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Microplastics analysis in Malaysian marine waters: A field study of Kuala Nerus and Kuantan

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ABSTRACT

The first report on the emergence of microplastic in Malaysian marine waters was documented in this study. Water samples were collected from two regions, namely Kuala Nerus and Kuantan port, as the representatives of different anthropogenic activities. Identification of microplastic was performed based on physical characteristics (colour, shape, density) and chemical characterisation (ATR-FTIR analysis) for a functional group of polymers. Fragment type, black or grey colour and high density ($> 1.02 \text{ g cm}^{-3}$) of microplastic were the most prevalent characteristics found in both areas. Two principal components (density and colour) rendered explained about 95.3% (Kuantan) and 95.6% (Kuala Nerus) of the total variance. Six possible polymer materials were identified, namely polyester, polystyrene, polyamide, polyvinyl chloride, polypropylene, and polyethylene. The findings of the study provided good baseline information on marine debris issue in Malaysia.

The occurrence of microplastic in aquatic environments has prompted public concerns worldwide. Major concern relies on its persistence, ubiquity, and role as vectors for mobility and exposure of persistent organic pollutants, especially hydrophobic contaminants (Gall and Thompson, 2015; Kwon et al., 2017; Ng and Obbard, 2006). The terminology of microplastic refers to a particle size $< 5 \text{ mm}$ (Andrady, 2011). There are two types of microplastics namely primary and secondary microplastics. The primary source came from the manufactured products built in microscopic size, while the secondary source generated after the breakdown of larger plastics (Cole et al., 2011). Distribution of microplastic in our natural environments relies on density, shape and degradation process of materials throughout the biological or chemical process (do Sul and Costa, 2014). Moreover, the availability of microplastic depends on different challenges compared to macro- or meso-plastic. Smaller plastics become more ingestible particularly for small organisms (Shim and Thomposon, 2015).

In Malaysia, environmental data linked to the abundance of microplastic have yet to be adequately addressed. The main work is now focussing on ingestion of microplastic by aquatic organisms. For instance, a study by Ibrahim et al. (2016) explained that microplastic was ingested by *Scapharca cornea* derived from polyethylene and polyamide materials. Recently, Ibrahim et al. (2017) explicated microplastic derived from polyamide and polyvinyl alcohol found in cage-cultured and wild *Lates calcarifer*, collected from Setiu Wetlands. In addition, four

edible fish tissues (*Rastrelliger kanagurta*, *Stolephorus waitei*, *Chelon subviridis* and *Johnius belangerii*) were analysed to evaluate the presence of microplastic. Findings of the study indicated polypropylene as dominant polymer materials (Karami et al., 2017).

Despite the main focus on the impact of microplastic ingested by organisms keeps vital and continuously ongoing, the analysis of marine debris in the water column is also crucial. In fact, the production of plastic-based materials in Malaysia was worth Malaysian Ringgit 15.8 billion in 2010. It is noteworthy to remark plastic as the highest contributor of solid waste composition (24%) in Malaysia or even the top producer among Asian countries (Malaysian Department of Housing and Local Government, 2011). Therefore, the aims of this research work are to identify and characterise the emergence of microplastic particles in marine waters. Physical and chemical analyses were performed to fill the knowledge gap about marine debris pollution in Malaysia. In the latest review article by Shim et al. (2017), both approaches were recognised as convenient methods of microplastic analysis.

Collection of water samples was carried out at two main locations on the east coast of Peninsular Malaysia, namely Kuala Nerus in Terengganu and Kuantan in Pahang. Kuala Nerus was chosen to represent the non-urban area, while Kuantan represented an urban area with port activities. Major activities in Kuala Nerus are linked to commercial fishing and tourism, while Kuantan port is known as one of the major multi-cargo ports in Malaysia. Five stations were located in

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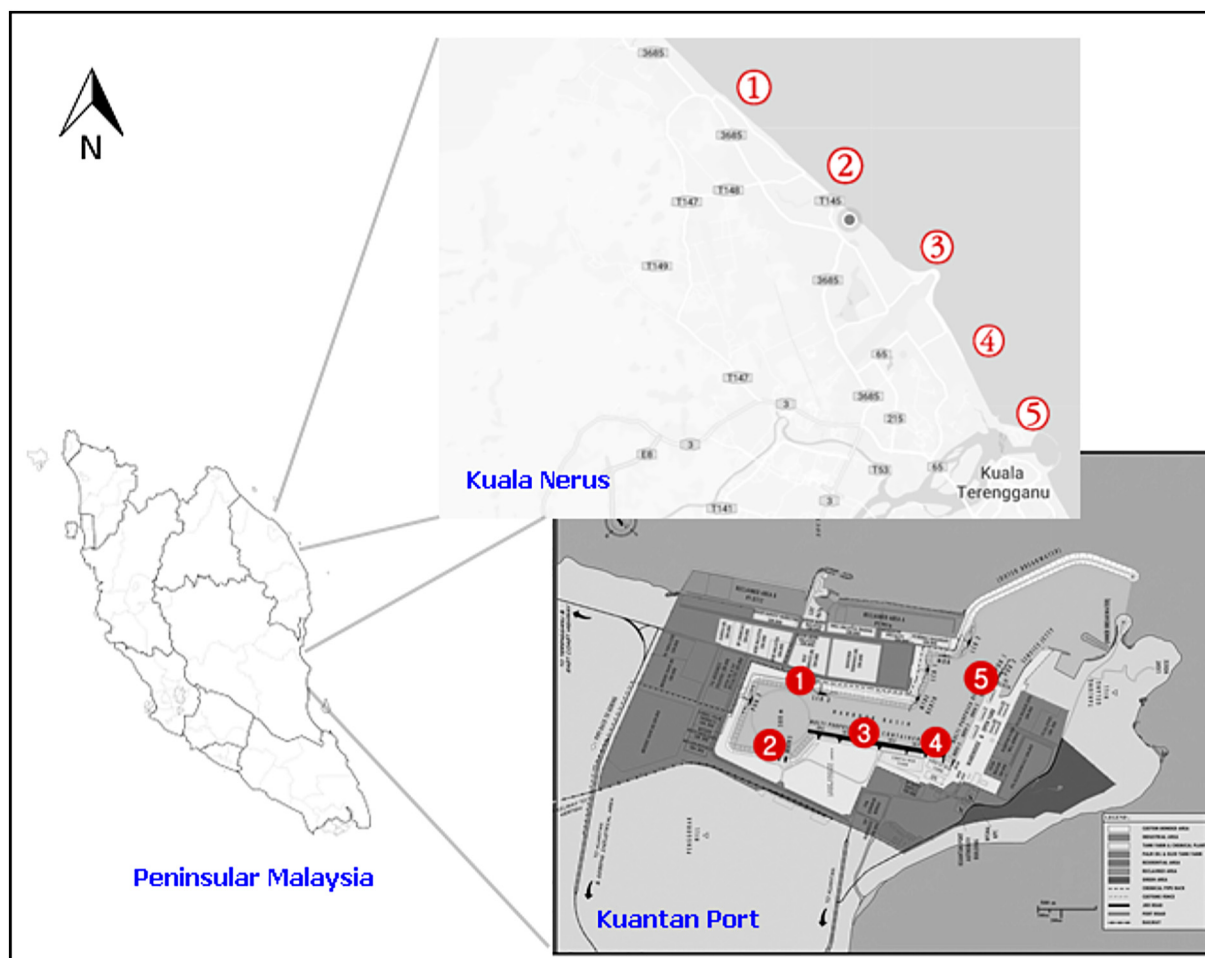


Fig. 1. The map of sampling station located in Kuala Nerus and Kuantan Port, Malaysia.

Kuala Nerus namely Batu Rakit (KN 1; KN2), Tok Jembal (KN3), Teluk Ketapang (KN4) and Seberang Takir (KN5). Sampling stations were positioned along the coastline area from Batu Rakit station to Seberang Takir station (14.96 km). Meanwhile, five stations in Kuantan port including the liquid chemical berth (KP1), multipurpose berth (KP2-KP4) and container berth (KP5). The location of the sampling station as illustrated in Fig. 1. Sampling activity was performed from September to October 2015.

Surface water samples were collected using a 5.7 L calibrated steel sampler. Samples were passed through 20 μm serial filtration net (length: 55 cm; length and wide mouth: 30 \times 30 cm) to trap microplastic. The process was done in 50 replicates. Debris in the cod-end of the net was transferred to a 250 mL acid treated glass bottle, refrigerated at 4 $^{\circ}\text{C}$ and returned to the laboratory for further examination.

Characterisation of microplastic was carried out based on their physical properties such as colour, shape, and density. Samples were screened and large debris (> 5 mm) was discarded through 200 μm mesh sieve. The particle was then resuspended in Milli-Q water, filtered by using GF/F 0.7 μm 47 mm \varnothing (Whatman, Kent, UK) to trap particles before transferring them into a petri dish. The filtered particles were oven-dried at 60 $^{\circ}\text{C}$ before getting ready for visual sorting. Next, the petri dish was placed under a stereoscopic microscope (Olympus SZX7, Japan) at 50 \times magnification and identified visually according to colour or shape. Identified microplastic was collected by using stainless steel tweezers and placed in 250 mL glass bottle, until further assessment.

For density separation, 250 mL glass bottles containing suspended

microplastic were placed on an orbital shaker for 30 min with speed controlled at 150 rpm. The supernatant was then carefully transferred to a glass petri dish. Microplastic abundance was observed under a dissecting microscope. Then, the particles were separated according to the suspended characteristic ($\leq 1.00 \text{ g cm}^{-3}$ distilled water) ($1.00 < x < 1.02 \text{ g cm}^{-3}$ seawater) or ($\geq 1.02 \text{ g cm}^{-3}$) seawater.

Confirmation of functional group of the polymer was performed using IRTracer-100 Fourier Transform Infrared spectroscopy (Shimadzu, Japan) equipped with attenuated total reflectance. The spectroscopic method has an advantage over the microscopic method which is more accurate for particle size < 1 mm (Song et al., 2015a, 2015b; Shim et al., 2017). The spectrum was recorded in the mid-range 4000–700 cm^{-1} using 20 scans/s for each analysis at 0.25 cm^{-1} resolution. Particles larger than 1 mm in diameter were used during the identification work. The analysis was conducted in triplicate. The spectrum obtained during the study was also compared with the literature studies.

Statistical data analysis was performed using Minitab Version 17 (Stat Inc., USA). Variations within inter- and intra-groups were assessed based on the ANOVA test. Values were reported as mean, and the differences were considered significant when $p < 0.05$. The principal component analysis was carried out using the eigen decomposition method. Agglomerative hierarchical cluster analysis was performed using the Ward method.

The total microplastic particles found in Kuala Nerus was relatively higher which were 1713, while Kuantan Port was 621 in filtered marine water. Only particles having a minimum 5 mm diameter were taken into account. Mean abundance was ranged from 0.13–0.69 pcs/L to

Table 1
Microplastic abundance in surface water of studied area.

Station	Total no. of microplastic	Station	Total no. of microplastic
KP1	115	KN1	111
KP2	110	KN2	300
KP3	120	KN3	270
KP4	121	KN4	446
KP5	155	KN5	586

0.14–0.15 pcs/L for Kuala Nerus and Kuantan Port, respectively. Microplastic found in surface water displayed no significant difference between stations ($F = 5.19$, $p = 0.71$) but significantly contributed between two areas ($F = 5.31$, $p = 0.02$). A strong correlation was observed between Kuala Nerus coastline and a total number of microplastic (slope = 109.6, $R^2 = 0.91$). For Kuantan port, the only moderate correlation was generated between the station and number of microplastic (slope = 9.1, $R^2 = 0.65$). Spatial homogeneity for microplastic abundance gave the idea that marine debris in the east coast area was uniformly distributed. Comparison between samples collected in this study and other reports was difficult, owing to different methodologies and quantitative measuring units. In terms of particle numbers, this study recorded low abundance compared to literature, such as 0.68 to 6.44 pcs/L in Yantai-Qinzhou coastline area (Qu et al., 2018) and 88 pcs/L in Jinhae Bay, Korea (Song et al., 2015a, 2015b). The abundance of microplastic in each station is tabulated in Table 1.

Microplastic is classified into three main shapes, namely filament, fragment and irregular (Table 2). For Kuantan port samples, the fragmented shape accounted for 50.8–66.1% of total microplastic. Filament and irregular shapes are recorded to vary within 20.9–38.3% and 10.8–19.1%, respectively. Data recorded from Kuala Nerus area constituted 76.2% were representative of the fragmented shape, while the rest of the materials (23.76%) were recorded as filaments. No significant difference was recorded for filament in relationship to the locality of the station ($F = 1.12$, $p = 0.44$) or area ($F = 1.48$, $p = 0.25$). Similar pattern also was calculated for fragment, $F = 3.19$, $p = 0.11$ (area) and $F = 0.76$, $p = 0.59$ (station). Compositions of microplastic showed consistency ratio in water, 1:2 (Kuantan) and 1:3 (Kuala Nerus), regardless the location of sampling sites. Thus, measuring the small scale of the coastline area does not clearly explain the multi-point source of marine debris pollutant existed in the east coast region of Peninsular Malaysia.

The high abundance of fragment type was also reported in other studies. A study performed by Maes et al. (2017) revealed that the largest contribution of microplastic in the North-East Atlantic was dominated by fragment type (63%) with high abundance remarkably found near the coast and river estuaries. The fragment was also found the highest from data analysis cruises across the Red Sea, it contributed 73% out of total item identified. Chemical characterisation suggested that particles were originated from polyethylene and polypropylene

Table 2
Classification of microplastic based on particle shape.

Station	Particle shape classification		
	Filament	Fragment	Irregular
KP1	24	76	15
KP2	30	59	21
KP3	46	61	13
KP4	28	79	14
KP5	40	87	28
KN1	96	15	NA
KN2	31	269	NA
KN3	228	42	NA
KN4	24	422	NA
KN5	28	558	NA

(Martí et al., 2017). In the Jinhae Bay water samples, the fragment was accounted 75% out of the total particles (Song et al., 2015a, 2015b). A comprehensive review by Hidalgo-Ruz et al. (2012) also explicated that fragment type was commonly found in the microplastic study. The fragment was identified coming from polyethylene and polypropylene materials. This was in close agreement with our study, which details of polymer identified are discussed in the next section. An example of microplastic visual under the microscope is shown in Fig. 2.

Particles of microplastic found were in eight different colours which are such as black, blue, brown, grey, red, orange, yellow and transparent (Table 3). Black coloured particles were the most dominant in Kuala Nerus coastline area with the frequency of detection reached to 65.5% (Kuala Nerus). Grey is the most frequent colour found in Kuantan port; 48.7% of the total microplastics. In fact, different colours may give an idea about the origin of the plastic materials; despite colour separation may not lead us any further confirmation. For instance, blue and black colours become prominent in fishery activities linked to a research study conducted at an Irish continental shelf (Martin et al., 2017). Meanwhile, a fragment in blue or green colour was found on six beaches in South Korea, identified as polyethylene. Materials reported were originated from aquaculture buoys or fishing nets (Jang et al., 2014). It indicated that similar pollutant sources also generated in the studied area. Therefore, chemical analysis such as the Fourier transform infrared spectroscopy was also carried out in order to strengthen our field study.

The density of microplastic particle was scaled according to ≥ 1.02 , $1.00 < x < 1.02$, and $\leq 1.00 \text{ g cm}^{-3}$. Each fraction is summarised as shown in Table 4. The high-density of the particle ($\geq 1.02 \text{ g cm}^{-3}$) was dominant in Kuantan port with a similar trend observed in all berths. A larger fraction of high density was also recorded in Kuala Nerus. It was dominant in each station with the frequency of detection in the range of 82.8–97.9%. It is noteworthy to highlight here that the large contribution of dense particle enhances the occurrence of microplastic in seafloor level. Beside degradation and leaching the additive, fragmentation is one of the process changes of original dense materials and reflects the pollutant distribution along the water column (Avio et al., 2017).

Two principal components explained about 95.3% (Kuantan) and 95.6% (Kuala Nerus) of the total variance in the water datasets from eigenvalue > 1 . Station KP5 and KN2 or KN4 were recognised as the most affected areas with microplastic pollutant. Biplot showed a correlation between spatial variation and parameter studied as illustrated in Fig. 3. PC1 had the highest positive loading of the density parameter for both regions with PC value of 0.53 (Kuantan) and 0.51 (Kuala Nerus). Meanwhile, PC2 which was the representative of colour, had positive loading with PC value accounted for 0.68 (Kuantan) and 0.56 (Kuala Nerus). Sampling stations were grouped into two clusters at $D_{\text{link}}/D_{\text{max}} \times 100 < 8$ for both studied areas. C1 represented KP1 and KP4; KN1 and KN3. Meanwhile, C2 represented KP2, KP3, and KP5; KN2, KN4, and KN5. Site similarities were considered high, thus, confirming that microplastic found in this study was uniformly distributed.

The FTIR spectra associated to microplastic as examples are shown in Fig. 4. Polymer identified in Kuantan port is namely, polyester, polystyrene, polyamide, polyvinyl chloride, and polyethylene. Meanwhile, polymer group found in Kuala Nerus was derived from polyamide and polypropylene materials. For polyester material, the absorption bands of $-\text{C}-\text{O}-\text{C}-$ ester linkage were observed at $\nu = 1113.88 \text{ cm}^{-1}$, together with the broader band at $\nu = 1164.16 \text{ cm}^{-1}$, thus, showed the characteristics of the ester group. The absorption peak appeared at $\nu = 1282.17 \text{ cm}^{-1}$ showed the $\text{C}=\text{C}$ linkage of polyester. Strong $\text{C}=\text{O}$ vibration band was shown at peak $\nu = 1644.86 \text{ cm}^{-1}$ indicating the presence of carbonyl compound. The symmetric $\text{C}-\text{H}$ stretching can be seen at $\nu = 2899.42 \text{ cm}^{-1}$, while the strong peak at $\nu = 711.62 \text{ cm}^{-1}$ attributed to $\text{C}-\text{H}$ bending for the benzene ring that can be found in a common polyester compound (Saleh et al., 2012).

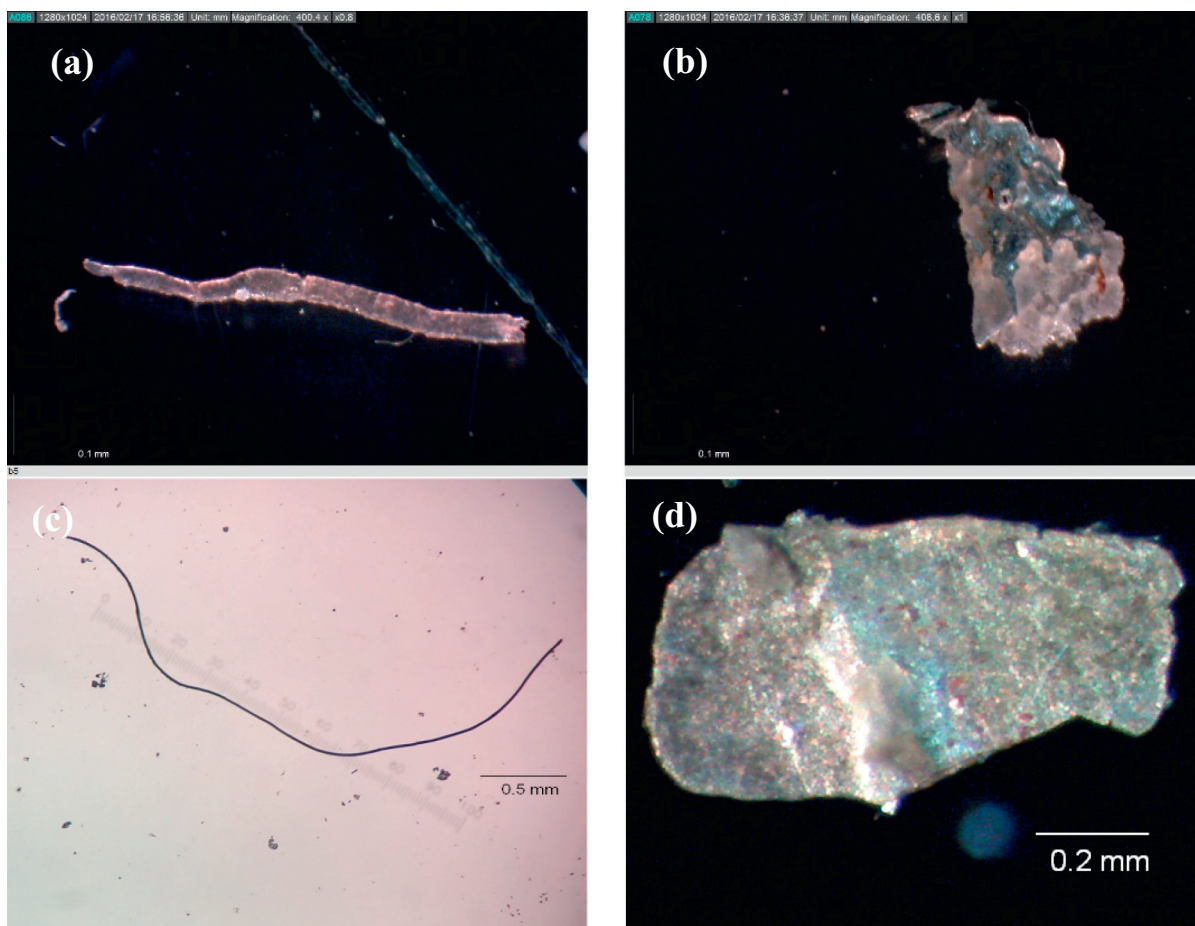


Fig. 2. Microplastic visual under microscope, (a and c) filament and (b and d) fragment.

Table 3

Classification of microplastic based on colour identification.

Station	Colour classification							
	Black	Blue	Brown	Grey	Red	Orange	Yellow	Transparent
KP1	56	2	28	20	1	5	0	3
KP2	54	0	28	11	0	12	0	5
KP3	58	0	28	20	0	14	0	0
KP4	51	0	36	0	0	34	0	0
KP5	69	9	48	1	1	26	0	2
KN1	65	6	3	7	12	0	1	0
KN2	27	0	1	268	1	0	0	2
KN3	180	16	14	15	21	6	0	0
KN4	17	0	1	354	3	0	67	3
KN5	21	0	11	544	1	2	0	0

For polystyrene materials, the absorption band of C=C aromatic stretching observed at $\nu = 1637.56 \text{ cm}^{-1}$ with additional weaker bands appeared around $\nu = 1525.64$ and $\nu = 1408.96 \text{ cm}^{-1}$. The absorptions of aromatic C-H stretching vibration for polystyrene were recorded around $\nu = 3200 \text{ cm}^{-1}$ – 3367 cm^{-1} with strong peaks of out-of-plane (oop) bending at $\nu = 746 \text{ cm}^{-1}$ – 910 cm^{-1} . For polyamide materials, two peaks observed at $\nu = 3423.65 \text{ cm}^{-1}$ and $\nu = 3321.42 \text{ cm}^{-1}$ for N-H stretching vibrations from the primary amines. Additionally, a small C-H aliphatic stretch absorption band can be seen around $\nu = 2899.42 \text{ cm}^{-1}$. The overtone of N-H bending shown at $\nu = 1632.85 \text{ cm}^{-1}$ and peak at $\nu = 1529.55 \text{ cm}^{-1}$ attributed to the C-H₂ bending. Strong C-N amine stretching was presented by the peaks around $\nu = 1028.06 \text{ cm}^{-1}$ – 1309.67 cm^{-1} .

For polyvinyl chloride, the absorption band of C-H asymmetric

Table 4

Classification of microplastic based on density materials.

Station	Density classification (g cm^{-3})		
	< 1.00	1.00–1.02	> 1.02
KP1	14	32	69
KP2	7	28	75
KP3	17	17	86
KP4	8	20	93
KP5	3	6	146
KN1	7	12	92
KN2	3	10	287
KN3	7	16	247
KN4	5	10	431
KN5	6	6	547

stretching was observed at $\nu = 2982.02 \text{ cm}^{-1}$ and C-H₂ deformation at 1350 cm^{-1} . The bending recorded at $\nu = 1584$ – 1600 cm^{-1} indicated the formation of polyene compound produced during the photo-degradation of PVC, and this range varied depending on the PVC stabiliser itself (Pimentel Real et al., 2008). In addition, the strong fingerprint absorption band of C-Cl vibration at $\nu = 840.67 \text{ cm}^{-1}$ indicated the main functional group for polyvinyl chloride. It was a close agreement with a study reported by Fotopoulou and Karapanagioti (2012) who indicated that vinyl group appeared at $\nu = 892 \text{ cm}^{-1}$.

For polyethylene, it was similar to the finding by Gulmine et al. (2002), the strong absorption band of C-H₂ stretching can be observed at $\nu = 2901.03 \text{ cm}^{-1}$. The C-H₂ bending deformation was shown at $\nu = 1482.91 \text{ cm}^{-1}$. Weak wagging and twisting oop deformations of

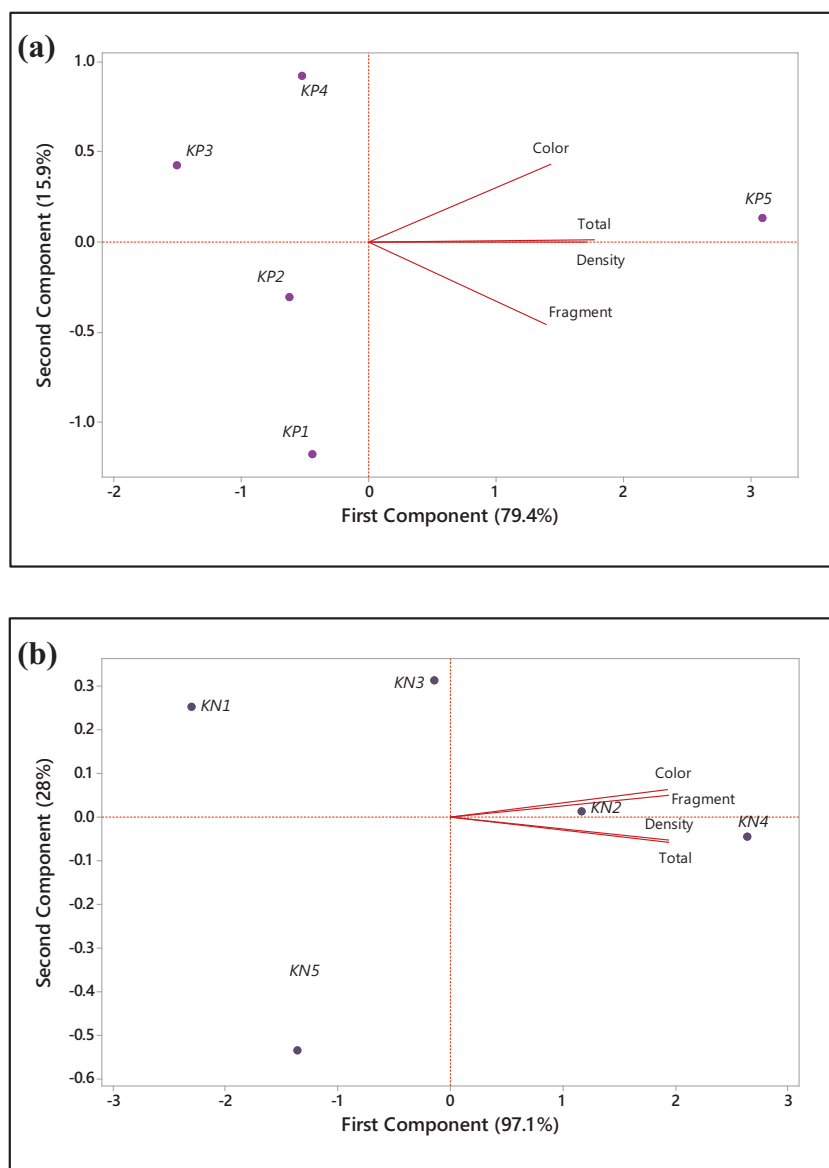


Fig. 3. Biplot interaction between station and parameter studied (a) Kuantan port and (b) Kuala Nerus.

–H–C–H– linkage closely appeared at $\nu = 1299.82 \text{ cm}^{-1}$ and 1041.32 cm^{-1} . In addition, the rocking deformation of the ethylene linkage can be observed by the presence of a very strong peak at $\nu = 847.89 \text{ cm}^{-1}$. The band of the hydroxyl group was suggested to appear at wave number $\nu = 3200\text{--}3900 \text{ cm}^{-1}$ (Waldman and De Paoli, 1998). Peaks around $\nu = 1711 \text{ cm}^{-1}$ on a polyethylene sample were indicative of oxidised material (Cooper and Corcoran, 2010). Veerasingam et al. (2016) suggested $\nu = 1715 \text{ cm}^{-1}$ linked to the presence of a ketone group. It was believed to occur due to thermal oxidation, solar radiation or biological process. Polyethylene is abrasion resistant and widely used in fishing materials. This material is not surprisingly found since fishery activities are one of the generic incomes for the local populations in this area.

For polypropylene, the absorption band of C–H₂ bending was observed at the higher frequency of wavenumber at $\nu = 1525.64 \text{ cm}^{-1}$ and $\nu = 1408.96 \text{ cm}^{-1}$. In comparison, FTIR spectrum with an observed peak was recorded by Nor and Obbard (2014) at wave numbers $\nu = 2860\text{--}2960 \text{ cm}^{-1}$, $\nu = 1452 \text{ cm}^{-1}$, and $\nu = 1375 \text{ cm}^{-1}$. The material was revealed as polypropylene but no detail of functional groups was reported. In fact, identified polymers such as polystyrene (1.02 g cm^{-3}), polyester (1.40 g cm^{-3}) and polyamide

($1.15\text{--}1.24 \text{ g cm}^{-3}$) were known to have higher density (Lusher et al., 2013). Hence, it indicated that pollutant may deposit on the seafloor and gauge environmental risk towards the benthic organisms became more crucial.

A field study conducted successfully gave an idea about the prevalence of marine debris in Malaysian marine waters. Even though the sampling of the studying area did not overlap exactly in terms of spatial-temporal scale, the outcome provided physical and chemical characteristics of microplastic. Anthropogenic dynamic activities between the two studied areas displayed no significant difference. Relatively, marine debris contribution is believed to be generated by local human activities. It is very interesting to extend the knowledge to study the impact of microplastic occurrence from the terrestrial area, especially hotspot connection to marine environments.

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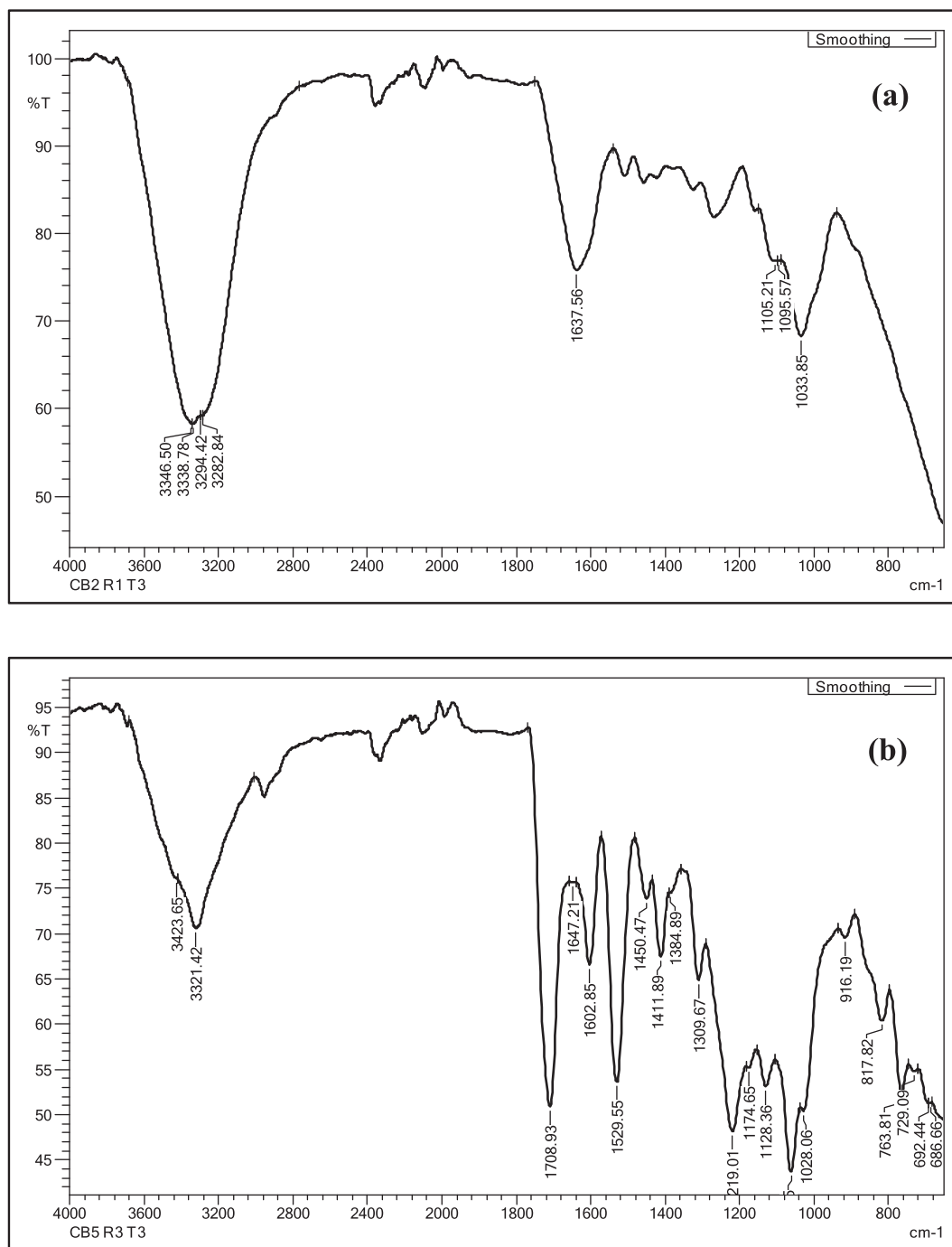


Fig. 4. FTIR spectra for analysis of (a) polystyrene and (b) polyamide.

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