



A multidimensional approach for microplastics monitoring in two major tropical river basins, Malaysia

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ABSTRACT

Microplastics (MPs) with the size of 1 μm –5 mm are pollutants of great concern ubiquitously found in the environment. Existing efforts have found that most of the MPs present in the seas mainly originated from land via riverine inputs. Asian rivers are known to be among the top in microplastic emissions. However, field data are scarce, especially in Malaysia. This study presents the distribution and characteristics of MPs in the surface water of two major river basins of Malaysia, namely Langat River (West Coast/Straits of Malacca) and Kelantan River (East Coast/South China Sea). Water samples were collected at 21–22 locations in Kelantan and Langat rivers, covering the river, estuary and sea. MPs were physically classified based on sizes, shapes, colours and surface morphology (SEM-EDS). The average of 179.6 items/L and 1464.8 items/L of MPs had been quantified from Kelantan and Langat rivers, respectively. Fibre (91.90%) was highly recorded at Kelantan, compared to Langat whereby both fibre (59.21%) and fragment (38.87%) were prevalence. Anthropogenic activities and urbanised areas contribute to high microplastic abundance, especially in the Langat River. Micro-FTIR analysis identified 14 polymers in Kelantan River, whereas 20 polymers were found in Langat River. Polypropylene, polyethylene, polyethylene terephthalate, nylon, phenoxy resins, poly(methyl acrylate), poly(methyl methacrylate), polystyrene, polytetrafluoroethylene, polyurethane and rayon were discovered in both rivers, although only polyethylene was significant (>1 ppm) when further analysed using pyrolysis-GC/MS. Correlation analysis and multiple linear regression were used to explain the relationship between water quality and MP abundance, suggesting only turbidity was positively significant to the microplastic occurrence. This comprehensive study is first to suggest a full-scale monitoring protocol for MPs in Malaysian riverine system and is significant in understanding MPs abundance in correlation to *in-situ* environmental factors. Consequently, this will allow the right authorities to develop mitigation strategies to address riverine plastic pollution in major river basins in Malaysia and the South East Asia.

1. Introduction

Microplastic (MP) refers to plastic particles less than 5 mm in size mostly appeared from manufactured plastic products (primary) and resulted from the breakdown of larger plastic (secondary) by water

turbulence, UV light or physical and chemical effects (Hildago-Ruz et al., 2012). When high concentrations of MPs are released into the aquatic environment, it may pose a significant risk of becoming food for the primary producers such as zooplankton, macroinvertebrates, fish, and mammals, which could then have an adverse effect on human health

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when consumed (Nan et al., 2020; Ibrahim et al., 2021). Noteworthy, MPs become a great concern when they serve as vectors for organic pollutants, metals, microorganisms, etc. (Amelia et al., 2021; Brennecke et al., 2016; Tang, 2021).

Over the past few decades, the presence of MPs in freshwater matrices has become a serious concern. The riverine system pathway could be the main route for 2.4 million tons of plastic waste into oceans annually (Han et al., 2020; Wang et al., 2021). Several studies demonstrated that the MPs in the marine environment originates from land-based sources, with rivers being the primary transporter of MPs pollution to the sea (Miejer et al., 2021; Wu et al., 2020). Previous works are anticipated on MPs variation, source identification, interaction at the water-sediment interface, potential impact, and possible chemical transfer to organisms (Eerkes-Medrano et al., 2015; Li et al., 2020; Meng et al., 2020). Nonetheless, quantitative analysis of the environmental fate of riverine MPs remains limited, leading to a lack of proper risk assessment. There are few significant hurdles, including non-harmonized sampling techniques, a large spatiotemporal variance of microplastics in rivers, a complex relationship between river, estuary, and ocean cycles, and different analysis protocols that are necessary to review and examine before allowing clear data comparisons (Bai et al., 2021; Bellasi et al., 2020).

Considerable attention should be given to the ever-increasing concentration of MPs by monitoring their occurrence, abundance and distribution to potentially formulate an effective mitigation strategy, whereby the desired monitoring work should focus on their occurrence, abundance, pattern distribution, and seasonality. For instance, the presence of MPs in the Malaysia rivers has been reported (e.g. Dungun, Cherating, Bara, Miri, Skudai and Tebrau rivers), but the data available is limited (due to limited sampling point and sample volume), with lack of detailed analysis especially on the polymer composition and relationship with water quality parameter, in which not fully understood. The water quality index relatively considered as the deterioration of water quality in particular rivers. Hence, fluctuations in MPs in these rivers may be resulted from the anthropogenic activities such as those connected to agriculture, residential sewage, the manufacturing of industrial plastic, and the fishing industry (Choong et al., 2021; Hwi et al., 2020; Liong et al., 2021; Pariatamby et al., 2020; Sarijan et al., 2018; Zaki et al., 2021).

In addition, there is still a research gap in the MPs research regarding the factors influencing the source, path, fate and prevalence of MPs and their degradation factors, especially in tropical countries. Efforts are also needed to understand the variation of MPs abundance and composition due to local activities. In this study, the Langat River and Kelantan River basins were selected as research sites due to population density around the area, thus leading to the potential contribution of microplastics from anthropogenic activities (polluted vs less-polluted rivers). For instance, the Langat River basin is known to supply water to more than 2 million people around the River basin water catchment in the Selangor state of Malaysia (Ahmed and Mokhtar, 2020), while the Kelantan River basin provides water supply to around 500 thousand population around the state of Kelantan (Yee et al., 2019). Furthermore, this study will be the first to report the comparative data of microplastic distribution in both important river basins in correlation with water quality parameters, with complete analysis from physical analysis (visual identifications by shape, colour and size), followed by chemical characterisation by Scanning Electron Microscope–Energy Dispersive X-ray spectroscopy (surface morphology and elemental analysis), micro-Fourier Transform Infrared spectroscopy (polymer identification) and pyrolysis-Gas Chromatography Mass spectrometry (polymer quantification). The results of this study not only fill the gap in current baseline data on MPs pollution, indeed, the data obtained would serve as reference for following studies in other tropical freshwater systems.

2. Materials and methods

2.1. Study area

The sampling locations selected cover seawater, estuary, and river areas (20 points with additional catchments) (Fig. 1). The Kelantan River is located on the east coast of peninsular Malaysia and could encompass the entire state of Kelantan. The river basin is 13,100 km² and reaches a maximum elevation of 2135-m. The Kelantan River originates in Kuala Krai (upstream) and empties into the South China Sea via the capital city of Kota Bharu. The river has a total length of 248 km (Wang et al., 2017). The study area was limited to a small portion of the river basin, specifically the downstream area of 20 km flowing out to the sea with a 1-km sampling interval for 20 sampling stations (SK01–SK20), with three distinct downstream zonal types were identified: river, estuary, and sea area. An additional sampling station at km-22 (SK22) was added to assess the surrounding factors affecting the distribution of MPs in the nearby tributary area.

Langat River is one of the four major riverine systems in Selangor, the most populous state in Malaysia, with an estimated total area of 2352 km² and approximately 200 km long (Amirabadizadeh et al., 2015). Langat River flows 182 km from the upstream zone in the Titiwangsa Range in the northern part and flows to the Strait of Malacca downstream (Ahmed and Mokhtar, 2020). Similarly to Kelantan site, sampling covered the river basin downstream of the Langat River with 20 stations (SL01–SL20) separated by a 1-km interval. Additionally, the sampling at this river includes the tributary area at SL22 and. It is worth noting that the water quality parameters were taken at both the ‘start’ and ‘end’ of towing activity, while the water sample was collected at the ‘end’ of each towing activity. For instance, SK00 represent the starting point of the first towing, whereby SK01 was the end point of this towing activity (transect SK01, Fig. 1).

2.2. Collection of water samples

Samples were collected using a Neuston net with a mesh size of approximately 300 µm (Hydro-Bios, Germany) and towed at a low speed of 1–2 knots against the flow (Sun et al., 2021). The surface sampling depths were found to be between 0.16 and 0.30 m, and towing activity lasted 10–20 min per tow, depending on the condition of the water. The water analysis of river and stream flow was conducted *in situ* using Hydrolab Multiparameter Sonde and ADCP, respectively. Bulk samples of river water were collected and stored in sampling bottles for further MPs analysis (MRIG laboratory) and water quality index (Water Quality Laboratory, NAHRIM).

A total of twenty samples of each river water were collected for the Kelantan River (SK01–SK20) and Langat River (SL01–SL20) during the research expeditions carried out in August and September 2021. The sampling design includes two stations at sea (SK01–SK02/SL01–SL02), one station in the estuary (SK03/SL03), and seventeen stations along the river (SK04–SK20/SL04–SL20) with a 1-km interval between them. Additional sampling sites (SK22, SL22, and SL23) represent water catchment areas within the tributary zone that connects to the main-stream. Water quality measurements were conducted immediately on the sampling day to ensure the representativeness of the data obtained. In brief, the Suspended Solid (SS) was analysed using APHA 2540D. The Ammoniacal Nitrogen (NH₃-N) was determined using APHA 4500-NH₃ and HACH Method-8038, Biochemical Oxygen Demand (BOD₅) was measured by APHA 5120B and APHA 4500-OG, and Chemical Oxygen Demand (COD) was analysed by HACH Method-8000.

2.3. Extraction of microplastics

In the laboratory, water samples were transferred to a sequential sieve with a mesh porosity of 5 mm, 1 mm, and 0.3 mm (Sun et al., 2021). While, the water sample residue (containing particle size <0.3

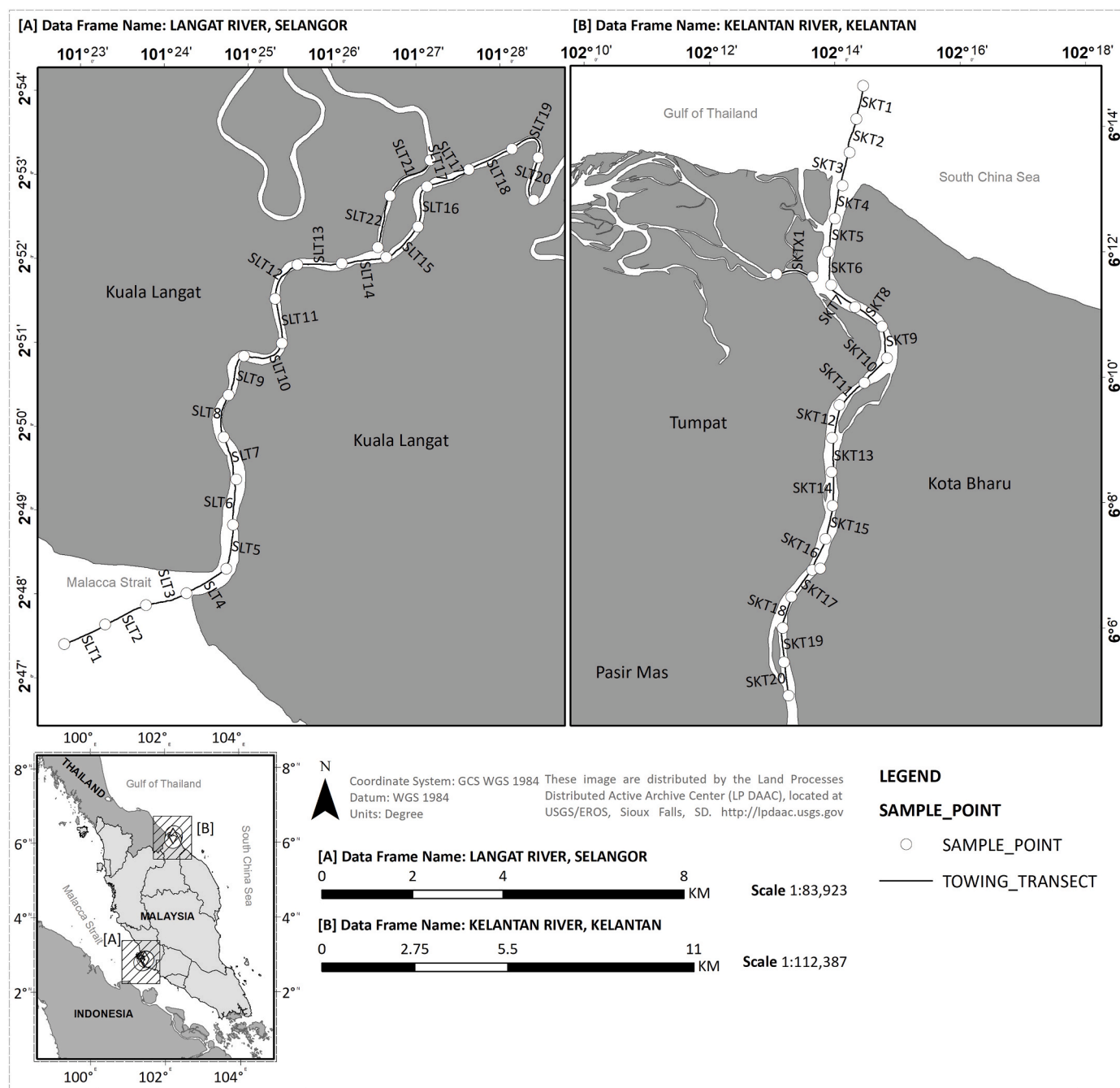


Fig. 1. The sampling locations selected basins cover seawater, estuary, and river areas of (a)Langkat River (b)Kelantan River.

mm) was subjected to the wet peroxide oxidation. A 20 mL of 30% H_2O_2 (analytical grade, Sigma Aldrich) solution was added to the beaker containing the water sample. The mixture was heated to 60 °C overnight and stirred at 180 rpm in a fume hood. In the presence of remaining organic material, 20 mL of 0.05M FeSO_4 (Sigma Aldrich) was added to the mixture, and the cycle of heating and stirring was repeated. After wet peroxide oxidation, the water sample was filtered using a GF/C filter paper (Whatman 47 mm, 1.2 μm pore size). The damped filter papers were placed in glass Petri dishes and covered with aluminium foil to prevent any potential airborne contamination, followed by drying in the desiccator for at least 24h before further analysis.

2.4. Microplastics characterisation

2.4.1. Visual analysis using microscope-axiocam

The dried, filtered samples were visually examined using a stereo-microscope (Olympus SZ51, Japan). MPs were observed and categorised based on their type and colour using small stainless-steel forceps. A hot needle test was utilised to verify the MPs materials since the MPs particles react to a high-temperature needle. The dimensions of the sorted MPs were determined using a ZEISS Axiocam208 microscope (Carl Zeiss, Germany) equipped with ZEN3.3 software.

2.4.2. Surface analysis using SEM-EDS

Scanning Electron Microscope–Energy Dispersive X-ray Spectroscopy (SEM-EDS, X-Max Oxford Instruments and XFlash Detector 610M Bruker) analysis was performed on representative MPs samples to

determine their morphology criteria and surface analysis. The particles were chosen randomly based on dominant shape found within the samples. Before the analysis, the sample was dried in a Universal Oven (Mettler GmbH, Germany) at 39.5 °C for 24h before it was transferred to a stub and coated with gold sputtering. The surface analysis was conducted at 15 kV, while element identification and surface mapping were subjected to EDS analysis.

2.4.3. Chemical identification using micro-FTIR

Samples were characterised and identified using micro-FTIR spectroscopy to identify the polymer of MPs (Nicolet iN10, ThermoFisher Scientific). The micro-FTIR has an ultra-fast motorised stage and a liquid nitrogen-cooled MCT detector. The solution containing MPs was filtered three times through a stainless-steel filter (13 mm, 20 µm pore size) and dried in a glass desiccator. Sample was analysed using Omnic Picta software and at a scan range of 4000 cm⁻¹ to 650 cm⁻¹ with 8 cm⁻¹ resolutions in transmittance mode. The spectral background was obtained on a clean part of the filter and the sample was considered a polymer if it matched the polymer in the instrument spectral library and work done by [de Frond et al. \(2021\)](#) and [Jung et al. \(2018\)](#).

2.4.4. Microplastic quantification using pyrolysis-GC/MS

Prior to pyrolysis-GC/MS analysis, a set of the calibration curve was prepared based on 12 common polymers, including PE, PP, PS, ABS, SBR, PMMA, PC, PVC, PU, PET, N6, and N66 (Microplastic Calibration Standard Set (MPCS), Frontier Lab). In brief, the microplastic samples were filtered through a pre-conditioned 13-mm stainless-steel filter, before it was folded into a pyrolysis sample cup for analysis. The analysis was performed with a pyrolyser (400 °C, Frontier Lab, Japan) attached to a gas chromatography-mass spectrometry (Agilent 6890N & 5973N, USA). Separation was carried out using a pre-column (Ultra Alloy*-5, length 30m, i.d. 0.25 mm, film thickness 0.5 µm) and column (Ultra Alloy*-50, length 2m, i.d. 0.25 mm, film thickness 10.5 µm). The sample blank was run in every sequence of 8 samples to evaluate the risk of cross-contamination while preparing the samples. To confirm the identity of each quantified MP, the minimum acceptable threshold for individual MPs is at 80% probability. The quantification was adopted based on the calibration and method as suggested by [Anuar et al. \(2022\)](#), [Frontier Lab \(2021\)](#), [Fischer and Scholz-Böttcher \(2017\)](#).

2.5. Contamination control

The contamination control during sampling activity was commenced as (i) procedure blank filter paper set for airborne MP control and (ii) field blank with filtered Milli-Q water placed in clean Schott bottles. The laboratory analysis was performed in a clean room with strict precautions were implemented to minimise the risk of contaminated samples. Any plastic materials is avoided, with only clean lab coats and cotton clothing were worn. The beaker and glass equipment of the vacuum pump system was rinsed three times with filtered Milli-Q water throughout each filtration cycle to prevent loss of MPs particles owing to surface contact with the glass beaker. Furthermore, any potential for secondary contamination via airborne contamination was evaluated by exposing clean filter papers to the air during sieving, filtration, and sorting/transfer procedures.

2.6. Statistical analysis

The data were analysed and interpreted using the R-Studio version 4.1.0 software (R Development, USA) to ensure the validity and integrity of the data obtained. Prior to data analysis, the normality (Shapiro-Wilk) test was run initially with a 95% confidence level ($p < 0.05$) (XLSTAT v2019, USA). To study the significant differences involving non-parametric variables, the Mann-Whitney *U* Test ($p < 0.05$, two-tailed) was performed to determine the significant differences between Kelantan River and Langat River for each water parameter.

Further analysis of correlation analysis (CA) and multiple linear regression (MLR) follows the parametric principle. A Pearson correlation matrix analysis was conducted for the identification of the correlation between MPs concentration for each sampling station, physical water parameters as well as water quality status. All correlation analyses were tested with a 95% confidence interval ($\alpha:0.05$). The correlation strength was defined into three different classes: strong ($r \geq 0.70$), moderate ($0.3 \leq r \leq 0.69$), and weak ($r \leq 0.29$) correlations ([Ibrahim et al., 2021](#)). For the final analysis, the data analysis is achieved using regression (XLSTAT). This study integrated the sensitivity analysis (SA) with MLR by forming the MLR-SA. MLR-SA has been widely used in deriving the simultaneous linear relationship between all dependent and independent variables based on the best value of the coefficient of determination (CoD), R^2 ([Arora and Keshari, 2018](#)). The R^2 result analysis is transformed into percentage contribution using SA to discover the least to most contribution to MPs abundance in both rivers.

3. Results and discussion

3.1. Abundance of microplastics

MPs abundance is varied on different geographical and input sources for comparison from a global perspective. As far as the Malaysian perspective is concerned, microplastic quantification and dispersion data in tropical surface water of various types (coastal, estuarine, tributary, and river) comparison have never been done in Malaysia. Thus this work may serve as a starting point for future monitoring program. Both rivers have a comparable number of sample stations, with each station separated by a kilometre. Additionally, 1 station was added in Kelantan River (S22) and 2 additional stations in Langat River to reflect the tributary portion. As such, the comparison of the total abundance of MPs in both rivers is shown in [Fig. 2](#), in which they were divided into four zones to emphasise the different water statuses. It was found that the abundance differed significantly between the station in both rivers ($p < 0.05$). On average, coastal recorded as low as 535.75 items/L, with estuarine recording the highest average (1133 items/L), followed by the tributary (1101 items/L) and river (830.59 items/L). Similarly, [Luo et al. \(2019\)](#) have identified that the coastal zone has a much lower MPs abundance than river.

In detail, there were 3772 pieces (average 179.6 items/L) of MPs in Kelantan River. MPs were only prominent in the Kelantan River between stations SK14 and SK20, with an abundance of 219–392 items/L. SK18 was the highest potentially contributed by the city effluent, particularly from the Kota Bharu district, where a massive monsoon drain is located around this sampling site. Apart from that, agricultural activities like coconut, rubber, corn, and sugarcane are cultivated along the river upstream. Additionally, SK22 is the additional station situated at the tributary of Kelantan River, and geographically, the tributary located in Tumpat River that has a narrower and slower flow compared to the mainstream of Kelantan River, resulting in a lower amount of MPs detected (143 items/L).

In contrast, Langat River offers the estuary area features a diverse environment, including mangrove areas downstream, but is densely industrialised along the river. Langat river has located approximately 21 km from Port Klang (i.e. the port with the highest traffic in Peninsular Malaysia). In Langat River, a total of 32,225 item MPs (average of 1464.8 items/L) was identified, in which 80% greater than Kelantan River. It is not surprising since Langat River is dense with heavy industry, which includes plastic waste recovery, steel production, haulage and logistics, vessel and tanker dock maintenance, metal material fabrication, metal waste generation, sand mining, wooden pallet manufacturing, and wastewater treatment plant. The rapid and poorly managed urbanisation and industrialisation in nearby areas have consequently deteriorated Langat River quality. However, contrary to Kelantan River, downstream sampling locations between SL03–SL10 are present with the most MPs, ranging from 1865 to 4240 items/L. By far,

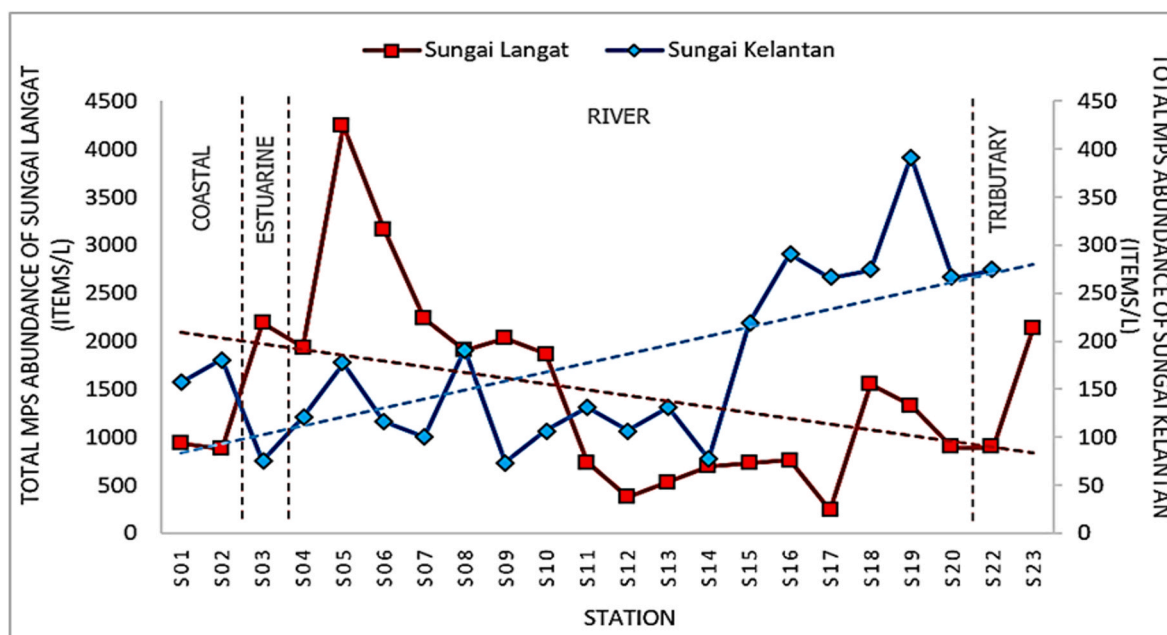


Fig. 2. Total Microplastic Abundance (items/L) for Kelantan river vs Langat river.

the abundance of MPs in Langat River is still lesser compared to the other rivers worldwide. For instance, the Qin River of China, was polluted with 16,670–611,110 items/L of MPs (Zhang et al., 2020),

whilst the Surabaya River accounted for 1470–43,110 items/L (Lestari et al., 2020). However, there was a situation where the MPs distribution was extremely low in other continents. For example, in East Africa, the

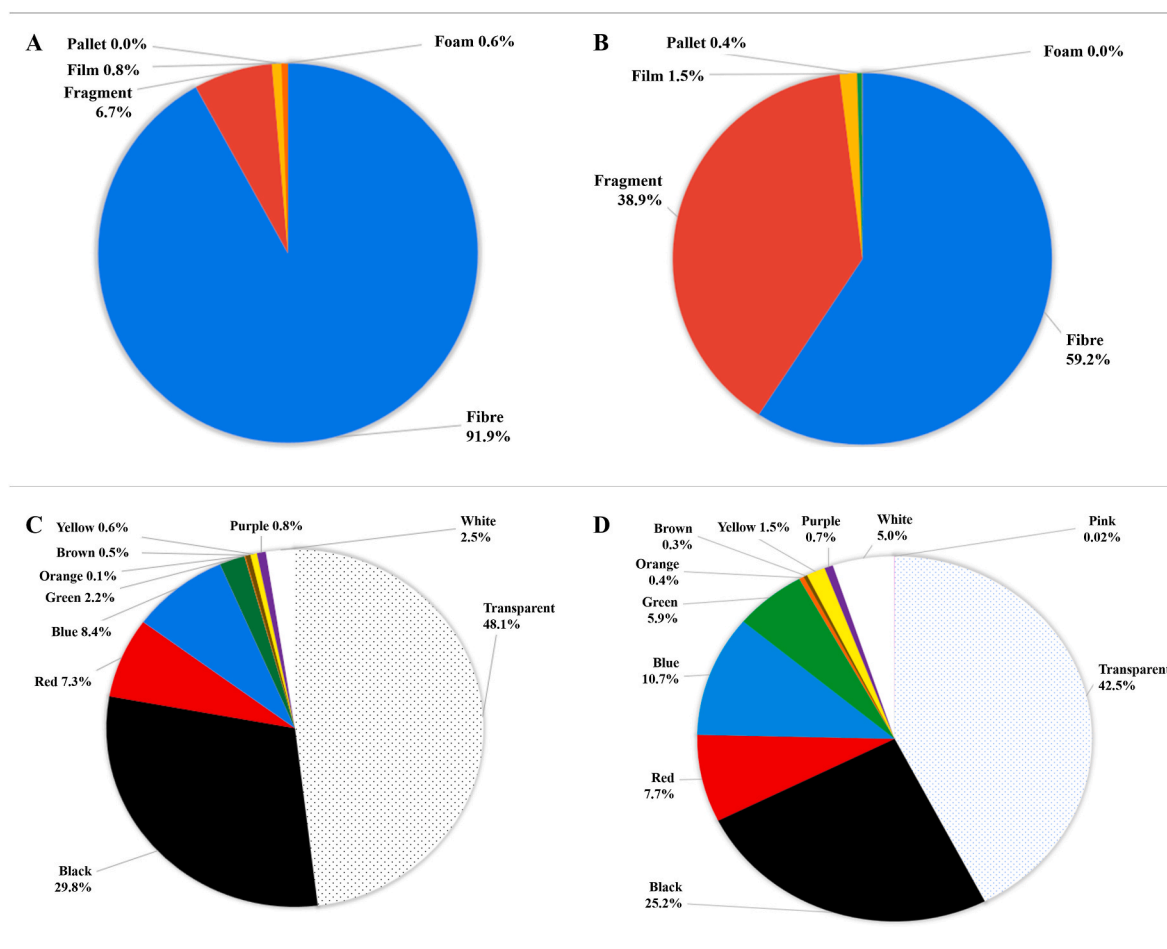


Fig. 3. Percentage distribution of shapes (a)Kelantan river (b)Langat river; and colours (c)Kelantan river (d)Langat river.

range of MPs in the Kagera River and Katonga River were recorded as 20–140 items/L (Egessa et al., 2020). Nevertheless, the size and flow rate of each river is critical in determining the amount of pollution carried to the sea. Thus, to determine whether there is any valid argument based on the differences in each river's physical characteristics, these aspects may be considered in the future as one of the primary views that should be mentioned with other environmental factors.

3.1.1. Type and colour

Five MPs were identified in water samples from the Kelantan River and Langat River: fibres, fragments, films, pellets, and foam (Supplementary 1), whereby eleven colours were recorded among the samples (Supplementary 2). Fig. 3 tabulates the percentage distribution of MPs by type and colour in both rivers. MPs abundance collected from Kelantan River was 91.90% fibre (3468 items/L), whilst both fibre and fragment were found prevalence in Langat River, accounting for 59.21% (19,080 items/L) and 38.87% (12,527 items/L), respectively. The findings are in-line with those reported in other countries where the most abundant MPs identified are fibre and fragment types in Mainland China (Liu et al., 2021; Lu et al., 2020; Luo et al., 2019; Wu et al., 2020; Zhang et al., 2020) and Indonesia (Wicaksono et al., 2021). Since the 1990s, Langat River has had a long history of aquatic pollution, which industrial activity has aggravated in recent years. Therefore, it is unsurprising that the MPs recorded in Langat River is 8.54 times higher than in Kelantan River. The fibre type was commonly found in the river and other water bodies that were possibly sourced from washing cloth, considering the wear and tear effect of plastics products (Wang et al., 2021); this most likely influenced the Kelantan River. Nonetheless, the highest fibre type found in Langat River is probably due to boat docking and maintenance hot spot area found at Langat River (Prata et al., 2021), and could also contributed by the mangrove area that has been acted as a plastic and microplastic trapper. It is well known that besides anthropogenic factors, MPs abundance and distribution could also greatly being influenced by environmental factors such as water flow, wave current, tides, cyclones, wind directions, temperature, and erosion effect of hard substance during transportation to environmental pollution (Song et al., 2018; Tibbetts et al., 2018).

In terms of colour, the transparent (48.1%) was the most common in the Kelantan River, followed by black where it was highly recorded at SK02 (coastal vicinity). However, the percentage contribution was

inflexible throughout all sampling locations, ranging from 0.79 to 12.70%, with a standard deviation of only 2.94. Similarly, transparent and black fibres accounted for most MPs detected in Langat River (67.7%), similar to those found by Lu et al. (2020). As suggested, the transparent MPs found in the Southeast Asia rivers might be originated from food containers (Lestari et al., 2020; Wicaksono et al., 2021). Whereas in contrast to Nan et al. (2020) and Wicaksono et al. (2021) only 10.7% blue were reported in Langat River, followed by red (7.7%) and green (5.9%). It is significantly less than 3% for colours such as orange, brown, yellow, purple, and pink in both rivers, whereas no pink colour was associated with the Kelantan River.

3.1.2. Size

MPs were classified according to their sizes: <300 µm (Type I), 300–500 µm (Type II), 500–1000 µm (Type III), and 1000–5000 µm (Type IV), as illustrated in Fig. 4 and Supplementary 3. A total of 24,189 items/L were discovered in both rivers with a mean range of 16.00–30.24 items/L (Kelantan) and 117.55–381.86 items/L (Langat) for all size ranges, respectively. The remaining MPs were lost due to the low magnification used during the measurement process. It is remarkable that Langat River contributed 8 times greater overall abundance than Kelantan, accounting for the majority of the input from all stations except SL01 and SL17.

In Kelantan River, there was consistency with low variance for all size types along SK1–SK20. A possible explanation for this may be the increased municipal activities in sub-city vicinity, as well as small local sand mining operations that actively participated in Sungai Kelantan during the sampling activities. Whilst in Langat River, Type IV (38.03%) > Type III (33.78%) > Type II (16.49%) > Type I (11.71%), with SL01 (coastal) and SL23 (tributary) being the lowest and highest regardless of the sizes. Fibre was found the only type (with all size types) polluting Kelantan River, but Langat River was dominated by 500–5000 µm fibre and fragments. Previously, Egessa et al. (2020), Lestari et al. (2020), and Zhang et al. (2020) pointed out that the overall MPs abundance in the riverine system was most likely dominated by the large-size particles with the size ranging from 1000 to 5000 µm, however the small size of MPs (100–1000 µm) has been reported to pose great impact on aquatic life (Ma et al., 2020; Tien et al., 2020; Wu et al., 2020). In this sense, the larger the surface area of microplastics can be the substrate for the other persistent organic pollutant and other inorganic contaminants, posing a

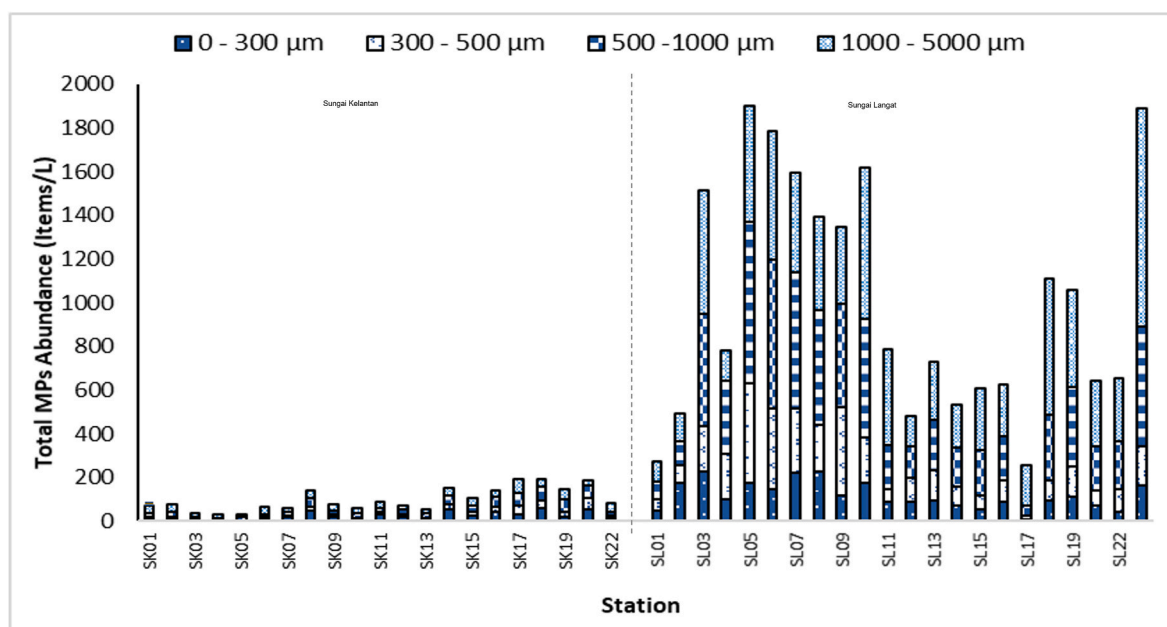


Fig. 4. Comparison of the Total Microplastic Abundance (items/L) based on size classes for Kelantan river vs Langat river.

higher risk of toxicity due to bioaccumulation and bioavailability of MPs in aquatic systems. The tiniest species discovered in Kelantan River was 29 μm while 25 μm was the smallest MPs size recorded in Langat River.

3.2. Characterisation of MPs

3.2.1. Micro-FTIR and pyrolysis-GC/MS

The type of polymer identified in the MPs samples using micro-FTIR of both river water varied in all locations (Fig. 5A). The result showed that 14 polymers were identified in the surface water of the Kelantan River, while 20 polymers were detected in the Langat River. Based on the micro-FTIR analysis, rayon, PTFE, and PE were the most detected from the MPs sample of Kelantan River. Meanwhile, PE, PP, PET and other polyolefins, as well as rayon are the most detectable in Langat River samples. In Kelantan River, the presence of other polymers was indicated as a minor contribution below 30%, while Langat River is more likely to be the “sink-and-source” area for many polymers. PE and PP can be considered the most abundant polymer type found in overall rivers because they can be transported by river flow due to low-density characteristics compared to water. Notably, PET, which was hardly detectable in Kelantan River, was dominant in Langat river, whilst PTFE (used mostly as non-stick material) was also prevailing in Kelantan River. PET is the common material for plastic packaging, water bottles, and drink containers. Furthermore, these products (in type macro/meso size plastic) were easily spotted and highly visible along the Langat River.

The micro-FTIR identification was made based on polymer characteristics and main functional groups of polymer profile, where in some cases such as complex environmental matrices with a smaller number of samples, it will be impossible. Furthermore, using micro-FTIR, smaller polymer particles were unable to be properly detected, thus can lead to incorrect polymer identification, whilst residual unprocessed organic matter may also hamper micro-FTIR identification. Hence, in this study, the relative mass estimation of polymer (in terms of the mass of polymer per volume of samples) was obtained by subsequent analysis using pyrolysis-GC/MS, utilizing the technique for all size range of samples that was unable to be detected by FTIR analysis. This provide the concentration of each individual polymers and allows the simultaneous determination of additives in polymer in the MP samples. For example, high concentration of PE was found in Langat River, especially for SL8 (31370.00 $\mu\text{g/L}$) and SL9 (22310.00 $\mu\text{g/L}$), while other stations in the range of 116.30–8990.00 $\mu\text{g/L}$. In some cases, the relative mass quantitation were ten to fifty times greater compared to Kelantan River samples (Fig. 5B). Regardless, PE was also abundant in Kelantan River, particularly in stations SK13 (3284.50 $\mu\text{g/L}$), SK14 (3085.50 $\mu\text{g/L}$) and SK19 (1983.00 $\mu\text{g/L}$), with the lowest that can still be detected in SK18

at 47.64 $\mu\text{g/L}$ (0.0476 ppm). A similar trend of magnitude also found in relative mass abundance (ppm or ppb level) of other types of polymers, for instance PP (4355.00 $\mu\text{g/L}$, SL9), N66 (485.00 $\mu\text{g/L}$, SL10), N6 (28.37 $\mu\text{g/L}$, SL8), PMMA, PS and ABS (30.36 $\mu\text{g/L}$, SL2). For PET, the concentration recorded was comparable in between the stations, in the range of 29.40 $\mu\text{g/L}$ – 142.60 $\mu\text{g/L}$ (LOD for PET: <14.30 $\mu\text{g/L}$). Overall, within all analysed water samples, PE is the most predominant polymer in both rivers, in agreement with the FTIR polymer identification. It is worth to note here that, PTFE, albeit commonly detected in Kelantan River samples by micro-FTIR, was actually not quantified by pyrolysis-GC/MS since the calibration for the analysis was prepared for only 12-common polymers. Nevertheless, the py-GC/MS is proven to be the complimentary technique for detail MPs measurement, by allowing the chemical structure of MPs to be fragmented and quantified, regardless of the complexity of the samples (Müller et al., 2020). The calculated microplastic concentration in both rivers based on the individual calibration curves prepared using pyrolysis-GC/MS are tabulated in the Supplementary 4, whilst FTIR spectra and pyrograms for representative polymers are shown in the Supplementary 5 and Supplementary 6, respectively.

Comparing the data for most analysed polymers (PE, PET, PP, PMMA) in Langat River, it is very interesting to see that the mass abundance of these polymers was found lower at sites closer to the open sea and mangroves, while the high amount of them was recorded in the river and tributary area. These polymers are usually found floating; thus, they will be affected by several factors in the riverine system, such as the tide and flow factor (Kataoka et al., 2019; Tibbetts et al., 2018), while polymers with higher density will tend to deposit at the riverbed. As such PE, PET, and PP plastics were widely used and contributed to more than half of global plastic production (Andrady, 2011). In Malaysia, these polymers are commercially produced by local factories and widely used as grocery bags, bottles, containers, furniture and household items. These microplastics occur around the sites in the middle of the river, for instance, in Kelantan River, to which the nearby agricultural work, human activity, and domestic effluents may contribute. Meanwhile, for the Langat River, these types of plastics, including PMMA (that are not quantified among Kelantan samples), may enter the river system due to the various industrial-based activities and human and domestic inputs. The concentration of MPs in the Kelantan River could result from sand mining activities in the upstream river. On the other hand, the concentration of MPs in Langat River may result from heavy industrial activities, such as sand mining transportation, vessel docking, steel production, fabrication, mill, and manufacturing works. This finding also revealed that the Langat River was more polluted than the Kelantan River due to the presence of industries nearby and human activity. Previously, it is suggested that pyrolysis-GC/MS could be used to

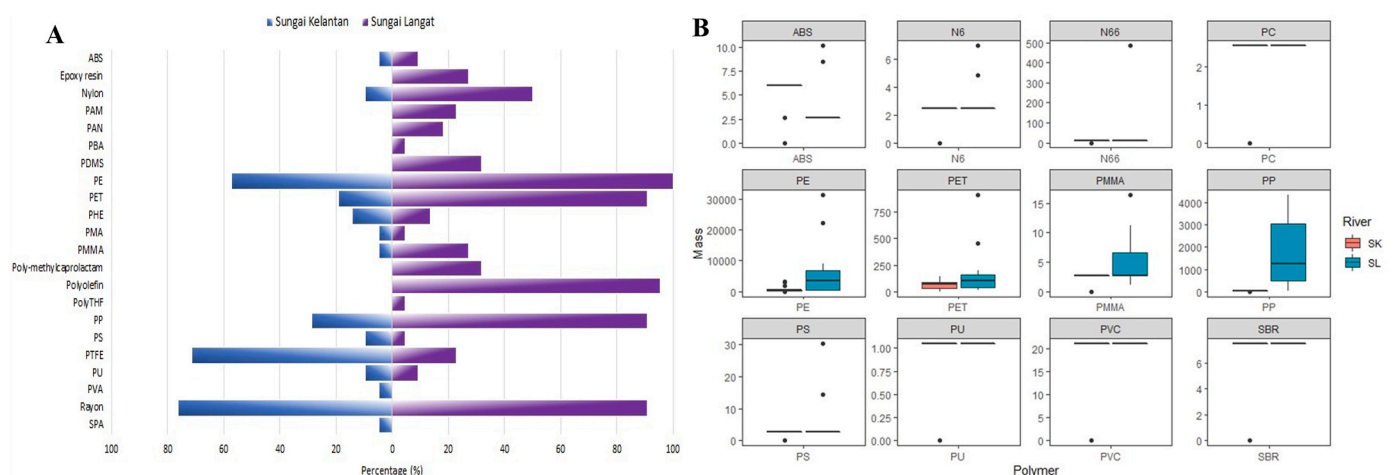


Fig. 5. (a) micro-FTIR analysis of microplastics; (b) polymer concentration based on pyrolysis-GC/MS.

identify persistent organic pollutant and organic plastic additives in microplastics, thus linking to the MPs accumulation and ecotoxicological study (Anuar et al., 2022; Schmidt et al., 2021). Recently, an ecological risk index assessment (PERI) of MPs of environmental matrices was investigated by considering the polymer hazard index (PHI) and pollution load index (PLI) for each polymers, suggesting that polymers such as PU and PVC are grouped among the highest hazard with hazard score of 13,844 and 10,001, respectively; followed by PA (47), PS (30), PE (11), PETE (4) and PP (1) (Shekoohiyan et al., 2022). Interestingly, PE and PP presumably could have higher PLI value based on the higher abundance in both Langat River and Kelantan River. Nonetheless, the potential toxic aspects of all 20 common polymers found in the current study should be further investigated in the future work.

3.2.2. SEM-EDS

SEM-EDS provides high-resolution imaging of microplastic surface structure and elemental composition signatures. Since fibre is the most dominant shape found in both rivers, only a representative of this shape was analysed using SEM-EDS. As such, Fig. 6A exhibited a relatively smooth surface and consistent cross-section size, indicating the possibility of manufactured fibres, while Fig. 6B demonstrates an edge crack characteristic but a smooth surface which may suggest a short period of environmental weathering process has occurred to the particles (Hwi et al., 2020). In contrast, Fig. 6C revealed characteristic surface cracks, irregular shapes of the surfaces, and evidence at the edges, suggesting that the primary plastic pieces experienced mechanical breakage and oxidative weathering on the plastic surface, causing the plastic to be brittle. A microfibre with a highly deformed margin and rough surface could be due to the accumulation of fine dirt particles on the surface created by environmental exposure, hence polymer ageing and longer exposure time can be observed in Fig. 6D by the formation of biofilm on the surface of the plastic particles. Additionally, EDS measurement shows significant number of carbon and oxygen elements (i.e., >45%) confirm the identified particles as a synthetic polymer with a carbon-hydrogen backbone such as PE, PP, or other polyolefins. The result is consistent with the micro-FTIR and pyrolysis-GC/MS. Based on SEM-EDS, oxygen comprises 8–25%, affirming them as environmental microplastics.

3.3. Water parameter

The overall water parameter distribution for Kelantan and Langat rivers including DO, pH, temperature, salinity, turbidity and conductivity were recorded to establish the correlation with environmental MPs and are shown in Supplementary 7, while other WQI profile were tabulated in Supplementary 8. Statistical analysis established a significant difference ($p < 0.0001$) for water parameters between both rivers. Based on the determined WQI (Department of Environment Malaysia, 2020), the Kelantan River is classified as slightly polluted (Class III), whereas the Langat River is classified as slightly polluted (Class III) to polluted (Class IV). The WQI status for both rivers is comparable to the previous studies done by Abdul Maulud et al. (2021) and Ahmed et al. (2022). In term of water quality parameters, the average DO for Kelantan River is 6.21 mg/L and shows a consistent increase with a small standard deviation across sampling stations from SK01 to SK20, 41.75% higher than that recorded in Langat River (2.72 mg/L). It is notable that in Langat, the DO level decreased from the sea (SL01) to the river (SL20), which would also indicate good river circulation in the Kelantan River compared to the Langat River. Meanwhile, Kelantan and Langat rivers generally have pH values ranging from 6.40 to 7.72 and 5.77 to 7.52, respectively. Both exhibit a similar pH gradient from the sea to the river and catchment. According to Malaysian National Water Quality Standard (NWQS), the pH level of Kelantan River meets the specifications for Class 1 water, whilst Langat River is classified as Class IIA. The anthropogenic factors along the Langat River lead to the depletion of dissolved oxygen levels due to the high organic matter, consequently inhibits phytoplankton activity and promotes microbial respiration, thus decreased DO and affecting the pH levels (Ustaoglu et al., 2020; Zhu-Wang et al., 2022).

In general, the turbidity profile in both rivers is divergent, however in Kelantan River, the turbidity level is rocketing starting at SK12. Unlike Langat River, there are a few local sand mining process areas in Kelantan River that might be presumed to be highly related to a higher turbidity level toward the end of the sampling station (upstream). Sand mining could lead to an increase in the resuspension of fine sediment and organic particles, as well as an increase in the total suspended solid, which would ultimately lead to an increase in the turbidity of river water (Ashraf et al., 2011). Instead of turbidity, the Malaysian Marine Water

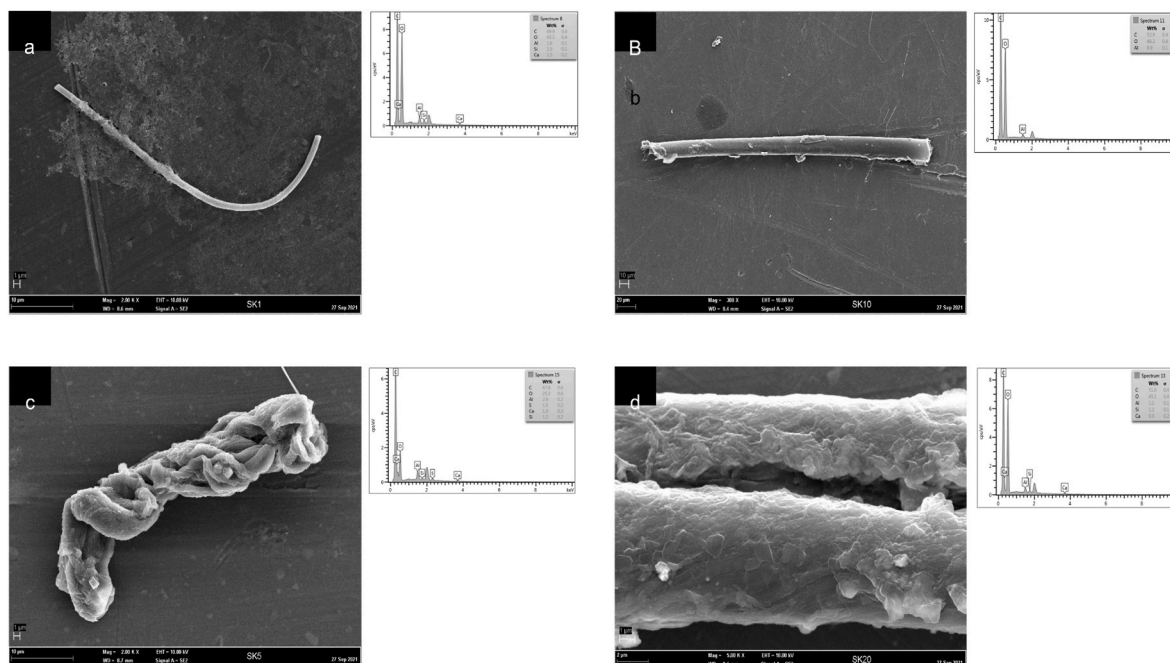


Fig. 6. SEM-EDS analysis of the surface morphology of microplastics.

Quality Standard (MMWQS) specifies total suspended solids (TSS) as a parameter for defining water quality. Both parameters were calculated and measured differently despite their similar appearance. In terms of salinity profile, it was decreases from the sea towards the upward of the riverbank for both rivers. The salinity level in Kelantan River was observed as being lower than 1 ppt starting at SK08, whereas the salinity level in Langat River began to decrease in SL12. Therefore, the Langat River must be 3-km longer than Kelantan River to be comparable in the 'freshwater' definition.

Meanwhile, the conductivity level is decreasing from the sea towards the upward of the riverbank. Even with a similar trend from the open sea toward the upward of the river, the Langat River is significantly higher in terms of conductivity than the Kelantan River. A significant decrease in conductivity was observed between stations in the estuary zone of both rivers. Due to the southwest-monsoon (SWM), which occurs annually in Malaysia from May to September (dry season), the temperature profile for surface water appears to be warmer in both rivers. A temperature range of 28.53–31.99 °C in the Kelantan River and 28.05–30.46 °C in Langat River established the influence of SWM during the sampling activity. As a result, in a tropical country like Malaysia, seasonal changes with varying precipitation rates may have an impact on the water quality of surface water (Abdul Maulud et al., 2021). Apart from that, other natural factors affecting river basin water quality include geology, soil erosion, hydrological condition (river flow and volume), climate, natural weathering of minerals, vegetation and flood runoff (Ahmed et al., 2022; Khatri and Tyagi, 2015; Ustaoglu et al., 2021). Therefore, both natural and human factors have an impact on the water quality of the Kelantan and Langat rivers.

3.4. Correlation between microplastics and water parameters

Water quality parameters (the average value of BOD, COD, SS, NH₃-NL, DO, pH, temperature, salinity, turbidity, and conductivity) were used to identify the influence of environmental factors towards microplastic distribution in the riverine system. Two hypotheses were developed to better comprehend the analysis: the null and alternative hypotheses. A total of 55 correlations were detected, of which 45 were evaluated to be significant (Fig. 7).

In Kelantan River, amongst the significant correlation, 11 were identified as strong, 27 moderate, and 7 were weak correlations (Fig. 7A). Strong correlations were found for conductivity/salinity ($r = 0.99$), conductivity/COD ($r = 0.96$), salinity/COD ($r = 0.96$), conductivity/pH and salinity/pH both with ($r = 0.85$), pH/COD ($r = 0.81$), NH₃-NL/SS ($r = 0.77$), Salinity/BOD ($r = 0.74$), conductivity/COD ($r =$

0.73), turbidity/pH ($r = -0.11$) and DO/BOD ($r = -0.27$). Among all tested water quality parameters, only turbidity has a significant impact ($p < 0.05$) on MPs abundance with a moderate correlation between turbidity/MPs ($r = 0.47$) especially towards to the upstream level. Other water quality parameters were deemed insignificant to the MPs abundance in the Kelantan River.

Significant correlations between factors were observed for 25 variables for the Langat River (Fig. 7B). Ten strong significant correlations were identified as conductivity/salinity ($r = 0.99$), DO/pH ($r = 0.94$), salinity/pH ($r = 0.93$), salinity/conductivity ($r = 0.93$), conductivity/SS ($r = 0.92$), conductivity/pH ($r = 0.92$), DO/salinity ($r = 0.91$), conductivity/DO ($r = 0.89$), DO/COD ($r = 0.89$) and pH/COD ($r = 0.88$). Only SS and temperature significantly influence MPs abundance in Langat River. In conclusion, a moderately significant relationship was shown between SS/MPs abundance ($r = 0.65$) whilst an inversely proportionate significant relationship between temperature/MPs abundance ($r = -0.40$). In another sense, the SS has a 65.00% influence on the MPs' abundance in Langat River, while the greater the temperature, the lower the MPs abundance in the river. While some physical evidence suggests a strong link exists between MPs and water quality data, Kataoka et al. (2019) stressed that the linear association between water quality parameters and MPs abundance might be problematic given the river's temporal and spatial variability. Thus, a subsequent multiple linear regression-sensitivity analysis (MLR-SA) detail study is presented to demonstrate the percentage contribution of the association between the assortment of river water quality parameters and the abundance of MPs for both river conditions (Supplementary 9). The MLR-SA both positive and negative proportions; any negative indicator indicates that the analysed parameter contributes nothing to the MP's abundance, whereas any positive indicator denotes a contribution. In Kelantan River, only 5 characteristics positively contributed to the MP's abundance: salinity, conductivity, pH, NH₃-NL, and suspended solids, with a proportion ranging from 9.34 to 40.76%, whilst in Langat River, the contribution of each parameter ranged from 12.53 to 56.09%. As such, salinity has consistently been identified as the most significant contributor, while indicators that heavily depend on oxygen, such as DO, BOD, and COD, have generally been the least significant correlation to MPs.

4. Conclusion

In general, due to the higher concentration of MPs (1464.8 items/L), Langat River was more polluted compared to Kelantan River (179.6 items/L). Fibre and fragment were the only two types that considered as

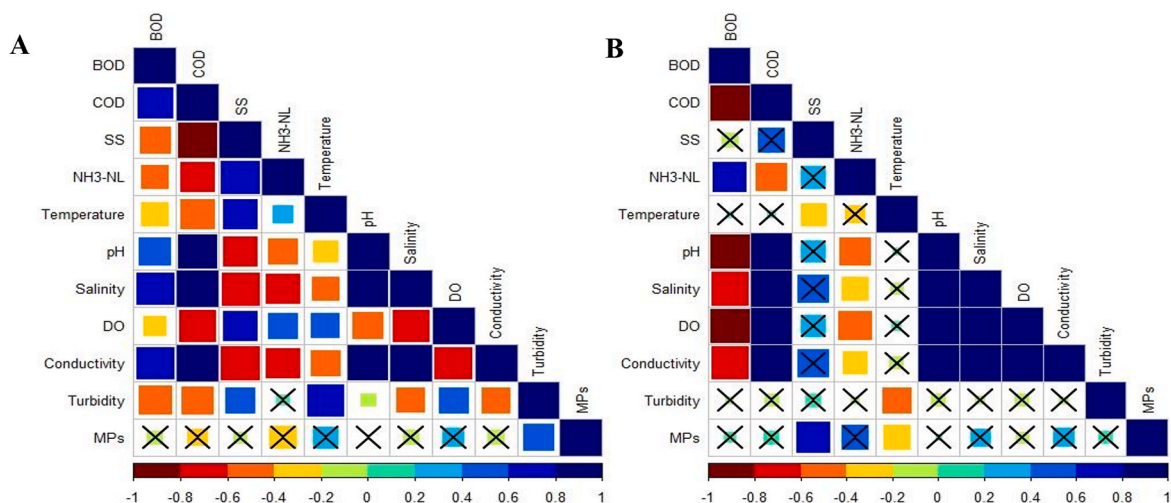


Fig. 7. 'Hot-map' correlation analysis using Multiple Linear Regression for multiple water quality parameters for (a) Kelantan river (b) Langat river.

major contributor to both rivers (>98%), with transparent MP represents the most prevalent colour in both river samples (>40%). Kelantan River shows better water quality than Langat River, despite Kelantan River having a higher turbidity level. Among the physical water parameters, only 3 showed a substantial monotonic association with MPs abundance: turbidity, suspended solids, and temperature. In Kelantan River, only turbidity had a moderate link with MPs, whereas suspended solids and temperature affected the MPs distribution pattern in Langat River. In this study, the total MPs abundance were compared using Mann–Whitney *U* Test for overall abundances found in the river. This approach also been applied to each variables analysed in this study. The result given by Mann–Whitney determined the significant differences ($p < 0.05$) between Langat and Kelantan rivers, proven that Langat River was significantly polluted compare to Kelantan River. Micro-FTIR analysis revealed that 14 polymers were identified in Kelantan River, while 20 polymers were detected in the Langat River, with PE, PP and rayon were majorly found in both rivers. Interestingly, PFTE was prevalence in Kelantan, while PET nylon and other polyolefins in Langat River, suggesting the contribution of different activities. The limitation analysis using micro-FTIR (in term of pollutant concentration) was overcome by pyrolysis-GC/MS, providing more information on chemical concentration and structures for MP polymers. The concentration of PE in Langat River samples was found exceeded the 1 ppm-unit level by pyrolysis-GC/MS and potentially regarded as the “sink-and-source” area for many polymers. However, this is not yet an indicator of the contamination degree until further investigations or longer monitoring program was carried out using the same sampling strategies combining river, estuary and marine waters with water quality. Further optimization on pyrolysis-GC/MS also need to be considered in the near future, especially on the analytical aspects (LOD, LOQ) and polymer ranges, especially for this technique to be able to be used in a more robust ecological risk assessment. Moreover, it should be noted that the comparison of MPs study between regions would be problematic if no compliance with sample and extraction protocols were adopted throughout the study. Hence, significant efforts are made to collect data from the sites (e.g., river, marine, or lake) to potentially establish a database as an important reference to other researchers in this field particularly for tropical climate. In addition to being one of the earliest studies to establish a database of MP occurrences and abundances in the riverine system, this research is significant for its preliminary investigation of MPs occurrence between polluted (Langat) and less-polluted (Kelantan) rivers in Malaysia. Nevertheless, the findings of this study will assist policymakers and related agencies, in proposing a standard sampling protocol and a better policy to deal with the problematic MPs in the riverine system, since the presence of MPs could threatens the entire river basin ecosystem.

Credit author statement

Conceptualization – Y.S.I & N.K.E.M.Y.; Methodology - Y.S.I, K.M.K. K.Y, S.T.A.; Data validation & formal analysis - Y.S.I, K.M.K.K.Y, Y.M.; Investigation (physical analysis) - S.T.A, Y.S.I, K.M.K.K.Y, Y.M.; Investigation (chemical analysis) – S.T.A., T.T.C. A.A.A., M.J., N.M., & W.M.A.W.M.K.; Writing original draft – S.T.A., N.S.A., K.M.K.K.Y & Y.S. I. Writing - Review & Editing – S.T.A & Y.S.I.; Project administration – N. K.E.M.Y.; Funding acquisition: Y.S.I & T.T.C.; All members carried out the field sampling works (S.T.A, N.S.A, K.M.K.K.Y., Y.M. A.A.A., M.J., N. M., W.M.A.W.M.K, Y.S.I.)

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Yusof Shuaib Ibrahim reports financial support was provided by National Water Research Institute of Malaysia (NAHRIM).

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2023.115717>.

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