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Microplastic contamination in the sediment of the Johor Strait Estuary, Malaysia

M M Zin^{1,2*}, S Azman¹, S H Anaziah³, N Khalid^{1,2}, S Jumali^{1,2} and N A Umaiban Yusof^{1,2}

¹ Department of Water and Environmental, Faculty of Civil Engineering, Universiti Teknologi Malaysia, Johor Bharu, Johor, Malaysia

² Civil Engineering Studies, College of Engineering, Universiti Teknologi MARA, Johor Branch, Pasir Gudang Campus, Masai, Johor, Malaysia

³ Chemical Engineering Studies, College of Engineering, Universiti Teknologi MARA, Johor Branch, Pasir Gudang Campus, Masai, Johor, Malaysia

*mazni9746@uitm.edu.my

Abstract. The issue of microplastics has garnered significant attention in light of their potential environmental hazards and possible health effects on higher trophic organisms and humans. However, the available information is restricted and knowledge about the interaction between microplastics in aquatic sediments and the microplastic-related impacts of aquaculture activities, and this area has not yet been adequately researched. This research looks at the occurrence and properties of microplastics in the bed sediment from *Perna veridis* aquaculture activity areas, in the Strait of Johor, Malaysia. Bed sediments were collected in replicates from five sampling sites using a box corer (Wildco). The extracted organic matter was digested with 30% hydrogen peroxide (H₂O₂) before being separated using NaCl and ZnCl solutions. The inspection of plastic debris was conducted via a Stereomicroscope (HSZ-600) at magnifications ranging from 40x to 45x and considered according to morphology, size, and colour. Microplastic polymers were validated using Attenuated Total Reflection Fourier Transferred Infra-Red (ATR-FTIR) spectroscopy. The sediment samples exhibited a varying range of microplastic abundance, with values ranging from 29 to 60 particles/ kg.d.w. Microplastics within the size range of 101-500µm (63%) predominated in all samples collected, with fragments (79%) and fibres (18%) being the main morphologies of MPs found. The dominant particles found in the sediment were polypropylene (PP) and polyamide. The findings suggest that aquaculture practises may have an impact on the presence of microplastics (MPs) in bottom sediment, as these locations are well-known aquaculture areas for *Perna veridis*.

1. Introduction

Plastics are remarkable because of their durability, lightness, and versatility. Market demand is forecast to increase, with worldwide plastic output expected to rise from 2 to 368 million metric tonnes (Mt) by 2019 [1]. Due to collection expenses and technical constraints, the recovery and ineffective management of plastic trash from entering aquatic ecosystems have resulted in a substantial volume of plastic garbage being discharged into the ocean [2]. Over a prolonged period, defragmentation of plastic waste by the culmination of natural and environmental interactions can



compromise the structural stability of plastic products, resulting in microplastics, which range in size from 1 μm to 5mm [3]. Biofouling and surface colonization by organisms increase particle density, leading microplastics to sink to the bottom [4].

Compared to surface waters, the distribution of sediments based on their stability may better reflect the long-term microplastic pollution situation in some locations, making sediments an ideal habitat for studying microplastic pollution [5]. [6] found that the abundance and characteristics of The presence of microplastics within bottom sediment can serve as an indicator for assessing the extent of microplastic pollution within the designated study area, since 70% of plastic waste is deposited on the ocean floor, riverbeds and bottoms of estuaries. However, the presence of microplastics in bed sediments exhibits variability and is predominantly influenced by their inherent characteristics and the surrounding environmental conditions [7]. The research conducted by [8], found that aquaculture activities can cause microplastic contamination in sediment, resulting in the accumulation of microplastics in farmed species. However, a notable knowledge deficit remains in existing scholarly on the occurrence of microplastics in sediments, particularly as a result of anthropogenic activities in the environment or plastic-based materials used in aquaculture infrastructure, prompting more comprehensive research [9]. Therefore, this study aims to determine the abundance of microplastics in the sediment, particularly in *Perna veridis* aquaculture areas in the Strait of Johor, Malaysia.

2. Methods

2.1. Sampling areas and procedures

The bed sediments were taken using a box corer (Wildco) in December 2021 from five sites in the Johor Strait estuary where *Perna veridis* aquaculture is practiced (Figure 1 and Table 1). The ideal subsample consisted of three samples per station taken in triplicate and mixed in a stainless-steel tray as one sample. The samples are then placed in containers and stored in a cool environment at 4°C in preparation for subsequent inspection. Sediment samples were covered with aluminium foil to minimize contamination from airborne plastic particles.

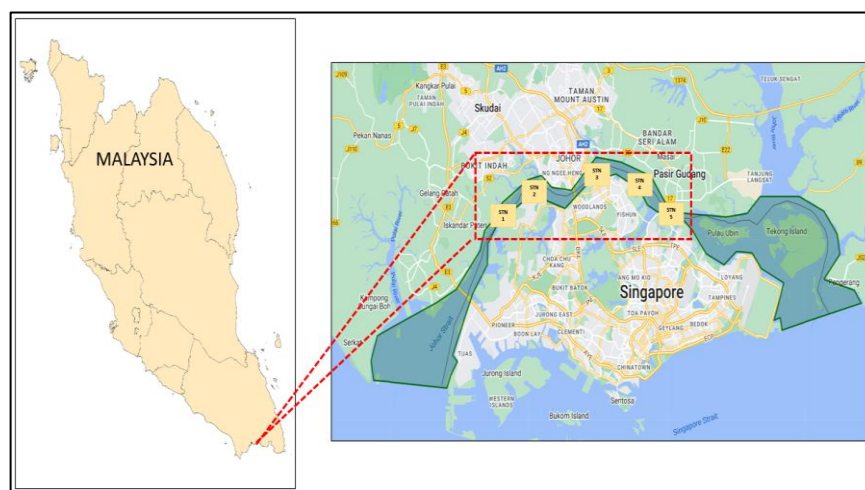


Figure 1. Location of the sampling sites.

Table 1. Coordinate of sampling location.

Sampling location	Latitude	Longitudes
STN1	1° 27' 29.8" N	103° 42' 37.0" E
STN2	1°27'21.7"N	103°42'23.1"E
STN3	1°27'45.7"N	103°51'17.2"E
STN4	1° 27' 59.7" N	103° 51' 17.244" E
STN5	1° 25' 48.9. " N	103° 56' 31.3" E

2.2. Pre-treatment of the sediment with 30% Hydrogen Peroxide (H_2O_2)

The sediment samples completed a drying process at temperature 50 °C for 48 hours. Digestion was performed three replicates. To eliminate organic matter, 50 g of dry sediments were weighed in a glass beaker, and 100 ml of 30% H_2O_2 was added. The solution was thereafter allowed to stand for a duration of 24 hours [10].

2.3. Density separation

Density separation followed sediment pre-treatment. A two-step extraction using different chemical densities was developed. The first solution separation process employed saturated NaCl (1.2 $g\text{cm}^{-3}$), while the second used zinc chloride (1.7 $g\text{cm}^{-3}$). The desiccated sediment was combined with 200 ml of NaCl, stirred for 15 minutes, and left overnight. A vacuum pump filtered the mixture via 1.2 μm Whatman GF/C. The filter paper was dried for further investigation. A constant weight was achieved by drying the sediment balance in a conical flask at 50°C for 3 days. ZnCl was added to the conical flask overnight for the second separation stage. The solution was then filtered with 1.2 μm filter paper and stored in a sealed petri dish for the next analysis.

2.4. Inspection and recognition of plastic debris

The microplastic particles that had been sorted were subjected to visual examination using a microscope (HSZ-600) with a magnification range of 40x-45x. Microplastics were recognised as particles with the characteristics of synthetic polymers (mouldable items with constant thickness and colour that do not break when squeezed with steel forceps). In case there was any uncertainty, a hot metal tip was placed on the object. The abundance of microplastic in sediment was expressed in items/kg d.w. The variation in microplastic abundance among the different sampling stations was assessed using a one-way analysis of variance (ANOVA) via Minitab Version 18 software. Differences at $p < 0.05$ were considered statistically significant. Ten percent of MPs selected from each sample group were used in order to ascertain the functional groups that are linked to the chemical characteristics of polymers using Attenuated Total Reflection Fourier Transferred Infra-Red (ATR-FTIR) spectroscopy (Bruker brand). The spectral range was set to 800 to 4000 cm^{-1} . The identification of polymers was accomplished by analysing the obtained spectra and comparing the adsorption bands documented in the existing literature, with a focus on the functional groups present. The spectra that matched more than 70% were considered reliable and were used to confirm the polymer types [11].

3. Results and Discussions

3.1. Occurance of microplastics in bed sediment

The highest concentration of MPs was at station 4 in Teluk Jawa (60 items/kg.d.w.), followed by 59 items/kg.d.at station 5 in Pasir Putih. Meanwhile, station 3 showed the lowest values (29items/kg.d.w.). In addition, station 1 consisted of 31 items/kg. d.w., and the MP concentration increased at station 2 to 43items/kg. d.w. (Figure 2). Statistical analysis using analysis of variance (ANOVA) at a 95% confidence level (Minitab 18.0) revealed significant differences ($P < 0.05$)

between sampling sites and the occurrence of microplastics in sediment samples. Most research found increased MPs concentrations in sediment in aquaculture locations such as Xiangshan Bay, China [12], Muara Kamal, Indonesia [13], and Ma'an Archipelago, China. MP sources and aquaculture conditions affect microplastic concentrations [14].

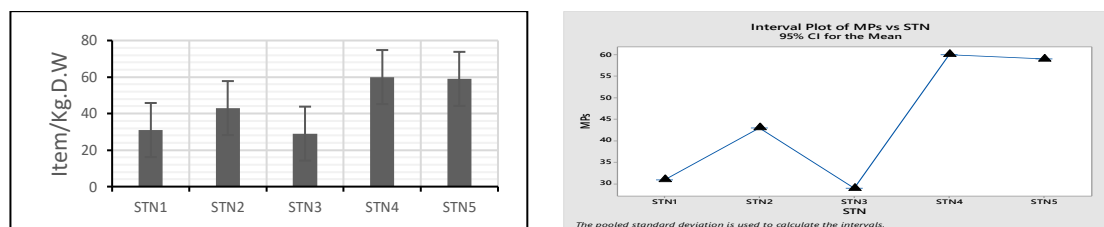


Figure 2. Microplastics abundance in bed sediment across station.

3.2. Characteristic of microplastics

3.2.1. Colour, morphologies and sizes of microplastics

The colour of the extracted MPs was classified as black, blue, red, yellow, transparent, and white (Figure 3B). Black (32%), red (32%), and transparent (25%) particles comprised the majority of observed MPs at all study sites. White and blue account for 1% and 9%, respectively. Yellow microplastics were absent from sediment samples. The findings of the study align with the outcomes reported in reference [15], who found that black was the most abundant colour in aquatic sediments. The colour may indicate the origin of the plastic waste and weathering at the study sites [16]. The current study found that *Perna veridis* aquaculture and anthropogenic activities along the estuary may contribute to the diversity of Mps colour. However, over time, UV radiation and weathering can also affect colour.

In addition, MPs were classified as fibres, fragments, foam, beads, and films (Figure 3A). Figure 4 shows the morphology of microplastics observed in this study. Fragments (79%), fibres (18%), and beads (2%) predominated. No film or foam particles were found in the sediment samples. In the aquaculture area situated within the Chao Phraya River estuary in Thailand, [17] discovered fragments were the most prominent in sediment samples. The majority of fragments in sediment are secondary sources from mechanical, weathering, and biological disintegration of larger plastic components. However, some experts claim that fibres are the most abundant form in sediment due to aquaculture activity in estuaries and inner bays [15].

Furthermore, in the current study, MPs exhibited a range from 0.1 to 5 mm (Figure 4C). The majority were 0.1–0.5 mm (63%), followed by 0.1–0.5 mm (15%). The assessment of the extent of MPs contamination in research sites and its potential ecological consequences heavily relies on the analysis of MP size distribution. Indeed, several studies have found that particle size affects the distribution of MPs in animal tissues [18][19].

3.2.2. Types of microplastics polymers in sediment

The ATR-FTIR spectrum at all five stations showed similar peak characteristics for polypropylene (PP) and polyamide (PA) respectively. Consequently, the predominant occurrence of MPs fibre, mainly composed of polyamide in sediment could be related to the above facts, originating from aquaculture or fishing gear in the estuary. This statement was reinforced by the study by [14], who claimed that plastic fishing gear represents a highly prevalent and significant contributor to the presence of microplastics in aquaculture ecosystems due to the aging of plastic fishing gear, such as fishing nets, which is thought to be a contributing factor. Furthermore, ATR-FTIR revealed that all fragments had a material quality identical to major plastic items, implying that several MPs may have originated from the same source.

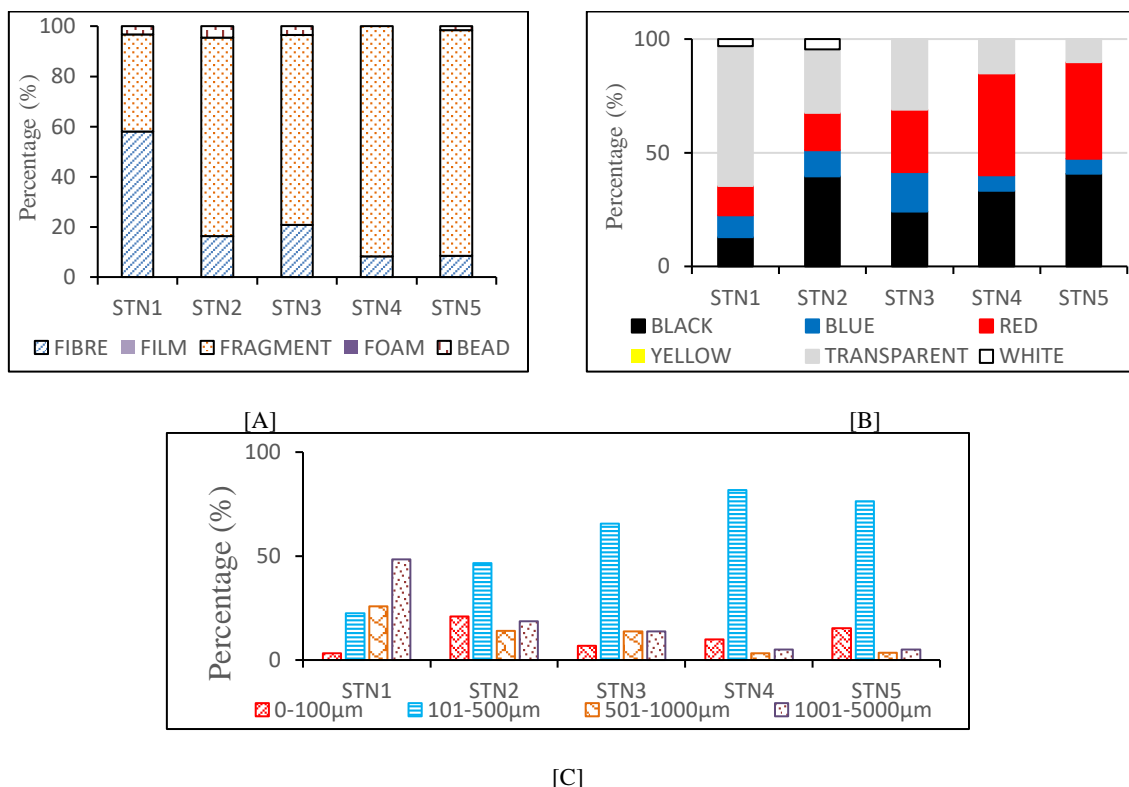


Figure 3. MPs Characteristics: Shape [A], Colour [B], Sizes and [D] Morphology of Mps found.

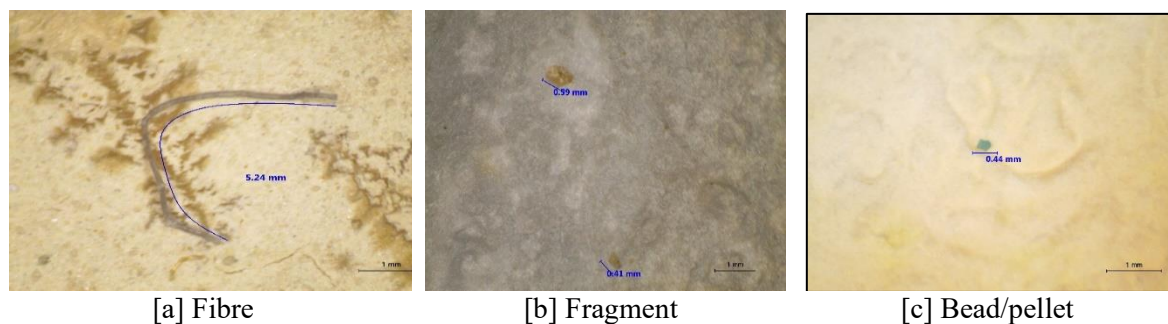


Figure 4. The morphology of microplastic observed under a microscope.

4. Conclusion

The highest concentration of MPs was found at station 4 with 60 particles/kg d.w while the minimum value was observed at station 3, with 29 particles/kg d.w. MPs size (0.1–0.5 mm) dominated all station samples. The morphology of the MP consisted mainly of fragments and fibres, suggesting that larger pieces of plastic degrade into secondary microplastics. The most abundant polymers were PP and polyamide, mainly from fishing gear and plastic packaging. The findings suggested that aquaculture may increase microplastics in estuary sediment due to the use of ropes and buoyant plastic in the mussel infrastructure.

Acknowledgments

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