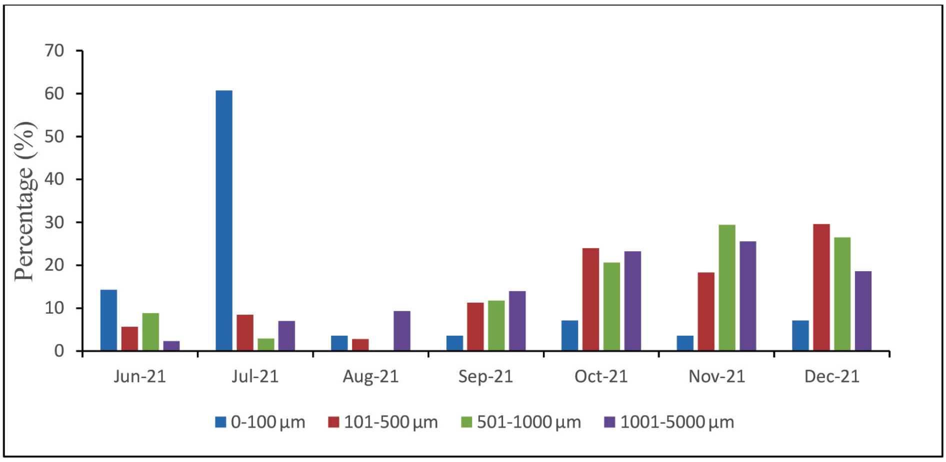
Figure 4: Microplastic by colour found in cultivated [A] and wild *P.veridis* [B]

with 54% in ST1 and 46% in ST 2, followed by red at 23% and 27% both in farmed and natural *P.veridis*. Blue 6% for both station, 1% and 2% yellow, transparent 16% - 17% and 3% white for ST 1. The various microplastic colour categories illustrated the diversity of their sources of pollution (Wang *et al.*, 2020). Ropes and nets in blue, black, white and red are widely used in fisheries and aquaculture in the Pasir Putih estuary. The percentage of yellowing or darkening caused by a rise in the carbonyl index and the level of ageing are utilised as indicators of weathering processes (Ta & Babel, 2020).

Microplastics vary in size, shape and colour which is particularly concerning because they are frequently mistaken for food by marine species. The presence of blue microplastics in the research area suggests that they are essentially secondary microplastics probably attributable to the waste-input coastal communities of the area.

Furthermore, different modes of mussel life might affect the occurrence rate of microplastic pollution. In the case of farmed mussel using a longline method for commercial purposes, high concentrations of blue microplastics have been linked to aquaculture infrastructures which derived plastic gear throughout the operation as a longline method using plastic bottles, some blue synthetic rope and blue HDPE drums as floating barrels preferential active ingestion by farmed mussels. This finding reinforces the notion that the aquaculture systems affect the microplastic pollution on farmed organisms as a result was similar to the study highlighted by Reguera *et al.* (2019) and also Zhang *et al.* (2020) claim the same finding on the issue. Moreover, the increased intake of blue microplastic was most likely due to extensive fishing activity in the Pasir Putih estuary, as outlined by Fang *et al.* (2019) and Zhu *et al.* (2020) in the Bohai Sea and Fujian province in China.

Microplastics in both wild and farmed mussels were divided into four size groups (Figure 5) based on their greatest dimension: < 0.1 mm, 0.1 mm-0.5 mm, 0.5 mm-1.0 mm and 1.0 mm-5.0 mm. Microplastics of more than 100 µm were present in all sampling months, concerning the size of microplastic found in the green mussel of our study. The most common size category was 0.1 mm-0.5 mm (59% in ST 1 and 49% in ST 2) were present in all sampling



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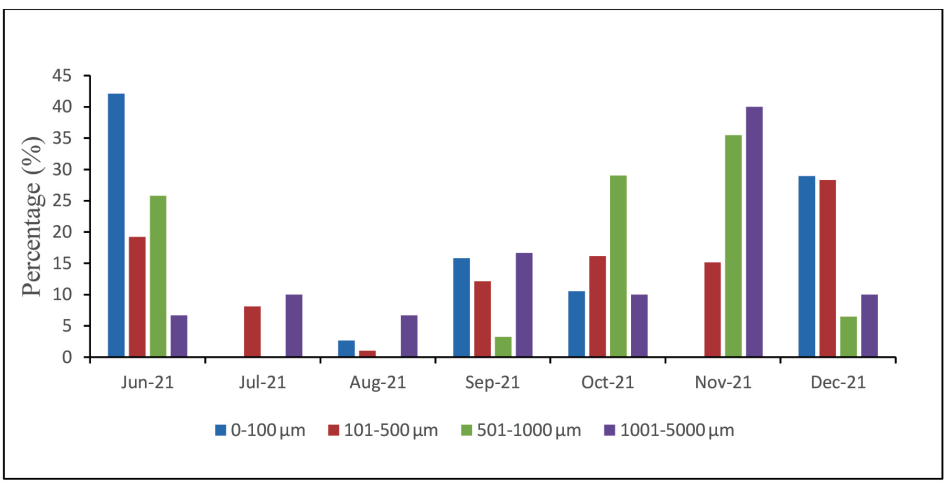
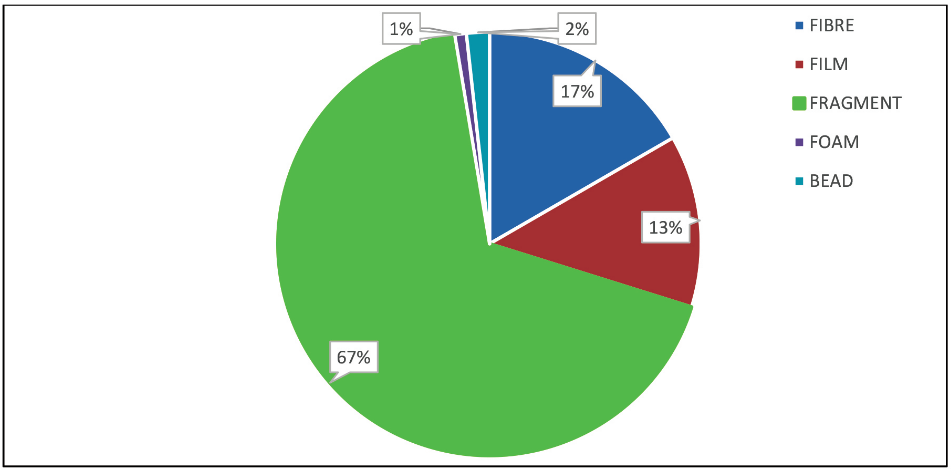


[B]

Figure 5: Microplastic particle based on sizes in cultivated [A] and wild *P.veridis* [B]

months, followed by particles < 0.1 mm for both wild and farmed *P.veridis* coinciding with the observations of Digka *et al.* (2018). The discrepancies and pattern in the distribution of MPs size can be linked to the sources of plastic debris and reflects the degree of weathering in the research locations (Ta & Babel, 2020). Overall, the number of microplastics reduced as size increased, indicating that mussel uptake of MPs is size-dependent. Besides, as pointed out by Patterson *et al.* (2021) and Li *et al.* (2021), the size of mussels tend to be a factor that influences MPs size uptake during the water filtering process. The fact that smaller-sized MPs appear to make up a more significant proportion of mussels suggests that mussels are more likely to consume tiny rather than larger microplastics.

Inconsistent with this finding, as shown in Figure 6, the most common shape category of microplastics discovered in both cultured and wild *P.veridis* are fragments indicating 68% at ST 1 and 76% at ST 2. However, ST 2 was found to have higher levels than ST 1. Similarly, in the study by Phuong *et al.* (2018), Digka *et al.* (2018) and Daniel *et al.* (2021) on mussels from the



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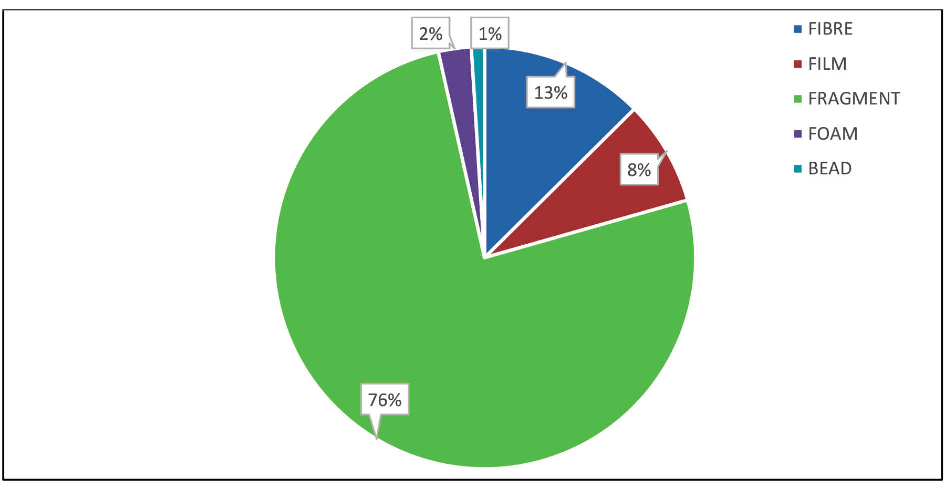

[B]

Figure 6: Shape of microplastic uptake by longline cultivated [A] and wild *P.veridis* [B]

French Atlantic coast Northern Ionian Sea and Kerala India, most of the microplastics observed were fragments reaching 82%, 77.8% and 69% which is close to our result (68% and 76% fragments). However, some comprehensive, globally studies on mussels such as Wakkaf *et al.* (2020) in Tunisia, Naidu (2019) in India, Li *et al*. (2018) on the coasts of England and Wales and Li *et al.* (2016) in China reported fibres are the most common form of MPs in mussels. As a study outlined by Li *et al.* (2019) and Chinfak *et al.* (2021), fibre and fragment are the most frequent microplastic morphology in mussels worldwide. Fibre has been discovered as the second most abundant type of MP in wild and cultivated *P.veridis,* with 17% and 13% in ST 1 and ST 2. The significant amount of fibres and fragments reveal the degradation of larger plastic products into secondary MPs through the combination of natural phenomena and mechanical forces, photolysis, thermal degradation and possibly via the biodegradation process (Mercogliano *et al.,* 2020). Microplastic fibres can be twisted or interlaced with food, increasing the consumption by organisms (Patterson *et al.*, 2021). The breakdown of larger plastics that are surface embrittled and fragmented by various degrees of physical activity such as photo-oxidative degradation, wave action in marine systems, physical wear and alternate freezing and thawing may result in a diversity of microplastic shapes (Patterson *et al.*, 2021).

Furthermore, the differences in morphology categories of ingested microplastics among green mussels might be attributed to varied sources and waste management practices in-country and sampling areas, tourism and marine activities (Chouchene *et al.*, 2020; Patchaiyappan *et al.*, 2020). In this scenario, microplastic uptake by green mussels can be attributed to sources such as urban inputs, fisheries and aquaculture. Variations in MPs morphology in aquatic habitats and living organisms may be caused by differences in polymer density, flexibility, stability and the degree of the ageing process resulting in a detrimental influence on the aquatic ecosystem.

Conclusion

This present study provides interesting results about the microplastic contamination in green mussels with different modes of life from Pasir Putih estuary in Johor, Malaysia. It contributes to the knowledge of microplastic contamination assessment through the finding obtained, allowing comparison between the same marine organisms with a different mode of living with an analysis of 378 individual *P.veridis* samples. Green mussel is also a commercially important food mollusc aquaculture in the Johor Strait. Our findings suggest that the concentration of microplastics in farmed green mussels harvested using a longline approach was greater than in wild green mussels. Microplastic concentration and type in green mussels were closely connected to microplastics in the surrounding environment. These study results would offer a piece of information for more studies on microplastics to help provide baseline data, especially on aquaculture perspectives and marine organisms. Most of the research studies have focused on aquaculture sectors as the source of microplastic pollution in farmed organisms. Hence, for future works, it would be interesting to study how the accumulation level of microplastics attribute to different types of aquaculture systems with different types of species.

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