

Investigating the Presence and Distribution of Microplastics in Wastewater Treatment Plants Systems

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ABSTRACT

Microplastic is a highly concerning emerging contaminant with the significant increase of municipal waste along with the elevating growth of population. Wastewater is one of the main routes contributing to the persistent microplastics presence in the environment since it originates from domestic, industrial, and even agricultural wastewater. These facilities are increasingly identified as potential contributors to the release of microplastics (MPs) into the environment. This study aims to examine the occurrence and distributions of MPs in both the influent and effluent of two districts sewage treatment plant (STP) in Kota Kinabalu (UMS STP) and regional sewage treatment plant Penampang (RSTP) Sabah Malaysia. The results showed that the abundance of MPs in UMS STP was higher in both influents and effluents than RSTP. The average size of MPs found varied from 1.0 mm to 125 μ m being the most abundant. Microplastics 2.0 mm is higher in influents, while effluents exhibit higher concentrations of smaller particles (63 μ m). In terms of microplastic morphology, fragments were identified as the most predominant shape in the sampled materials from both sewage treatment plants (STPs), whereas microplastic beads were the least frequently detected. The distribution of colours across the influent and effluent samples was consistent, with a variety of colours identified, though green, blue, and red predominated. Polymer analysis revealed a diverse array of microplastic types present in the samples, including polypropylene (PP), nylon (PA), and polyethylene terephthalate (PET/PETE), indicating a variety of sources and characteristics of the microplastics entering the treatment systems. These polymer types were found in both influent and effluent, suggesting that certain microplastics may persist through the treatment process. Overall, this study offers a comprehensive analysis of the physical and chemical properties of microplastics in wastewater treatment systems, providing valuable insights into their distribution, persistence, and the potential implications for the fate of microplastics within wastewater treatment processes. The findings contribute to a better understanding of microplastic behavior, highlighting key factors influencing their occurrence and removal efficiency in sewage treatment plants.

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1. Introduction

The occurrence of microplastics (MPs) worldwide has been increasing rapidly each year, drawing significant attention from both researchers and the general public. This growing issue is one of the particular concern due to its potential to disrupt ecological systems, as microplastics enter the environment and ultimately the food chain. The transport and fate of these particles pose further risks, potentially threatening the health of living organisms and the environment [1]. Microplastics can be classified into two different classes of primary and secondary microplastics where primary microplastics are the one that is produced readily within the given size of 5mm and below. Meanwhile, as for secondary microplastics, they are microplastics generated due to the breakdown of larger plastics items into smaller pieces. Microplastic is a major concern due to various potential pathways that it may have as an emerging contaminant. The pathways of microplastics in wastewater would be starting from industrial and residential sources which are gathered within one system of treatment known as sewage treatment plant. After sewage are being treated from the system, it will then be released into the environment such as river, streams, or sea [2]. Through these pathways, it will contribute towards the increasing presence of microplastics within the environment [3]. Sewage treatment plants (STPs) are facilities designed to treat wastewater, which contains a variety of contaminants. Given the diverse nature of pollutants present in sewage, the treatment process typically involves multiple stages, each targeting specific contaminants. The objective of STP is to treat the wastewater effectively, ensuring that the effluent meets regulatory standards before being discharged into the environment, thereby minimizing the risk of water pollution [4]. However, based on previous studies, STP act as a major contributor of microplastics within the environment, particularly, in the marine and freshwater ecosystem. Liu *et al.*, [5] identified sewage treatment plants (STPs) as significant contributors to microplastic pollution in marine ecosystems, particularly due to the high abundance of fibre-shaped microplastics. These microplastics are thought to originate primarily from the fragmentation of synthetic fibres, which result from domestic anthropogenic activities, especially laundry processes [5]. Other studies have also shown that wastewater treatment plants (WWTPs) or STPs play a role in introducing microplastics into the environment, with higher concentrations typically found in influent compared to effluent [6]. The possibility of microplastics passing through the different treatment systems could be due to shear and stress during different treatment process stages or even because lacking technology efficiency in micro-sized pollutants removal [3]. There is a need for deeper understanding of how sewage treatment plant (STP) capacity influences microplastic removal efficiency, as well as the downstream fate and accumulation of microplastics in aquatic ecosystems post-treatment. Additionally, limited attention has been given to analyzing removal efficiency based on specific microplastic types (e.g., fibers, fragments) or materials, as well as the impact of temporal and seasonal variations on microplastic concentrations in wastewater. Therefore, in this study the occurrence and distribution of microplastics in different capacity of sewage treatment plants system, University Malaysia Sabah's sewage treatment plant (UMS STP) and regional sewage treatment plant Penampang (RSTP) Kota Kinabalu, Sabah will be investigated to evaluate the potential for microplastics to bypass treatment systems due to shear, stress, or insufficient technological efficiency in removing micro-sized pollutants. This investigation will contribute to a better understanding of the fate of microplastics in wastewater treatment systems of varying scales, providing valuable insights into the effectiveness of current treatment technologies in mitigating microplastic pollution at both local and regional levels.

2. Methodology

2.1 Experimental Design

The research methodology in evaluating microplastics occurrence from two different sampling locations, RSTP and UMS STP, was conducted according to three steps with the order of (a) sampling collection, (b) laboratory analysis as well as by conducting quantification, classification, and characterization of microplastics. Sample collections will be retrieved from the influents and effluents of the different sampling sites. It will then be stored in suitable conditions to preserve it before conducting the laboratory works for the following analyses. Laboratory analysis involves sample pretreatment and density separation to extract and isolate microplastics from the sewage samples. The determination of physical and chemical morphologies of microplastics retrieved from the samples will be done through microscopy and stereoscopy analyses.

2.2 Microplastics Sampling Collection

The samplings were conducted by taking samples from influents and effluents from each of the different treatment plants. The samples were taken by using a 10 L stainless-steel bucket with the volume of 10 L [7]. The depth of the samples taken from the sampling area were at 50 cm from the surface water [8]. The frequency of samplings were four times within the period month of September until late of November 2023. The samples were then brought back into the laboratory for preparations and preservation [9].

2.3 Sample Preparation and Preservation

Samples were stored in a total of ten 1000 mL Schott's glass bottles for each influents and effluents which were covered by aluminium foils. The samples were then stored in the chiller to refrigerate the samples in a dark surrounding with the temperature of approximately 4 °C [10].

2.4 Quality Control and Quality Assurance

To minimize sample contamination, plastic materials were avoided from being utilized in the experiment. Sampling tools, equipment, and lab wares for microplastics extraction were also ensured to be cleaned with ultra-pure, Milli-Q water or by using distilled water at least three times which were then let dried before used [11]. All of samples, solutions, and lab wares that were used were also wrapped with aluminium foil when they are not in use to prevent from any airborne contamination of microplastics. During experimental laboratories work, blank controls were also prepared prior to spectrophotometry analysis to ensure that the FTIR is reliable for the analysis [10]. Other than that, all work surfaces will also be cleaned with 70% ethanol three times prior to laboratory analysis. Tape lifting technique as in forensic science laboratories will also be applied to get rid of fibre and other particle contaminations around the laboratory analysis area [12].

2.5 Sample Pretreatment

In this study, samples obtained that require pretreatment underwent the wet peroxidation (WPO) process with the use of hydrogen peroxide (H₂O₂) along with the presence of 0.05 M Iron (II) as catalyst to treat the various types of organic constituents within the sewage samples [13]. The volume of 30% hydrogen peroxide that will be used is 20 mL of and the same volume will also be

used for the Fenton's reagent, 20 mL. The Fenton's reagents were prepared by adding 7.5 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, which is equal to 278.02 g/mol, to 500 mL of water and also 3 mL of concentrated sulphuric acid. The exact 20 mL each of (H_2O_2) and the Fenton's reagent will be added to the sample. The mixture was placed on a hot plate set to 75 °C to allow reactions to execute all of the organic material. After all organic materials have reacted, the mixture will be left in a partially closed fume hood to allow all of the H_2O_2 mixture to fully evaporate [9].

2.5.1 Density separation

The density separation technique was carried out by using saturated sodium chloride (NaCl) salt solution. The density of the solution is 1.2 g/cm³ and can enable the floatation and extraction of low density microplastics particles such as PP (0.82-0.90 g/cm³), PE (0.92-0.97 g/cm³), PS (1.05-1.06 g/cm³), or PA (1.13-1.15 g/cm³) [5].

2.6 Quantification, Classification, and Characterisation of Microplastics

Microplastics collected from the different sampling sites of sewage treatment plants (STP), RSTP and UMS STP will be characterized based on their abundance of size, colours, shapes, and polymer types. These parameters can be classified through quantifications, identifications, and validations through microscopy and stereoscopy analyses.

2.6.1 Quantifications of microplastics

The enumeration of microplastics is a fundamental step in microplastic research, typically carried out following sample filtration using the vacuum filtration method. Laboratory containers holding the liquid sample are rinsed three times with distilled water, with filtration conducted after each rinse to ensure thorough sample processing. A 0.45 µm gridded membrane filter paper (Whatman) is employed during the filtration process. After filtration, the gridded membrane filter papers are examined under a stereomicroscope for microplastic identification. The physical characteristics of the microplastics, including size, shape, and colour, are recorded. Size is roughly measured using stainless steel sieves of varying mesh sizes (2.0 mm, 1.0 mm, 125 µm, and 63 µm). Microplastics are further classified based on their shape and colour, following established classification protocols [14]. Finally, the quantification of microplastics in the sewage samples is determined using the formula provided below:

$$\text{Abundance of Microplastic} = \frac{\text{Number of microplastics particles}}{\text{Volume of Sewage sample (L)}} \quad (1)$$

2.6.2 Microplastics physical identification

The identification of the physical morphology of microplastics in the current study involves the observation of size, shape, and colour using a stereomicroscope [15]. Visual identification through stereomicroscopy will allow for the classification of microplastic particles into distinct shapes, including fragments, fibres, films, beads, and others, as well as the identification of colours such as white, black, red, and blue. These morphological characteristics are then used to categorize the microplastics based on the observed variations. The diversity in shape and colour provides valuable information about the potential polymer type, sources, or pathways of the microplastics. This visual identification step is performed prior to spectroscopic analysis using Fourier-transform infrared

spectroscopy (FTIR), which is essential for distinguishing plastic particles from other anthropogenic solids or organic materials [13].

2.6.3 Identification and validation of microplastics polymer type

In this study, Fourier-transform infrared spectroscopy (FTIR) will be used, as it is a widely applied method for identifying microplastics in sewage treatment plants. FTIR analysis involves comparing the spectral features, particularly the peaks, of the microplastic samples with those of reference spectra to identify the polymer types [13,16].

2.6.4 Statistical analysis

Statistical analysis was performed using Microsoft Excel to conduct a Two-way Analysis of Variance (ANOVA) and to evaluate significant differences in microplastic characteristics and associated parameters. This analysis facilitated the identification of correlations and significant variations in the size, shape, color, and polymer type of microplastics relative to their abundance in influent and effluent samples collected from various sampling locations.

3. Results and Discussion

3.1 Microplastics Occurrence and Distribution

Figure 1 below depicts the bar charts that illustrates the mean occurrences of microplastic per litre for 10 L in different sampling points influents, effluents of UMS STP and RSTP. The results showed that University Malaysia Sabah's sewage treatment plant (UMS STP) has higher concentrations of microplastics in both influents and effluents compared to regional sewage treatment plant (RSTP), Penampang.

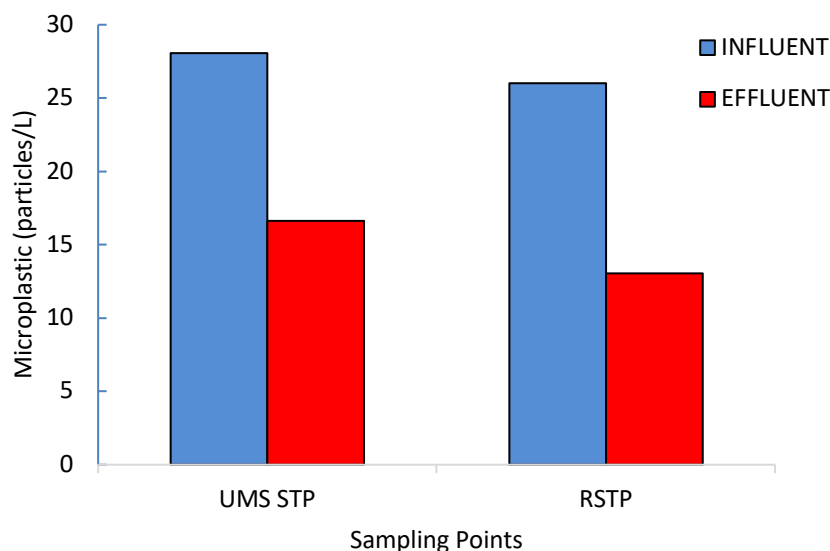


Fig. 1. Microplastics mean distributions per L of 10 L samples from influent and effluent samples of UMS STP and RSTP

The mean sum of microplastics identified in 10L of water samples for influent as well as effluent are 294 and 169 for UMS STP while RSTP are 258 and 128 respectively within the current study. The observed differences in microplastic concentrations between UMS STP and RSTP may be attributed

to variations in their facility capacities. UMS STP is a smaller treatment plant serving localized areas, including the University Malaysia Sabah (UMS) campus and the USIA 1Borneo residential college. In contrast, RSTP is a larger facility capable of serving broader regions, including Penampang, Kota Kinabalu, Putatan, and surrounding areas. Smaller facilities, such as UMS STP, are likely to have limited microplastic transport due to their smaller scale, which may result in relatively higher concentrations of microplastics within the plant [17]. Additionally, smaller treatment plants require more frequent maintenance, as sediment can accumulate in the sedimentation tanks if not regularly serviced. This accumulation may contribute to the higher abundance of microplastics observed at UMS STP. In summary, smaller sewage treatment plants may accumulate and release higher concentrations of microplastic particles compared to larger facilities, due to both operational constraints and maintenance needs.

3.2 Physical Characterisation of Microplastics

As shown in Figure 2, the bar charts illustrate that microplastic sizes (2.0 mm, 1.0 mm, 125 μm , and 63 μm) were generally found in higher concentrations in the influent compared to the effluent samples. Among these sizes, the 125 μm and 1.0 mm categories, represented by yellow and red bars respectively, exhibited the most pronounced trends, indicating that these two size ranges were the most abundant in both influents and effluents across the sampling sites.

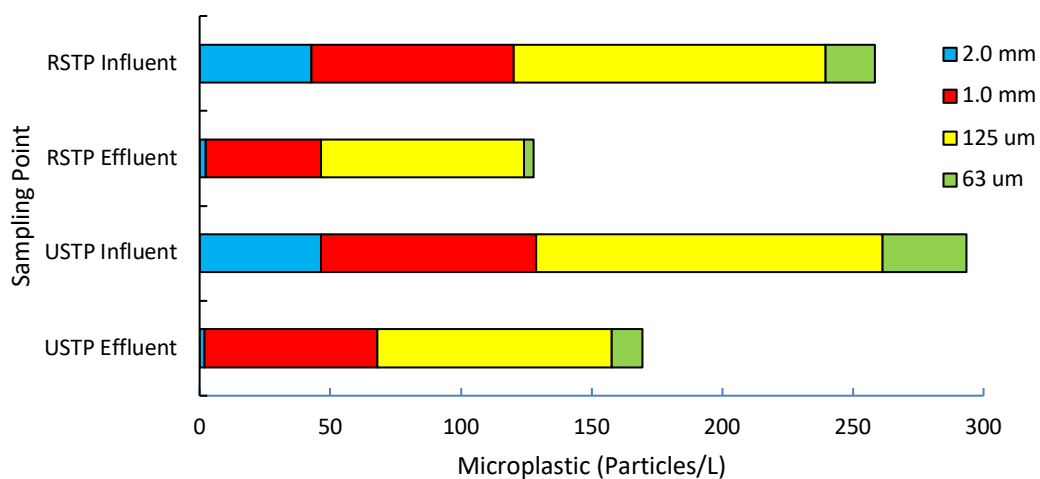


Fig. 2. Size distribution of microplastics throughout UMS STP and RSTP influents and effluents

The variation in microplastic particle size distribution between influents and effluents reflects differences in the abundance of microplastics across the available size ranges. For the 2.0 mm and 63 μm sizes, both were observed in lower quantities compared to the other two sizes. Specifically, the blue bars representing 2.0 mm particles were more abundant in the influent than in the effluent, while the green bars representing 63 μm particles were found in higher concentrations in the effluent than in the influent at both sampling sites. It can be concluded that majority of the microplastic found were within medium-ranged size (1.0 mm and 125 μm) [18]. It is important to note that larger microplastic particles (2.0 mm) are more prevalent in the influent samples, while smaller microplastic particles tend to be more abundant in the effluent, although the 63 μm particles were still present in relatively low quantities. This pattern can be explained by the fact that influents consist of wastewater from multiple sources, including domestic, industrial, and agricultural effluents, which introduce larger microplastic particles, such as larger plastic fragments, into the treatment plant [19].

Consequently, the concentration of 2.0 mm microplastics is higher in the influent. In contrast, the lower concentration of 2.0 mm particles in the effluent may be attributed to the screening process, which is typically the first stage in wastewater treatment. This process is designed to remove larger objects and debris from the incoming sewage, effectively reducing the number of larger microplastic particles in the effluent. Figure 3 illustrates the available size ranges of microplastics within the retrieved samples.

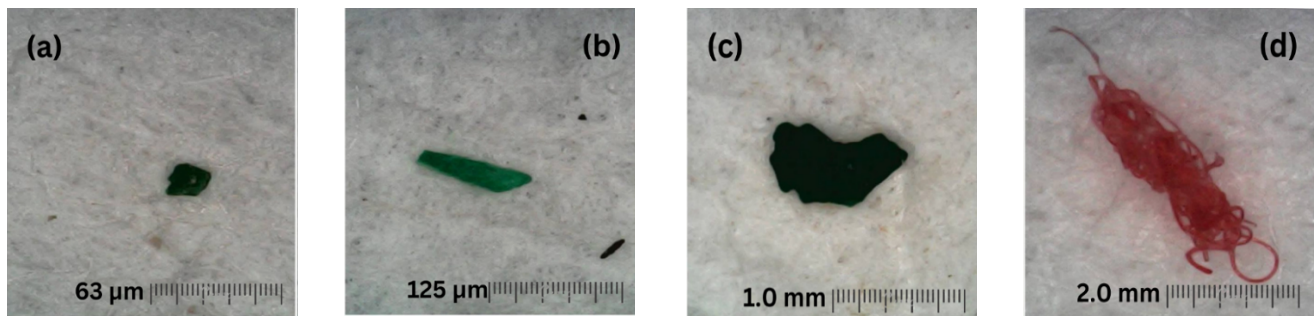


Fig. 3. Different sizes range for obtained samples within the study, (a) 63 µm, (b) 125 µm, (c) 1.0 mm, and (d) 2.0 mm

Microplastic shape analysis is crucial for identifying the potential sources of microplastics, as well as for distinguishing between primary and secondary microplastics. Figure 4 presents a stacked bar chart depicting the distribution of microplastic shapes from 10 L sewage samples collected from both influents and effluents at the University Malaysia Sabah Sewage Treatment Plant (UMS STP) and the Regional Sewage Treatment Plant (RSTP) in Penampang.

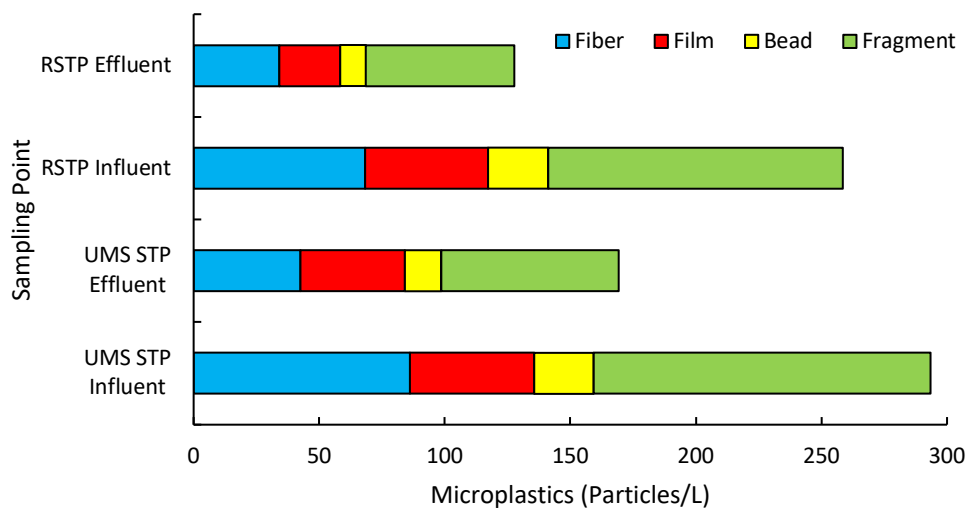


Fig. 4. Shape distribution of microplastics throughout UMS STP and RSTP influents and effluents

The chart reveals four distinct microplastic shapes, with fragments being the most abundant, followed by fibres, films, and beads. This distribution pattern (fragment > fibre > film > bead) was consistent across both influent and effluent samples, indicating a similar composition of microplastic shapes in both stages of treatment. Fragments shape microplastics is the most abundant while the least is bead shape. This could be to the fact that wastewater treatment plants receive wastewater from diverse sources such as industrial, daily anthropogenic activities, agricultural and so on [20]. Potential fragments microplastic can originate from many possible factors such as secondary

microplastic fragmentations of plastic items like plastic baskets, plastic bottles, plastic product packaging, and many more [21]. Hence, secondary microplastics due to fragmentation could be the possible factors that contribute to the major generation of microplastic fragment shape, which also applies to film or even fibre shape of microplastic particles occurrences within influents and effluents of UMS STP and RSTP. Bead-shaped microplastics were found in very small quantities in both the influent and effluent samples from UMS STP and RSTP. Beads are considered primary microplastics, as they are originally produced in a spherical shape and are commonly used in personal care products, such as exfoliants, to assist in the removal of dirt and pollutants from the skin [22]. However, due to growing awareness of microplastic pollution, the use of bead-shaped microplastics in hygiene products has been increasingly banned. While microplastics can be found in various forms, the most common shapes identified in this study include fragments, films, beads, and fibres, as illustrated in Figure 5.

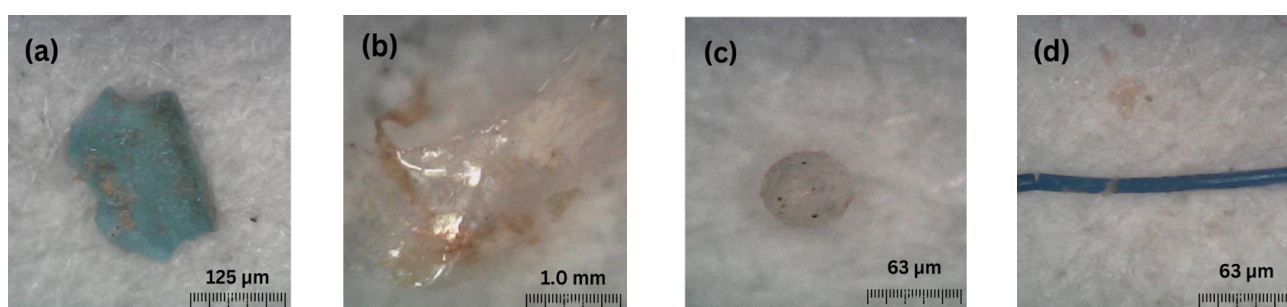


Fig. 5. Different shapes of microplastics from obtained samples (a) fragment, (b) film, (c) bead, and (c) fibre

Microplastics are synthetic materials produced in a wide range of colours, which aids in their identification. The distinct colours of plastic particles facilitate the detection of microplastics in influent and effluent samples from UMS STP and RSTP, Penampang, as the contrasting colours make it easier to observe their presence.

As shown in the pie charts (Figure 6), the distribution of microplastic colours in the influent and effluent samples from both UMS STP and RSTP reveals that "other" colours represent the largest proportion at all sampling points. This category likely includes a variety of colours such as white, black, pink, purple, yellow, and others. Among the individual colours, blue, green, and red were the most predominant[23]. The green colour was the least abundant among the three primary colours, while blue and red were found in nearly equal proportions across both influents and effluents. Specifically, in the pie charts (a) for UMS STP influent and (d) for RSTP effluent, the colour distribution follows the pattern of "Other" > Blue/Red > Green. Similarly, in pie charts (b) for UMS STP effluent and (c) for RSTP influent, the distribution shows a similar trend, but with blue slightly more abundant than red (Other > Blue > Red > Green).

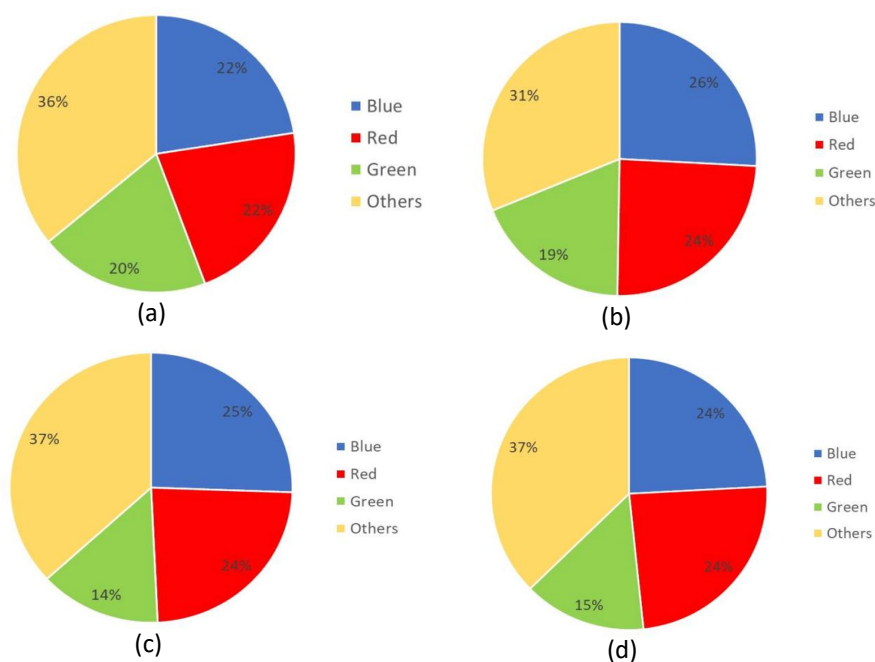


Fig. 6. Colour distribution of microplastics throughout UMS STP and RSTP influents and effluents, (a) UMS STP influent, (b) UMS STP effluent, (c) RSTP influent, and (d) RSTP effluent

3.3 Microplastics Polymer Type Distribution

The distribution of polymer types is illustrated in the pie chart in Figure 7. Based on the chart, polypropylene (PP) is the most abundant polymer, constituting 20% of the total polymer types identified in the samples through FTIR spectroscopy. This is significant because polypropylene is commonly used in a variety of applications, such as packaging materials, textiles, and household products, making it a likely candidate for microplastic contamination in wastewater [24].

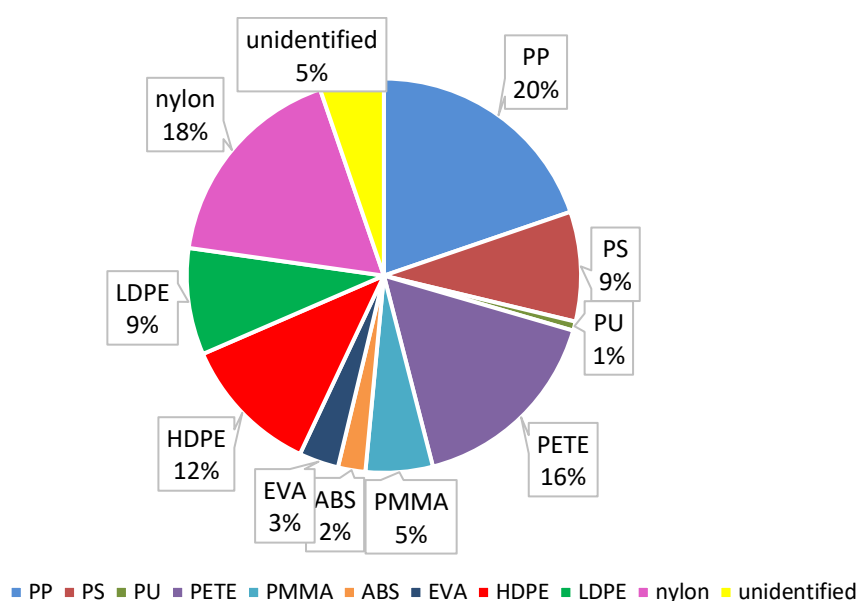


Fig. 7. Distribution of different polymer types of microplastics that are present within all the samples

In contrast, acrylonitrile butadiene styrene (ABS) is the least abundant polymer in the samples, representing only 2% of the total. ABS is often used in the manufacturing of durable goods, such as electronic housings, automotive parts, and household appliances, but it is less commonly found as microplastic debris in the environment. The FTIR analysis identified several polymer types in the microplastic samples, including nylon (PA), polyethylene terephthalate (PETE), high-density polyethylene (HDPE), low-density polyethylene (LDPE), polystyrene (PS), poly(methyl methacrylate) (PMMA), and ethylene-vinyl acetate (EVA). These polymers are prevalent in a range of products, from textiles (nylon) to packaging (PETE, HDPE, LDPE, PS) and household items (PMMA, EVA)[25]. The wide variety of polymers found in the samples suggests that microplastics in wastewater are derived from multiple sources, including domestic, industrial, and commercial waste. However, a small proportion of the polymers (5%) could not be identified accurately. This lack of identification was likely due to insufficient peak intensity or interference in the FTIR spectra. In FTIR analysis, the ability to identify a polymer depends on the clarity and distinctiveness of the absorption peaks corresponding to the polymer's molecular vibrations. If the sample contains low concentrations of the polymer or if the spectra are contaminated by other compounds (such as organic matter, additives, or impurities), the identification can become less reliable, leading to challenges in determining the exact polymer type.

3.4 Statistical Analysis

The analysis of influent microplastic particles of varying sizes between UMS STP (University Malaysia Sabah Sewage Treatment Plant) and RSTP (Regional Sewage Treatment Plant) revealed a statistically significant difference, as indicated by a p-value less than 0.05 ($p < 0.05$). This finding highlights that the distribution of microplastics in the influents from the two plants is influenced by factors beyond random variation, such as differences in wastewater sources or the operational characteristics of the treatment plants. Furthermore, a significant variation was also observed among different microplastic size categories in the influents, suggesting that the occurrence of microplastics of specific sizes is likely governed by source-specific or process-related factors. A similar pattern was noted in the effluents, where significant correlations ($p < 0.05$) were found between the two sampling locations and across the different microplastic size categories, indicating that the treatment processes at both plants impact the size-specific distribution of microplastics. Regarding the shape of microplastics, significant correlations ($p < 0.05$) were also observed between the shapes and their presence in the influents and effluents at both plants. This suggests that microplastic shapes are not randomly distributed but are likely influenced by the treatment processes. However, the statistical analysis of the polymer types in the influents and effluents showed no significant correlation ($p > 0.05$). This lack of correlation may be due to the sampling methodology, where only a fixed number of randomly selected samples were analyzed using Fourier Transform Infrared Spectroscopy (FTIR). As such, the analyzed samples may not adequately represent the diversity of microplastic polymers present across all samples. Furthermore, the polymer type analysis primarily focused on validating the presence of microplastics rather than assessing the full range of polymer distributions. These limitations suggest that future studies should consider more representative and comprehensive sampling strategies to better understand the role of polymer types in microplastic distribution in wastewater treatment systems.

3.5 Potential Intervention and Future Changes of Microplastics Removal in Sewage Treatment Plants

In effort of mitigating the arising microplastics issue, it is crucial for every party to be responsible to contribute effectively. Overcoming the issues of microplastics requires a comprehensive approach

so that it can be more efficient in solving the problem. One of the initiatives that can be implemented to tackle the microplastic issue is by reducing the production and usage of plastics. Nowadays, plastic consumption can be seen all around the world where single-use plastic is one of the major contributors to the enormous number of plastic wastes in the environment. As previously stated, larger sized plastics materials can undergo a weathering process where the plastic will be broken down into smaller fragments due to physical, chemical, and biological interaction as a result of exposure to sunlight, wind, water, as well as other environmental factors [5]. With the aim to reduce plastic production and consumption, governments should implement policies and regulations to ensure that plastic production could be reduced while the consumption of single-use plastics should be banned. Although ban of plastics could be sudden and impulsive, the government could practise soft and slow approach by encouraging the use of sustainable alternatives like promoting the 3Rs practice or encourage to bring their own drinking bottles and food containers, or even by having their own grocery bag.

The introduction of circular economy concept which is an economic system that promotes the reuse and regeneration of materials or products via sustainable and environmental way which can also contribute to minimizing plastic waste generation [26]. Besides, governments should establish and enforce regulations specifically targeting microplastics. Industries that generate products with microplastics should be confronted by taking stricter action and enforcing the regulation. For instance, there should be restrictions or ban against the production and usage of products containing microplastics especially in facial cleansers, body scrubs, other personal care products, detergents, and many other products. Guidelines for wastewater treatment facilities should also be stricter to prevent large loading rate of microplastic discharge into the environment. Other than that, international regulations and conventions should also be established or enforced as an effort to address the microplastic issue collectively. As an example, MARPOL Convention which is an international convention that aims to resolve or reduce pollution in marine environments caused by ships either due to accidental incidents or occurrences from routine operations. From the convention, six annexes are available as references to the types of pollutants being discharged or emitted from ships. The most important annex from the MARPOL convention that would contribute to mitigation of microplastic issues would be the Annex V where the annex deals with pollution by different types of garbage from ships whereby a complete ban on the disposal of all forms of plastics into the sea is imposed.

4. Conclusions

In conclusion, this study examined the occurrence and distribution of microplastics (MPs) in influent and effluent samples from two sewage treatment plants (STPs) in Kota Kinabalu, Sabah. Results showed that UMS STP had higher MP concentrations compared to PNP STP, with fragments being the most common shape and polypropylene (PP), nylon (PA), and polyethylene terephthalate (PET/PETE) identified as the main polymers. Smaller MPs (less than 2.0 mm) were more prevalent in effluents, indicating treatment challenges for finer particles. The findings highlight the persistence of microplastics through treatment processes and emphasize the need for improved technologies to effectively remove microplastics, particularly smaller ones, from wastewater before they enter the environment. The study underscores the importance of further research to assess the environmental impact of microplastics and develop strategies to mitigate their spread in aquatic ecosystems.

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