DETECTION OF MICROPLASTICS IN BOTTLED DRINKING WATER

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DETECTION OF MICROPLASTICS IN BOTTLED DRINKING WATER

by

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Thesis submitted in partial fulfilment of the requirements for the degree of Bachelor of Science (Honours) (Forensic Science)

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CERTIFICATE

This is to certify that the dissertation entitled "Detection of Microplastics in Bottled Drinking Water" is the bona fide record of research work done by Farah Binti Mohd Noor during the period from October 2024 to February 2025 under my supervision. I have read this dissertation and that in my opinion it conforms to acceptable standards of scholarly presentation and it fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Science (Honours) (Forensic Science).

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purpose.

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Farah Binti Mohd Noor

Date: 27th February 2025

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LIST OF ABBREVIATIONS

MP	Microplastics
PET	Polyethylene Terephthalate
RT	Room Temperature
PT	Peak Temperature
SRT	Shaking Room Temperature
SPT	Shaking Peak Temperature
RPM	Revolutions per Minute

PENGESANAN MIKROPLASTIK DALAM AIR MINUMAN BERBOTOL

ABSTRAK

Pencemaran mikroplastik (MP) daripada botol air minuman sekali pakai telah muncul sebagai kebimbangan utama, terutamanya mengenai potensi implikasinya terhadap keselamatan produk dan kesihatan pengguna. Tujuan kajian ini adalah untuk menilai pembebasan MP di bawah pelbagai keadaan, termasuk variasi suhu pada suhu bilik (25 ± 2°C) dan suhu puncak (60 ± 2°C), tekanan mekanikal pada dua kelajuan getaran (30 RPM dan 60 RPM), dan penggunaan botol air minuman secara berulang. Selain itu, kajian ini juga mencirikan morfologi MP yang dibebaskan. Keputusan menunjukkan bahawa suhu tinggi dan peningkatan tekanan mekanikal secara signifikan meningkatkan pembebasan MP, dengan pembebasan tertinggi diperhatikan pada suhu 60°C dan kelajuan 60 RPM. Penggunaan berulang turut meningkatkan pelepasan MP, menunjukkan bahawa degradasi termal dan mekanikal memainkan peranan penting dalam pembebasan MP. Bentuk MP yang paling kerap diperhatikan ialah gentian, serpihan, pellet, filamen dan filem dengan lut sinar MP mendominasi semua ujian, menggambarkan bahan botol. Kajian ini memberikan kesedaran kepada pengguna mengenai kesan kritikal terma, tekanan mekanikal dan penggunaan botol air minuman secara berulang kali melalui ujian suhu pada dua suhu berbeza (25 ± 2 and 60 ± 2°C), ujian getaran pada 30 RPM dan 60 RPM, dan ujian kebolehgunaan semula, terhadap pelepasan MP daripada botol air minuman sekali pakai.

DETECTION OF MICROPLASTICS IN BOTTLED DRINKING WATER

ABSTRACT

Microplastics (MPs) contamination from single-use drinking bottled water has emerged as a significant concern, particularly regarding its potential implications for product safety and consumer health. This study aimed to evaluate the leaching of MPs under different conditions, including temperature variations at room temperature (25 ± 2°C) and peak temperature (60 ± 2°C), mechanical stress at two shaking speeds (30 RPM and 60 RPM), and repeated usage of drinking bottled water. Additionally, the study characterized the morphology of the leached MPs. Results revealed that peak temperatures and increased mechanical stress significantly enhanced MPs release, with peak leaching observed at 60°C and 60 RPM. Repeated use further amplified the release, indicating that thermal and mechanical degradation play critical roles in the fragmentation of MP particles. The most commonly observed MP shapes were pellets, fibers, and filaments, with transparent MPs dominating across all tests, reflecting the material of the bottles. This study highlighted the awareness to the consumer on the critical impact of thermal, mechanical stress and repeated use of drinking bottled water through temperature test at two different temperatures (25 ± 2 and 60 ± 2°C), shaking test at 30 RPM and 60 RPM, and the reusability test, on the release of MPs from single-use bottled drinking water.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

According to United Nation Environmental Program (UNEP), one million plastic bottles are purchased every minute worldwide, raising concerns on the environmental and health impacts of microplastics. In Malaysia, sales of bottled water exceeded USD 167.39 million in 2019 and it is forecasted to transcend USD 322.30 million in 2025 (Praveena et al., 2022). Single-use bottled drinking water has become an everyday commodity due to its convenience, lightweight, durability, and stability (Makhdoumi et al., 2021). However, this convenience comes at a significant environmental and health cost due to the leaching of microplastics from plastic containers into the water.

Microplastics (MPs) are defined as plastic particle ranging from 1 µm up to 5 mm, typically originating from different environmental stress factors such as heat, physical movement and material degradation. These particles are generated as larger plastics break down through processes like photo-oxidation and chemical weathering. Due to their smaller size, MPs can bypass the gastrointestinal tract and migrate to other parts of an organism's body, posing potential health risks (Makhdoumi et al., 2021).

In Malaysia, the market is dominated by two types of single-use bottled water: natural mineral water and bottled drinking water. Natural mineral water, typically sealed with coloured caps like blue or green, contains naturally dissolved minerals and undergoes minimal treatment to meet water quality standards. In contrast, bottled drinking water, often sealed with white caps, is usually treated tap water derived from rivers and subjected to additional filtration processes such as reverse osmosis, distillation, or deionization before being packaged (Praveena et al., 2022). This study focused exclusively on single-use bottled drinking water due to its widespread consumption and the potential for repeated use by consumers, which may exacerbate MPs contamination.

While the presence of MPs in single-use bottled drinking water in Malaysia is now well-documented (Praveena et al., 2022; Wong et al., 2021), the factors that influence the release of MPs under real-world conditions, such as temperature variations, physical agitation, and bottle reuse, remain underexplored. In Malaysia's tropical climate, high temperatures may accelerate the fragmentation of plastic materials, leading to increased MPs migration from bottle caps, necks, and the packaging itself. Additionally, mechanical stress such as shaking during transport and handling may further promote the release of MPs (Kankanige & Babel, 2020; Lin et al., 2022). Moreover, the repeated use of plastic bottles, often practiced as a cost-saving measure, may contribute to the increased of MPs contamination over time (Licciardello, 2024).

Given these concerns, this study aimed to examine how temperature, mechanical stress, and bottle reusability contribute to MPs released in single-use bottled drinking water. By analysing the physical properties such as quantity and shape of the released MPs, this study seeks to provide a better understanding of the factors that influence MPs contamination. The findings will not only contribute to the body of knowledge on MPs contamination in bottled water but may also serve as a basis for improving drinking water safety standards and consumer practices. These insights can help develop more effective guidelines and policies for the safe use and reuse of plastic bottles, ultimately promoting better environmental and public health outcomes.

1.2 Problem Statement

With the increasing consumption of single-use bottled drinking water in Malaysia, concerns over the release of MPs from plastic bottles into the water have intensified. Studies have revealed that MPs, which result from the degradation of plastic bottles, are present in bottled drinking water. Despite growing evidence of MPs presence in bottled drinking water (Hossain et al., 2023; Kankanige & Babel, 2020; Makhdoumi et al., 2021; Oßmann et al., 2018; Praveena et al., 2022; Wong et al., 2021; Zhou et al., 2021), there is a lack of systematic studies investigating how environmental and usage factors influence the release and properties of MPs.

This study aimed to address this gap by assessing the effects of temperature, mechanical stress, and reusability of single-use drinking water bottles on MPs release. By investigating the relationship between these factors and examine the physical properties of MPs such as quantity and shape, this study aims to investigate these factors to develop a safer packaging standards and better consumer practices.

1.3 Objective

General Objective:

To evaluate the influence of temperature, mechanical stress (shaking speed), and reusability on the quantity of MPs released from single-use bottled drinking waters, and to characterize the morphology of the MPs under these varying conditions.

Specific Objectives:

1. To determine the quantity of MPs released on different temperature (25 ± 2°C and 60 ± 2°C).
2. To determine the quantity of MPs released on the impact of mechanical stress at two different shaking speeds (30 RPM and 60 RPM).
3. To determine the quantity of MPs released on effect of repeated use of drinking bottled water.
4. To characterise the morphology (shape) of released MPs.

1.4 Significance of Study

The findings of this study would enhance the understanding of factors influencing MP release from the single-use bottled drinking water with a focus on the effects of temperature, mechanical stress (shaking speeds), and the repeated use of the single-use drinking bottled water. By highlighting how these conditions affect MPs release, the study will contribute to the development of safer packaging standards and promote better consumer practices. This research underscores the need for public awareness and regulatory measures to mitigate the environmental risks associated with MPs.

CHAPTER 2

LITERATURE REVIEW

2.1 Microplastics

Over the past 70 years, global plastic production has surpassed 8 billion tons, with projections suggesting that by 2050, annual production of primary plastics could exceed 34 billion tons (Li et al., 2024). As plastics degrade through physical, chemical, and biological processes, they fragment into smaller particles of varying sizes. Among these, MPs, ranging from 0.1 µm to 5 cm, have garnered significant attention due to their widespread presence and potential ecological and health impacts. By 2024, the European Drinking Water Directive plans to include MPs on the 'watch list' of emerging compounds, reflecting the growing public concern over MPs and their impacts on human health (Dettori et al., 2022).

Kankanige & Babel (2020) identified numerous pathways exist for human exposure to MPs. These include the transfer of plastic particles through the food chain via marine organisms, consumption of seafood, MP-contaminated sea salt, MP-contaminated beer, and various drinking water sources. Of particular concern is the detection of MPs in bottled water, which highlighted direct and prolonged exposure to plastic particles.

Due to their light weight, high plasticity and flexibility, thermal and electrical insulation, chemical resistance, durability and low cost, the bottled water industry has steadily expanded over the years, with an annual global production exceeding 6 billion gallons (Luo et al., 2018). According to Praveena et al. (2022) the sales of plastic bottled water in Malaysia has reached over USD 167.39 million in 2019 and are projected to surpass USD 322.30 million by 2025. Given the increasing trend of bottled water consumption in Malaysia, assessing bottled water quality, in particular, the presence and quantity of MPs is critical in understanding the potential human health risks.

2.2 Microplastics In Bottled Water

MPs in bottled water vary widely in their size, shape, and polymer composition, reflecting their diverse origins. These particles can be broadly categorized into primary MP and secondary MP, based on their origin. Primary MP include microbeads from personal care products and industrial manufacturing pellets whereas secondary MP result from the degradation of larger plastics into smaller particles (Oßmann et al., 2018). Both categories contribute to the contamination of MPs in bottled water, raising concerns about human exposure and the long-term effects on health and ecosystems.

The global prevalence of MPs in bottled water has become a well-documented issue, with studies consistently reporting contamination across various brands and geographical regions. For example, Mason et al. (2018) conducted a global study involving 259 PET water bottles, purchased from 19 locations across the world. Their finding revealed an average concentration of 325 particles/L (ranging from 6.5–100 µm) and 10.4 particles/L (≥100 µm). Similarly, a study by Oßmann et al., (2018) focused on German brands of single-use PET bottled water, reporting an average MPs concentration of 2649 ± 2857 particles/L with particles as small as 1 µm detected.

In Malaysia, MPs contamination in bottled water is also a growing concern. Praveena et al. (2022) analysed eight major bottled water brands and found concentrations ranging from 8 to 22 particles/L, with an average of 11.7 ± 4.6 particles/L. The predominant particle sizes ranged from 100 to 300 µm, accounting for approximately 31% of the detected microplastics. Fragments were the most frequently identified type, with transparent particles being the most common. Moreover, a study by Cox et al. (2019), found that bottled water contains 94 items/L of MPs, a concentration over 20 times higher than the 4.2 items/L found in tap water. This suggests that this contamination is a global phenomenon, influenced by manufacturing practices, packaging materials, and environmental conditions.

2.3 Drinking Bottled Water

Bottled water, as defined by Mahmood et al. (2018) refers to any types of waters intended for human consumption that is marketed in bottles. Depending on the source and treatment process, bottled water can be subdivided into spring water, natural mineral water, and treated water (Zhou et al., 2021). Drinking bottled water, which falls under treated water will be our focus in this study. Packaged drinking water originates from treated tap water, typically sourced from rivers will undergo further processes, such as reverse osmosis, distillation, or deionization, before being bottled and marketed as drinking water (Praveena et al., 2022).

Polyethylene terephthalate (PET) has been the material of choice in the bottling industry since the 1970s, owing to its numerous advantageous properties. As a durable and strong semi-crystalline copolymer, PET can be made clear, colourless, or coloured, providing versatility in bottle design. With a glass transition temperature of around 76 °C and a melting temperature of approximately 250 °C, PET is suitable for a range of applications. Its density (1.3-1.4 g/cm^3) and minimum internal viscosity (0.7 dL/g) contribute to its robustness. PET is also almost impermeable to gas, ensuring the integrity of its contents. Additionally, it is inexpensive to produce and fully recyclable, making it an environmentally friendly option. These characteristics have led to its widespread use in manufacturing water bottles, transparent films, and textile fibers, among other products (Vasylius et al., 2023).

However, despite these beneficial properties, the widespread use of single-use PET in bottled water packaging introduces significant concerns regarding MPs contamination. According to Sekar et al. (2024), the quantity of MPs in bottled drinking water is affected by various handling factors, as depicted in Figure 2.1, which include: (i) bottle manufacturing, (ii) the bottling process, (iii) mechanical stress, and (iv) chemical degradation.

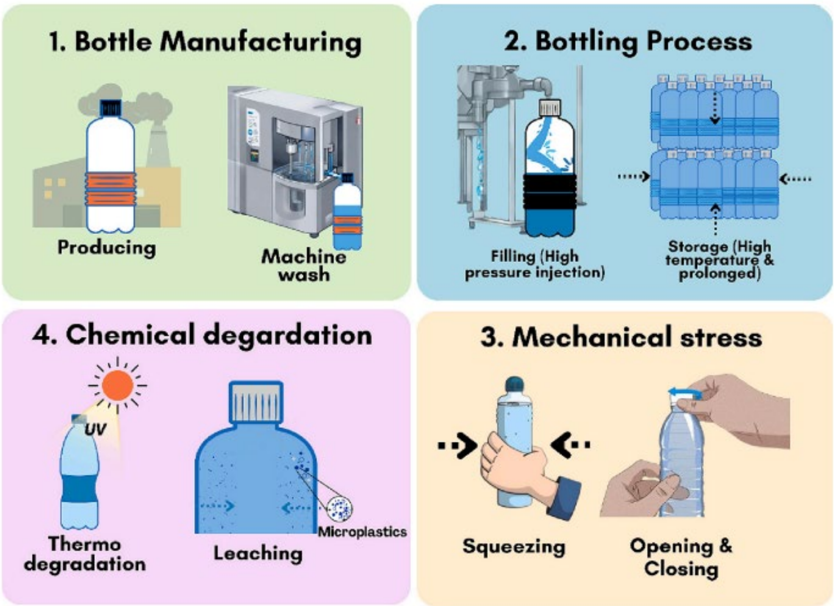


Figure 2.1: MPs fragmentation from the bottle into bottled drinking water (Sekar et al., 2024)

MPs can be inadvertently introduced during the production and processing of bottled water. As noted by Zhou et al. (2023), the manufacturing process, which involves heating and moulding PET resin, can result in the release of small plastic particles as a byproduct. These particles may adhere to the inner surfaces of the bottles (Winkler et al., 2019). The quality of plastic used in the manufacturing process plays a crucial role, as lower-grade plastics are more prone to impurities that can generate MPs particles (Sekar et al., 2024). In addition, certain manufacturing processes use mould release agents to help remove bottles from moulds. These agents can sometimes contain MPs particles, potentially adding to the contamination.

In addition to the manufacturing process, mechanical stress during the production and washing stages can also introduce MPs into bottled water. As indicated by Hee et al. (2022) and Oßmann et al. (2018), abrasion caused by machine parts during these stages (Figure 2.1, 1) can cause poorly moulded plastic components, such as flash (the excess plastic formed during injection moulding), to break off and enter the water. Moreover, the shear stress during the bottle filling process, caused by hydrodynamic and high pressure, can also release MPs particles into the water (Makhdoumi et al., 2021).

Additionally, the physical handling of plastic bottles can lead to the release of MPs. The process of storage and distribution of drinking bottled water as depicted in Figure 2.1 (2), can lead to the release of MPs due to the stress on the plastics surface. The manufacturing of bottle caps and seals can also result in the generation of microplastics. When these components come into contact with the bottle's neck or opening, friction can release small plastic particles.

A study by Song et al. (2021) further highlighted the role of consumer behaviour and product design in the release of microplastics. They found that different brands of plastic bottles released varying amounts of microparticles after repeated opening and closing cycles. Notably, some brands released significantly more MPs due to design differences, such as cap abrasions, illustrating how both product design and consumer interactions can influence microplastic contamination levels.

The release of MPs in bottled drinking water is further influenced by chemical leaching and exposure to ultraviolet (UV) radiation. High temperatures, for instance, can accelerate the leaching of plasticizers and other additives from the bottle into the water. Ahmed et al. (2021) found that heating plastic bottles increased the leaching of contaminants, including MPs, into the water. Additionally, Ghanbarian et al. (2022) noted that bottled water exposed to intense sunlight during transport could experience leakage of plastic monomers due to the UV radiation from the sunlight, hence further contributing to microplastic contamination.

The combined effects of manufacturing processes, physical handling, and environmental conditions highlight the complexity and multifaceted nature of microplastic contamination in bottled drinking water. Addressing this issue requires robust and accurate detection methods to quantify and analyse microplastics present in bottled drinking water.

2.4 Analysis Methods for Microplastic

The analysing of MPs in bottled water involves a series of meticulous methods, including sample preparation, visual identification, and chemical analysis. Each stage employs a range of techniques to ensure accuracy and reliability in identifying MP particles.

The first critical step in MP detection is sample preparation, which is crucial for isolating MPs from bottled water. A widely adopted method is filtration, where water samples are passed through filters to capture MPs particles. This approach is favoured for its ability to concentrate MPs from large volumes of water (Zainuddin & Syuhada, 2020). Membrane filters, commonly used in this process, allow water and smaller particles to pass through while retaining MPs. These filters are characterized by their pore size, which determines the size of particles that can be trapped. Typically, membrane filters are made from materials such as polyethersulfone, cellulose, and polytetrafluoroethylene, with pore sizes ranging from 0.45 μm to 1 μm (Adhikari et al., 2022). The choice of pore size is crucial, as it must be small enough to capture MPs while allowing for the passage of water and smaller contaminants.

After sample preparation, visual identification serves as the first qualitative assessment step in MP detection. This process generally uses optical or stereomicroscopy to examine the size, shape, and colour of the particles. For instance, Syuhada et al. (2023) in their study employed digital microscope because it can enhance the visibility of MPs and helps distinguish them from other organic materials. Meanwhile Hossain et al. (2023), Makhdoumi et al. (2021) and Praveena et al. (2022) utilised stereomicroscopes, which provide a three-dimensional view of the samples, making it easier to identify the morphology and structural characteristics of the particles. Additionally, stereomicroscopes offer a larger field of view than other microscopes, allowing a broader area of the sample to be examined at once, which is particularly useful for detecting MPs dispersed across filters.

In addition to visual identification, staining techniques, such as the use of fluorescent dyes like Nile Red, are also commonly employed. In this method, a fluorescent dye binds to hydrophobic plastic particles, making them visible under a fluorescence microscope. This method according to Kang et al. (2020) and Stanton et al. (2019) can avoid misidentification and allow for visualization of smaller particles besides it is also used for polymer identification. Studies by Kankanige & Babel (2020), Makhdoumi et al. (2021), and Mason et al. (2018) has employed dyes such as Nile Red and Rose Bengal to enhance the visualization of particles, particularly smaller ones, and to aid in polymer analysis. However, this approach may result in false positives if the dye binds to non-plastic hydrophobic particles.

Imaging techniques like Scanning Electron Microscopy (SEM), provide high-resolution images of particle surfaces, making them useful for assessing the shape, texture, and structural features of microplastics particles. This capability is essential for distinguishing between different types of MPs based on their physical characteristics which can provide insights into their sources and degradation states. Understanding these characteristics is crucial for assessing the environmental impact of MPs. However, while SEM provides valuable physical characterization, it cannot identify the chemical composition of the particles. Therefore, SEM is often combined with other methods, such as FTIR or Raman spectroscopy, to achieve a comprehensive analysis.

These visual identifications of MPs are limited as they cannot confirm the chemical composition of the particles. Hence, spectroscopic methods like Fourier Transform Infrared Spectroscopy (FTIR) and Raman Spectroscopy are employed since it provides more detailed analysis by identifying the polymer types. These methods can identify various polymers such as polyethylene (PET), polypropylene (PP), and polystyrene (PS), by detecting functional groups within polymers, which are commonly found in bottled water. For example, Raman spectroscopy has been utilized by Kniggendorf et al. (2019) to provide detailed chemical fingerprints of the MPs polymer. While FTIR is effective in identifying particles up to 20 µm, micro-Raman spectroscopy can detect even smaller particles, down to 1 µm (Oßmann et al., 2018).

Thermal analysis, such as Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) are another advanced technique used to identify polymer types by measuring the changes in physical and chemical properties of materials as a function of temperature. DSC technique measures the heat flow associated with phase transitions in materials as a function of temperature. For MP, DSC can be utilized to determine melting points, glass transition temperatures, and crystallization behaviours of different polymer types present in bottled water samples. The thermal profiles obtained can help identify specific types of MPs based on their thermal characteristics. Studies by Zainuddin & Syuhada (2020) highlight the effectiveness of DSC in determining glass transition temperatures and crystallization behaviours of MPs.

Similarly, TGA measures the mass change of a sample as it is heated, cooled, or held at a constant temperature. This method is particularly useful for assessing the thermal stability and composition of microplastics. By analysing the weight loss at various temperatures, researchers can infer the presence of different polymers and additives within the MPs, providing insights into their degradation and potential environmental impacts (Zainuddin & Syuhada, 2020).

The detection of MPs in bottled drinking water involves a combination of sophisticated methods, each providing critical insights into the presence and characteristics of these particles. From preparation and visual observation to advanced spectroscopic analyses, these techniques have laid the groundwork for understanding the scale and nature of MPs contamination.

While these methods help quantify and understand MPs contamination, it is equally important to evaluate their potential health implications. The widespread presence of MPs in consumables, such as bottled water, underscores the urgency of assessing their effects on human health. Exploring how these particles interact with the human body is vital for understanding the long-term risks associated with exposure.

2.5 Microplastics Effects to the Human Health

The discovery of MPs in bottled water has underscored their ubiquitous presence in the environment and raised pressing concerns about their potential health risks. As shown in Figure 2.2, humans are exposed to microplastics primarily through ingestion, inhalation, and dermal penetration, with ingestion being the dominant pathway. It is estimated that individuals consumed 39,000–52,000 MPs particles annually through food consumption alone. For those relying exclusively on bottled water as their primary source of hydration, Cox et al. (2019) reported that an additional 90,000 particles could be ingested per year, compared to around 4,000 particles for individuals consuming only tap water.

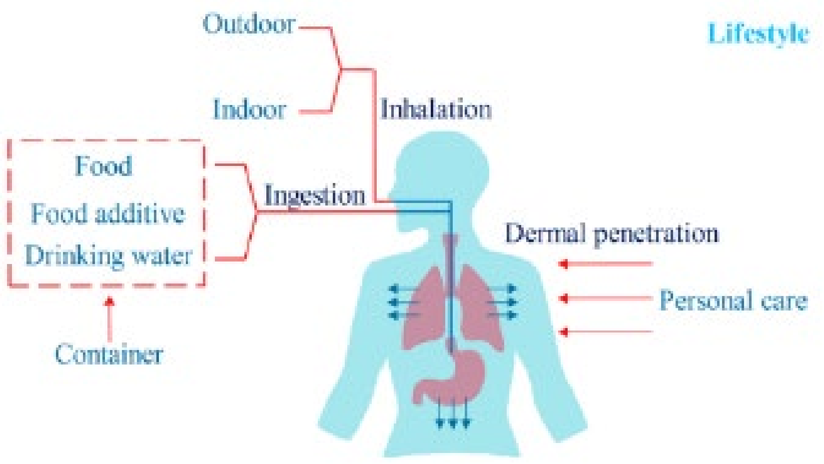


Figure 2.2: Exposure routes of microplastics to human (Cox et al., 2019)

The ingestion of MPs has been confirmed through human biological samples. Luqman et al. (2021) detected MPs in eight out of eleven stool samples collected from a fishing community, while Leslie et al. (2022) and Ragusa et al. (2021) demonstrated their presence in human blood and placental tissue. This evidence suggests that MPs can enter systemic circulation and cross biological barriers, emphasizing the need to evaluate the risks of long-term exposure.

MPs are not inert particles; they carry various additives such as plasticizers, stabilizers, and pigments, which can leach into human tissues and potentially cause harmful effects. These chemicals can distribute within lipophilic tissues and provoke inflammatory responses, cytotoxicity, oxidative stress, and even carcinogenic behaviour. Studies suggest microplastics could disrupt the gastrointestinal and immune systems, and their ability to accumulate in critical organs like the liver, spleen, and kidneys through the lymphatic and circulatory systems raises further health concerns (Cox et al., 2019; Praveena et al., 2022). According to World Health Organisation (WHO) certain polymers, including PE, PET, and PS have shown cytotoxic effects at the cellular level in the human gastrointestinal tract (WHO, 2019).

Additionally, the size, shape, and composition of MPs play a critical role in their toxicity. (Kankanige & Babel, 2020). Particles a few microns or smaller can be directly absorbed by cells in the lungs or gut, while particles up to 10 µm may be taken up by specialized cells in the Peyer's patches of the ileum. Particles as large as 130 µm can enter tissues through paracellular transport via persorption, although the rate of particle transfer to blood over 24 hours may be as low as 0.002% (Cox et al., 2019). Although MPs of various sizes have been shown to penetrate and accumulate in human organs, the exact size thresholds remain undefined, and data limitations on size classes in consumed items make it unclear how estimates of MP consumption pose risks to human health (WHO, 2019).

Despite growing evidence of the potential health risks, the long-term health effects of MP exposure remain poorly understood. Much of the current research relies on animal models or in vitro studies, leaving significant gaps in knowledge about human impacts. Further studies are essential to determine dose-response relationships, the bioaccumulation behaviour of MPs in the human body, and their chronic effects on health.

2.5 Microplastics Effects to the Environment

The persistent presence of MPs in the environment not only raises concerns about their potential impact on human health but also highlights their far-reaching consequences on ecosystems. MPs, whether originating from bottled water containers or other plastic sources, pose a significant threat to the delicate balance of natural systems. These particles are resistant to degradation and persist for long periods, exacerbating pollution levels. Once in the environment, particularly in the ocean, plastic is subjected to mechanical, physical, and biological forces. Exposure to UV rays, low temperatures, and mechanical abrasion from waves and sand causes plastic to degrade and break down into smaller pieces. As a result, secondary MPs are formed from the fragmentation of larger plastic items (Ziani et al., 2023).

One of the most concerning environmental effects of MPs is their ability to act as carriers for harmful pollutants. Their high surface area-to-volume ratio allows MPs to adsorb persistent organic pollutants (POPs) and heavy metals, which increases their toxicity (Chen et al., 2021). These contaminated MPs enter the food web when ingested by marine organisms, such as bivalves, posing risks to both biodiversity and food safety. The accumulation of these toxic substances in marine organisms raises concerns about bioaccumulation and the potential transfer of hazardous chemicals to humans through seafood consumption (Albazoni et al., 2024).

MPs also disrupt ecological processes and degrade habitats. In aquatic ecosystems, they alter the physical and chemical properties of sediments, affecting microbial communities that are critical for nutrient cycling and overall ecosystem health. Marine organisms ingesting MPs may experience physical harm, reduced growth, and behavioural changes, which can cascade through the food web, ultimately impacting marine biodiversity. Beyond these environmental consequences, MPs also infiltrate the human food chain through contaminated water and food, presenting potential public health risks (Li et al., 2023). The effects of MPs in the aquatic ecosystem are depicted in Figure 2.3.

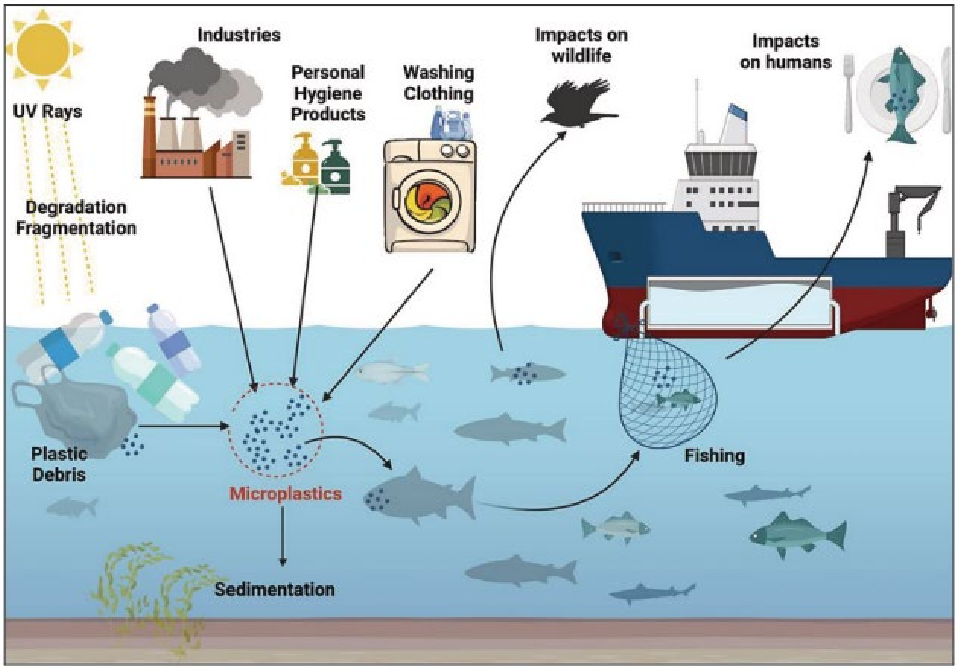


Figure 2.3: Effects of microplastics on the aquatic ecosystem (Ziani et al., 2023)

In terrestrial environments, the fragmentation of larger plastic debris, such as plastic bags, contributes to soil MP contamination. These particles accumulate through various pathways, as illustrated in Figure 2.4. One common route involves the breakdown of larger plastic debris, like discarded bottles, into MPs due to environmental factors such as sunlight, wind, and weathering. Additionally, MPs are introduced into soils through the use of contaminated products like fertilizers and irrigation systems (Albazoni et al., 2024; Ziani et al., 2023).

MPs can alter soil's physical properties, impacting its structure, porosity, and water-holding capacity. Their presence can increase soil compaction, hindering water and air infiltration, which negatively affects root growth and nutrient availability for plants. Furthermore, MPs can form aggregates that destabilize soil structure. (Albazoni et al., 2024). Soil organisms, such as earthworms, may ingest these particles, causing intestinal damage and reducing their survival rates. As earthworms play a crucial role in soil health and nutrient cycling, their impairment could have cascading effects on soil fertility and ecosystem functionality. (Ziani et al., 2023).

MPs can also reach terrestrial ecosystems through atmospheric deposition, where wind or rain carries particles from degraded plastic bottles and deposits them on land. Bees have also been implicated in spreading MPs, as they inadvertently collect particles on their bodies during foraging (Ziani et al., 2023). These particles are transported to hives, contaminating products like honey, wax, and royal jelly. Studies have found synthetic polymers such as polyester and polyethylene in honey, highlighting the role of bees in microplastic accumulation within terrestrial ecosystems (Diaz-Basantes et al., 2020; Liebezeit et al., 2015).

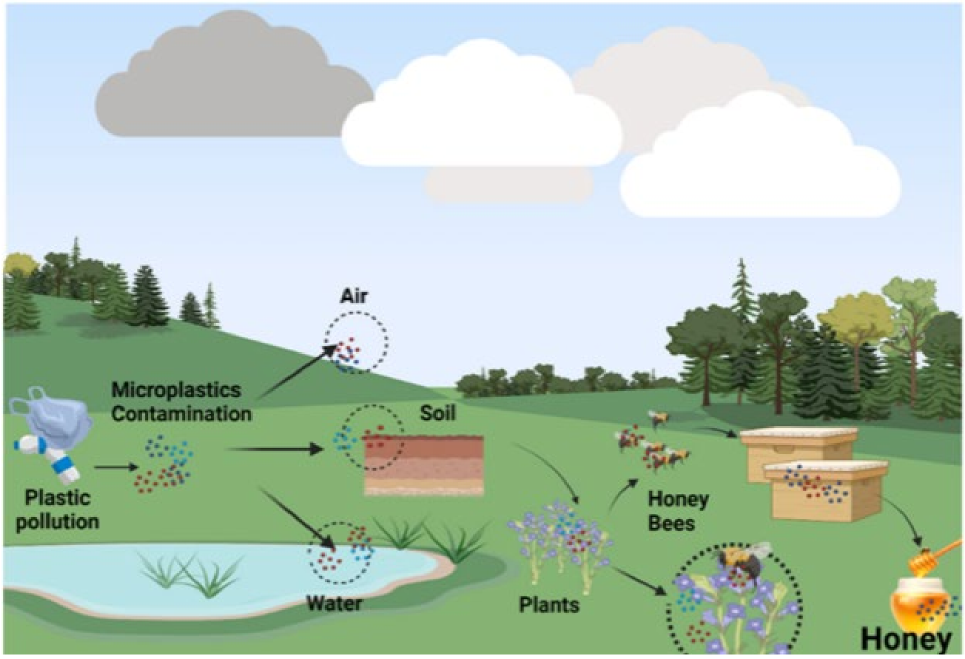


Figure 2.4: Microplastics in the terrestrial environment (Ziani et al., 2023)

MPs pose significant environmental threats by contaminating soil and marine environments. They degrade physical properties of soil, affect marine life through ingestion, and act as carriers for harmful pollutants, posing risks to human health through the food chain. Their persistence in the environment underscores the urgent need for improved waste management and regulatory measures.

While national regulations have been proposed or established to reduce MPs in the environment, they primarily address primary MPs. Based on the reviewed reports, San Francisco is the first American state to ban plastic water bottles as one of the alternatives to combat the plastic pollutions (Sharma & Chatterjee, 2017). However, the lack of regulations specifically targeting secondary MPs, which are fragments from larger plastic items, are still concerning (Karbalaei et al., 2018). This gap is significant because secondary MPs can release into bottled drinking water due to factors such as heat, physical movement and material degradation (Sekar et al., 2024). Therefore, this study will evaluate the impact of thermal stress, mechanical stress, and repeated use on the single-use drinking bottled water to understand how these factors contribute to the release of MPs into drinking bottled water.

CHAPTER 3

METHODOLOGY

The quantity of MPs with the regards to the temperature, shaking speeds and the reusability of the drinking bottled water were carried out. For the temperature, two conditions were conducted: room temperature (25 ± 2°C) and peak temperature (60 ± 2°C). For the shaking test, two shaking speeds were conducted: 30 RPM and 60 RPM. The bottled waters were left in respective conditions for 4 hours to simulate realistic conditions that bottled drinking water might experience during typical storage and transportation scenarios. To study reusability, both the temperature and two shaking tests at different speeds were repeated three times. The properties of the MP such as quantity and shape were examined under stereomicroscope.

3.1 Sample Collection

A total of 18 drinking water bottles (250 mL volume) from only one brand were purchased from Kubang Kerian, Kelantan for MP testing and the characteristic of the drinking bottled water brand were recorded. All bottles were stored at room temperature after purchased. Then, screening test was carried out all the samples.

3.2 Materials

The filtration of samples was conducted using a vacuum filtration apparatus (vacuum flask and Buchner funnel) equipped with a vacuum pump. Nylon-66 microporous membrane filter (Ø47 mm,0.45 µm pore size) was used to retain the MPs particles. The retained MPs were placed in a clean glass petri dish covered with a lid. A stereomicroscope (Motic SMZ-168 BH LED) with 5x objectives lens was used to observe the filters and enumerate the particles.

3.3 Screening Test

All the 18 drinking bottled water underwent screening test to observe for initial MP particles in the drinking bottled water. Before analysis, the exterior of each bottle was thoroughly cleaned using detergent and then rinsed with distilled water and were air-dried in a controlled, clean environment. The 250 mL water then was filtered from the bottle using filtration apparatus connected to the vacuum pump with tubing to collect for microplastic. The bottle was rinsed with the filtered water. The filter paper containing the filtered particles were transferred into a clean petri dish and covered with its lid. After that, the petri dish was dried at 60ºC in the oven until dry. Lastly, the filter paper was examined under a stereomicroscope to observe for the presence together with the morphologies of the MPs. The filtered water was transferred back to the previous rinsed bottle for the next study of temperature, shaking speed and reusability test.

3.4 Temperature Test

Six bottles were labelled, three for room temperature test (RT1, RT2, RT3) and another three for peak temperature test (PT1, PT2, PT3). The bottles assigned for room temperature were let at room temperature (25 ± 2°C) for 4 hours. Meanwhile, the other bottle assigned for peak temperature were placed in water bath at 60 ± 2°C for 4 hours.

After 4 hours, the water was filtered to collect MPs. The bottle then rinsed with the filtered water to ensure any MP residue inside the bottle were collected. The filter paper containing the filtered particles were transferred into a clean petri dish and dried at 60ºC until dry. The filter paper was examined under the stereomicroscope to observe for the morphology of the MPs.

3.5 Shaking Test

Another six bottles were labelled in this test. Three for room temperature (SRT1, SRT2, & SRT3) and another three for the peak temperature (SPT1, SPT2 & SPT3). The bottles assigned for room temperature were placed in the shaking water bath at 30 RPM for 4 hours at room temperature (25 ± 2°C). Meanwhile for peak temperature, the bottles were placed in the shaking water bath set at 60 ± 2°C for 4 hours at 30 RPM speed.

After 4 hours, the water was filtered to collect for MPs. The bottle then rinsed with the filtered water to ensure any MP residue inside the bottle were collected. The filter paper containing the filtered particles were transferred into a clean petri dish and dried at 60ºC until dry. The filter paper was examined under the stereomicroscope to observe for the morphology of the MPs.

For the shaking test at 60 RPM, the same process was repeated with the only differences being the shaking speed increased to 60 RPM. The labelled for this test: room temperature (SRT4, SRT5, & SRT6) and peak temperature (SPT4, SPT5 & SPT6)

3.6 Reusability Test

Reusability test was conducted after the completion of all samples from Sections 3.4 and 3.5. The samples from these sections underwent the same process as mentioned in Sections 3.4 and 3.5 for three times, demonstrating that the bottled waters were reused three times.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Sample Collection

The characteristics of the single-use drinking bottled water were recorded in Table 4.1.

Table 4.1: Characteristics of the drinking bottled water

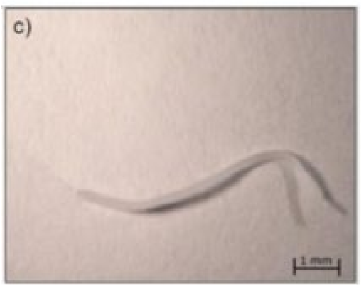
Picture	Characteristic	Observation
	Volume	250 mL
	Water source	Treated tap water supply (Reverse Osmosis Process)
	Bottle packaging type	PET
	Cap color	White

4.2 Screening Test

All the 18 drinking bottled water underwent screening test to observe the presence of initial MPs particles in the bottle. The exterior of each bottle was thoroughly cleaned using detergent and rinsed with distilled water to prevent from any potential external contaminants and then air-dried in a controlled, clean environment. The 250 mL water was filtered using 0.45 µm microporous membrane filter to collect for MPs particles in bottled water. Microplastics are generally defined as plastic particles smaller than 5 mm, with some studies focusing on MPs down to the micro meter scale. A 0.45 µm filter allows the capture of very fine particles, including those in the lower micro meter range. Moreover, 0.45 µm filter was chosen to align with established methods in microplastic research in drinking bottled, ensuring consistency and comparability of results with other studies (Kankanige & Babel, 2020; Praveena et al., 2022; Wong et al., 2021).

Using the filtered water from the plastic bottle, the bottle was rinsed to ensure any MP residue inside the bottle was collected. The filter paper containing the filtered particles were transferred into a clean petri dish and closed with its lid to avoid any airborne contamination. After that, the petri dish was dried at 60ºC in the oven until completely dry. The filter paper was examined under a stereomicroscope with 5x objectives lens to observe for the morphologies of microplastics. Stereomicroscope was chosen in this study because it has a larger working distance compared to another microscope (Li et al., 2022) which is particularly suitable for counting large quantities of microplastics. Given the focus on quantifying the microplastics for each assessment, the stereomicroscope's capability to handle and analyse bulk samples made it an ideal choice for this study. The morphology of the MPs was characterised and compared with the previous studies (Muhammad Husin et al., 2021; Rosal, 2021; Singh et al., 2021). The data were presented in Table 4.2.

Table 4.2: Morphologies of MPs observed from the screening test compared with previous studies

Shape	References	Current study
Fiber	 Very thin, straight or fibrous plastic particles (Singh et al., 2021)