DETECTION OF MICROPLASTICS IN COMMERCIAL BOTTLED MINERAL WATER

NURANNISAH BINTI OLONG

UNIVERSITI SAINS MALAYSIA

2025

DETECTION OF MICROPLASTICS IN COMMERCIAL BOTTLED MINERAL WATER

by

NURANNISAH BINTI OLONG

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

February 2025

CERTIFICATE

This is to certify that the dissertation entitled “Detection of Microplastics in Commercial Bottled Mineral Water” is the bona fide record of research work done by Ms. Nurannisah binti Olong during the period from October 2024 to January 2025 under my supervision. I have read this dissertation and that in my opinion it conforms to acceptable standards if scholarly presentation and is dully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Science in Forensic Science

Supervisor,

________ ________

Dr. Noor Zuhartini Md Muslim

Lecturer

School of Health Sciences

Universiti Sains Malaysia

Health Campus

16150 Kubang Kerian

Kelantan, Malaysia

Date: 24 February 2025

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 purposes.

____   ____

(Nurannisah binti Olong)

Date: 24 February 2025

ACKNOWLEDGEMENT

Alhamdulillah, I am deeply grateful to Allah SWT for granting me opportunity to embark on this meaningful journey for the first time in my life. I am also truly moved by the unwavering support of those who have stood by me throughout this process. Their guidance and assistance have been invaluable, and without them, completing this masterpiece would not have been possible. First and foremost, I would like to extent my deepest gratitude to my supervisor, Dr. Noor Zuhartini Md Muslim, for her invaluable guidance and continuous support throughout this study. Her profound knowledge, insightful advice, and constant encouragement have played a crucial role in developing and enhancing the quality of this study. I am sincerely thankful for her patience, dedication and the time she has devoted to helping me refine my concepts and achieve my objectives. I am truly grateful to all academic and administrative at Universiti Sains Malaysia Health Campus for providing the necessary resources and environment conducive to my academic growth. Their professionalism has made my learning experience truly meaningful. I would like to acknowledge and express my gratitude to of Mr. Baharuddin Zakaria, Ms. Hasnita Zulkifli, and Ms. Syazwani Che Hussin for their technical support during the laboratory analyses. Their assistance was essential in ensuring that the experiments were conducted smoothly and efficiently. I am immensely gratitude to my family and friends for their continuous support throughout this entire process until this masterpiece was completed. I am especially thankful to my mother, Anni Angkayau, whose unwavering love, understanding, and encouragement have been my greatest source of strength during this journey. I would also like to express my greatest appreciation to my friend, Farah Mohd Noor, who was conducting her study under the same supervisor. Her intellectual support throughout

this challenging journey, along with the experiences we have shared, has greatly enriched this journey and added significant meaning to it. Once again, I would like to convey my heartfelt gratitude to myself for the resilience I have shown and for the patience I have had in overcoming setbacks. I admire my own determination, my ability to remain focused, and my continuous willingness to kept learning and growing. As a result, I am proud as I have proven to myself that hard work and perseverance can lead to great accomplishment. This thesis stands as evidence of my commitment, and I take pride in the person I have grown into this journey.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

RT	Room temperature
PT	Peak temperature
SRT	Shaking room temperature
SPT	Shaking peak temperature
RPM	Revolutions per minute

PENGESANAN MIKROPLASTIK DALAM AIR MINERAL BOTOL KOMERSIAL

ABSTRAK

Mikroplastik bersaiz antara 1 µm hingga 5 mm adalah bahan pencemar dalam botol air mineral, mencetuskan kebimbangan terhadap kesihatan. Kajian ini bertujuan untuk mengesan kehadiran mikroplastik dalam air mineral botol komersial. Sebanyak 18 botol air mineral tempatan telah digunakan sebagai sampel dalam kajian ini. Enam sampel dianalisis melalui ujian suhu, dengan tiga sampel didedahkan kepada suhu bilik (25°C ± 2°C) dan tiga lagi didedahkan kepada suhu puncak (60°C ± 2°C). Seterusnya, 12 sampel lagi sedang menjalani ujian goncangan. Dalam ujian goncangan, sampel didedahkan kepada dua suhu berbeza (suhu bilik dan puncak) dan kelajuan (30 dan 60 RPM). Setiap tiga sampel didedahkan kepada suhu bilik pada 30 RPM, suhu puncak pada 30 RPM, suhu bilik 60 RPM, dan suhu puncak pada 60 RPM. Selepas ujian suhu dan goncangan selesai, sampel menjalani ujian kebolehgunaan semula di mana sampel-sampel itu menjalani ujian masing-masing untuk tiga kali penggunaan berulang. Untuk melihat kehadiran mikroplastik, sampel ditapis melalui penapis membran nilon-66 bersaiz liang 0.45 mikron. Mikroplastik akan diperhatikan di bawah stereomikroskop untuk menentukan kuantiti, bentuk, dan warna mikroplastik. Keputusan menunjukkan bahawa sampel yang terdedah kepada tegasan haba yang tinggi (60°C ± 2°C) dan tekanan mekanikal (30 dan 60 RPM) membebaskan lebih banyak mikroplastik. Dari segi bentuk mikroplastik, serpihan, gentian, filamen, dan filem dikesan dalam sampel, dengan didominasikan oleh serpihan mikroplastik. Warna mikroplastik yang dikesan dalam sampel adalah jernih, hijau, dan kelabu gelap, dengan warna jernih yang paling dominan. Penggunaan botol berulang kali tidak

menyebabkan peningkatan kepada pembebasan mikroplastik. Sebaliknya, bilangan mikroplastik yang dikesan berbeza-beza secara tidak konsisten dalam setiap botol. Kesimpulannya, kajian ini dapat meningkatkan kesedaran dalam kalangan pengguna untuk mengambil langkah berjaga-jaga bagi memgurangkan potensi pencemaran mikroplastik dalam air mineral botol mereka.

DETECTION OF MICROPLASTICS IN COMMERCIAL BOTTLED MINERAL WATER

ABSTRACT

Microplastic ranging in size from 1 μm to 5 mm are contaminants in bottled mineral water, raised significant health concerns. This study aims to detect the presence of microplastics in commercially bottled mineral water. A total of 18 local bottled mineral water were used as sample in this study. Six samples were analysed using temperature test, in which the three samples were exposed to room temperature (25°C ± 2°C) and another three were exposed to peak temperature (60°C ± 2°C). Another 12 samples were undergoing shaking test. In shaking test, the samples were exposed to two different temperatures (room and peak temperature) and speeds (30 and 60 RPM). Each three samples were exposed to room temperature at 30 RPM, peak temperature at 30 RPM, room temperature 60 RPM, and peak temperature at 60 RPM. After temperature and shaking test were completed, the samples underwent reusability test where they underwent their respective tests for three repeated uses. To observe the presence of microplastic particles, the samples were filtered through nylon-66 membrane filter 0.45-micron pore size. The microplastics were observed under a stereomicroscope to determine the quantity, shape, and colour of microplastic particles. The results highlighted that samples that were exposed to high thermal stress (60°C ± 2°C) and mechanical stress (30 and 60 RPM) leached more microplastics. In terms of the microplastic shape, fragment, fibre, filament, and film were presented in the samples and predominantly by fragment microplastics. The colour of microplastics detected in the samples were transparent, green, and dark grey with transparent colour predominantly. Repeated use of bottles did not lead to an increase in microplastic

leaching. Instead, the number of microplastic particles detected varied inconsistently in each bottle. To conclude, this study may raise awareness among consumers to take necessary precautions to minimise the potential microplastics contamination in their bottled mineral water.

CHAPTER 1

INTRODUCTION

1.1 Study Background

The increasing popularity of commercial bottled water has driven the growth of the bottled water industry, particularly in Malaysia, where it is categorized into two main types which Natural Mineral Water and Drinking Water (Ng, 2015). According to the U.S. Food and Drug Administration (FDA), mineral water differs from drinking water in which it is regulated to preserve its original physicochemical properties, often contains naturally occurring compounds, making it a preferred choice for health-conscious consumers. However, concerns have emerged regarding microplastic contamination in bottled mineral water, raising questions about its safety.

Microplastics are tiny plastic particles, 1 μm to 5 mm in size, resulting from the degradation or leaching of plastics (Ziani et al., 2023). They are classified by origin as primary (intentionally produced) or secondary (formed from larger plastic debris) and by shape into categories such as fragments, fibres, films, filament, foam, and beads (Browne et al., 2010). Factors such as high temperatures (Li and Tang, 2022), agitation during transportation (Kour et al., 2023), and repeated use of plastic bottles contribute to the leaching of microplastics into the water (Muhib et al., 2023).

Moreover, there are potential health risks of consuming microplastics include digestive issues, endocrine disruption, respiratory problems, and immune system compromise (Lee et al., 2023). As evidence of these risks grows, the detection and analysis of microplastics in bottled mineral water have become critical areas of study to address public health concerns and ensure consumer safety.

1.2 Problem Statement

The widespread use of commercial bottled mineral water has raised concerns about the potential leaching of microplastics from plastic packaging. Factors such as temperature, agitation during transportation, and repeated use of the bottles may influence the rate at which microplastics are released into the water. This study aims to investigate whether these factors significantly influence the leaching process. Hence, it is crucial to detect and identify the physical properties of microplastics in commercial bottled mineral water to ensure its safety for consumption.

1.3 Objective

The general objective of this study is to detect the presence of microplastic in commercial bottled mineral water.

Specific objectives:

1. To investigate whether temperatures influence the leaching of microplastic from commercial bottled mineral water.
2. To investigate whether shaking motion applied on the commercial bottled mineral water affect the leaching of microplastic.
3. To investigate the effect of different shaking speed on the leaching rate of microplastic from commercial bottled mineral water.
4. To determine the colour, shape, and quantity of microplastic present in commercial bottled mineral water.
5. To investigate whether repeated use of the bottle of commercial bottled mineral water increases the release of microplastic over time.

1.4 Significance of the Study

Commercial bottled mineral water has emerged as a particular focus because of its global consumption, making it one of the primary avenues through which microplastics enter the human body. Microplastics give a negative effect to human body system. The potential release of microplastics from bottled mineral packaging may come from the exposure to high temperature, mechanical stress, and repeated use of the bottles. By addressing this issue, the study may raise awareness among consumers to store and handle the bottled mineral water appropriately.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Microplastics

Microplastics is defined as small plastic particles that leach from the degraded of plastic materials, have become a significant environmental pollutant (Shruti et al., 2022). These synthetic materials are composed of high polymer content, are solid particles with the size of 1 μm to 5 mm, insoluble in water, and non-degradable, though they break down into smaller fragments over time (Dobaradaran et al., 2018). Microplastics are categorised into two main types based on their origin; primary microplastics which intentionally produced as microparticles, and secondary microplastics, which are formed through the fragmentation of larger plastic debris that more than 5 mm (Browne et al., 2010).

Microplastics can also be classified according to their shape, with the most common categories being fragments, fibres, films, foam, and beads (Kooi and Koelmans, 2019). Fragments have irregular shapes and result from the breakdown of larger plastic items, a form of secondary microplastic. Fibres are cylindrical in shape and typically originate from synthetic textiles. Films are thin, sheet-like particles derived from the breakdown of plastic bags, packaging materials, and other flexible plastics. Foam microplastics come from the degradation of foam products, such as Styrofoam, while beads are spherical and are often found in personal care products like exfoliating scrubs and cosmetics. As the colour of microplastics, it is typically determined by the colour of the plastic material from which they originate. The presence of microplastics can be found in commercial bottled water.

2.2 Commercial Bottled Water

Bottled water is categorized into two main types which are Natural Mineral Water and Drinking Water (Ng, 2015). According to the U.S. Food and Drug Administration (FDA), mineral water differs from drinking water in that it contains a minimum of 250 parts per million (PPM) of total dissolved solids, originating from a geologically and physically protected underground water source.

To be labelled as ‘mineral water,’ no substances may be added, ensuring the preservation of its original physicochemical properties. Given its underground origin, the water must be confirmed to be microbiologically safe for consumption without the need for further treatment. Additionally, mineral water may contain elevated levels of minerals and other naturally occurring compounds, such as calcium, magnesium, sodium, bicarbonate, sulphate, chloride, and fluoride (Quattrini et al., 2017).

A study published in the UTAR Agriculture Science Journal (2015) by Casey Ng, consumers of bottled water are typically well-educated, middle-class adults who demonstrate a high level of awareness regarding health and wellness, particularly in matters related to food and drink. This preference is largely driven by public concerns over the quality of tap water, with bottled water being perceived as a safer and healthier option due to its content of essential minerals (Williams, 2024). However, despite these perceived benefits, growing concerns have emerged regarding the contamination of bottled mineral water by microplastics as the release of microplastics poses potential health risks when consumed. Oβmann et al. (2018) and Zhou et al. (2021) reported the presence of microplastics in bottled water, suggesting that these particles could originate from packaging materials, bottle caps, and the bottling process itself.

2.3 Microplastics Health Risks

Microplastics, ranging in size from 1 μm to 5 mm, can enter the human body through various pathways, including inhalation via the respiratory system, ingestion through contaminated food and water, or dermal penetration from contact with cosmetics and clothing (Winiarska et al., 2024). Inhalation of microplastics may result in oxidative stress in the airways and lungs, leading to respiratory symptoms such as coughing, sneezing, and shortness of breath due to inflammation and tissue damage. Additionally, it can cause fatigue and dizziness, potentially linked to reduced blood oxygen levels (Lee et al., 2023). Moreover, the gastrointestinal tract is believed to be a primary entry point for microplastics into the human body, primarily through the ingestion of contaminated foods and beverages such as seafood, milk, beer, honey, sugar, salt, and bottled water (Kwon et al., 2020; Kutralam-Muniasamy et al., 2020; Liebezeit and Liebezeit, 2013, 2014, 2015; Yang et al., 2015; Mason et al., 2018). Once ingested, microplastics can cause inflammation in the gastrointestinal tract due to physical irritation (Lee et al., 2023). This irritation arises from microplastics altering the intestinal microbiome, disrupting the balance between beneficial and harmful bacteria, which can lead to symptoms such as abdominal pain, bloating, and irregular bowel movements (Bouwmeester et al., 2015). Additionally, microplastics may contribute to chemical toxicity by adsorbing and accumulating environmental pollutants, including heavy metals and polycyclic aromatic hydrocarbons. When these toxins are introduced into the body, they can trigger symptoms such as nausea, vomiting, and abdominal discomfort (Abbasi et al., 2021).

Microplastics have been also linked to endocrine disruption, potentially causing a range of endocrine disorders, including metabolic, developmental, and reproductive issues, by interfering with hormone production, release, transport, metabolism, and elimination (Lee et al., 2023). Studies by Kutralam-Muniasamy et al. (2023) and Zhao et al. (2023) have identified microplastics of various colors and sizes (≥800 nm to 5 mm) in several human biological samples, including the lungs, breast milk, liver, spleen, placenta, blood, sputum, colon, saliva, feces, urine, testes, and semen. However, no microplastics were detected in the kidneys or lungs of stillborn infants. Prolonged exposure to microplastics has been shown to induce chronic inflammation and disrupt homeostasis in animal experiments (Lee et al., 2023). Besides, research by Détrée et al. (2018) indicates that microplastics can activate innate immunity in human lung cells by influencing the expression of genes and proteins involved in the immune response. However, the potential health risks are depending on the level of exposure and intake.

2.4 Estimated Daily Intake (EDI) of Microplastics

A study on microplastics in Malaysian bottled water by Praveena et al. (2022) estimated the Estimated Daily Intake (EDI) based on low (8 particles/L) and high (22 particles/L) microplastic particle concentrations in bottled water. For adults, the EDI ranged between 0.068 and 0.19 particles/kg/day, while for children, it ranged from 0.089 to 0.25 particles/kg/day. These calculations were based on the ingestion rate provided by Vieux et al. (2020). In contrast, EDI estimations by Makhdoumi et al. (2021) were significantly lower, at 0.015 particles/kg/day for adults and 0.065 particles/kg/day for children. The variation in EDI estimations may be attributed to differences in the number of microplastic particles leaching into the water. Therefore, identifying and addressing factors contributing to microplastic leaching is crucial for minimizing their presence in bottled water.

2.5 Factors Influencing the Presence of Microplastics

Several factors contribute to the leaching of microplastics into bottled water, ranging from production processes to environmental conditions. One of the factors is related to the production and packaging of bottled water. During the bottling process, packaging materials may release microplastic particles (Hossain et al., 2023). The microplastics generated in this process are classified as primary microplastics, as they are intentionally created in small sizes for use in production. These particles can directly enter the water during the packaging process, contributing to the overall contamination.

Environmental and physical stress during transportation and storage can also lead to the leaching of microplastics. For example, physical stress during transportation where the shaking and bumping of bottles while being moved or stored, can cause the plastic to degrade. This degradation process results in the release of microplastic particles into the water (An et al., 2024). The storage conditions of bottled water are also crucial in determining the extent of microplastic leaching. Exposure to high temperatures during storage, particularly when bottles are stored in warm environments or direct sunlight and moisture, can accelerate the degradation of plastics (Li et al., 2023; Mejjad et al., 2023).

Another factor that contributes to microplastic release is the repeated use of bottles (Amir, 2023). Each time a bottle is reused, the plastic material undergoes further wear and tear, causing the plastic to degrade over time. In particular, the frequent opening and closing of bottles can cause friction between the cap and bottle, generating even more microplastic particles. According to Hossain et al. (2023), this constant friction can cause microplastics to be released more frequently, further contributing to the contamination of the water with microplastics.

Microplastics may not only come from the bottle itself but also from the water source (Hossain et al., 2023). Bottled mineral water is typically sourced from underground water, which may already be contaminated with microplastics. Furthermore, the plastic equipment used in the water purification and distribution processes can also contribute to microplastic contamination. As these plastic materials age or degrade over time, they may release microplastic particles into the water. This can be especially true if the purification and distribution systems are not regularly maintained or replaced, leading to the high deterioration of plastic components and the eventual release of microplastics into the water (Mejjad et al., 2023).

2.6 Analysis of Microplastics

A study conducted by Praveena et al. (2022) analysed eight major bottled water Malaysia brands for microplastics. Each bottled water brand was swirl by shaking the bottle, and 500 mL of water was taken for testing. The water was filtered three times through a 0.45 μm filter paper using vacuum filtration to collect microplastics. The filter papers were then dried at 60°C until they reached a constant weight. Blank samples, using deionized water, were also tested in the same way for comparison. After that, the microplastics captured on the filter paper were photographed using Nikon Eclipse E200LED MV RS microscope with a magnification of 40 and a BestScope International Limited camera. The results showed that microplastic concentrations ranged from 8 to 22 particles/L, with an average of 11.7 ± 4.6 particles/L. The dominant particle sizes were between 100 and 300 μm, and fragments with transparent colour were the most prevalent. The polymers identified, such as polyethylene terephthalate (PET) and polypropylene (PP), were consistent with prior findings that microplastics in bottled water are mainly derived from packaging materials and bottle caps (Praveena et al., 2022).

Weisser et al. (2021) examined four mineral water bottling facilities in Germany to investigate the sources of microplastic contamination in glass bottled mineral water but the capping was made from plastic. They analysed the entire bottling process, from raw water extraction to the final bottled product. The research involved standardized sampling and analysis techniques to monitor microplastic contamination. The study focused on critical stages of the bottling process, including groundwater extraction, water treatment (deferrization), bottle cleaning, filling, and capping. Raw water was sampled directly from the well, and after undergoing deferrization and filtering, further water samples were collected to assess changes in microplastic concentrations during treatment. To evaluate the effect of bottle cleaning, returnable glass bottles were sampled after leaving the bottle washer. This cleaning process involved exposure to caustic soda and water jets to ensure cleanliness. After the filling process, additional water samples were collected before and after capping to determine whether the capping process introduced microplastic into the water. They also collected samples from the caustic cleaning solutions used to clean the returnable bottles, to assess whether they were a potential source of microplastic contamination. After that, the samples were analysed using advanced imaging and spectroscopy techniques, primarily Fourier-transform infrared (FTIR) imaging. This method allowed for the identification and quantification of synthetic and natural polymer particles in each sample. The results were then extrapolated to determine microplastic concentrations per litre of water and to identify the predominant polymer types at each stage of the bottling process. One of the first findings was that raw groundwater, even before any industrial processing, contained detectable levels of microplastic. On average, the raw water samples contained 97 microplastic per cubic meter (m^3), suggesting that environmental pollution had already introduced microplastic into the natural water sources. However, the study showed that water treatment processes, such as deferrization (the removal of iron), were somewhat effective in reducing microplastic concentrations. After treatment, microplastic levels dropped slightly, though trace amounts of microplastic remained in the water, indicating that filtration and deferrization systems are not completely capable of eliminating microplastics. The study found that the freshwater jetting step after cleaning effectively removed microplastic, resulting in concentrations below detection limits in the bottles. The most critical finding of the study was the sharp increase in microplastic contamination after the bottles were capped. The majority (81%) of microplastics resembled the polyethylene (PE), indicating abrasion from the sealings as the main entry path for microplastics into bottled mineral water.

Wong et al. (2021) aimed to investigate the presence of microplastics in 40 samples from three different local bottled water brands in Malaysia. The study followed two main experimental procedures. A library database was created as a reference for identifying microplastic particles. The control samples included four types of microplastics such as fibre, fragment, film, and bead, which were produced by cutting a synthetic towel, plastic bag, and facial product into small pieces, and grinding a plastic bottle into smaller fragments. These particles were then mixed with distilled water and prepared for analysis. For the filtration process, a vacuum system and a microporous membrane filter (Ø47 mm, 0.45 μm pore size) were used. The filtered samples were examined with a digital microscope (Nikon Eclipse LV150N) using a 5x objective lens. The microscope, equipped with Nikon NIS-Elements D Imaging software, enabled digital imaging of microplastic particles ranging from 1 to 300 μm, with particles smaller than 5 μm excluded from the study. The study highlighted that 66.7% of detected microplastics were smaller than 20 μm, which raises concerns about their bioavailability and potential health effects. Smaller particles are more likely to penetrate biological membranes and enter systemic circulation, which could lead to adverse health outcomes as suggested by various toxicological studies. The characteristics of microplastics such as size, shape, and density can influence their behavior in aquatic environments and their removal efficiency during water treatment processes.

The study by Oßmann et al. (2018) analysed 32 samples from 21 different brands of mineral water purchased in Bavarian food stores. The samples were packaged in various types of bottles which are 12 reusable PET, 10 single-use PET, and 10 glass bottles. To treat the samples, EDTA was added to dissolve calcium and magnesium carbonate particles, followed by the addition of sodium dodecyl sulphate (SDS) to help suspend plastic particles. The samples were then filtered through a polycarbonate membrane filter. Micro-Raman spectroscopy was used to identify and quantify the particles on the filter surface. A microscope captured images of specific areas on the filter, and the Particle Finder Module of the software was used to identify the particles. Spectra were recorded for the particles and compared to standard references of different synthetic polymers. Classical least squares (CLS) analysis helped determine the polymer types, and any unclear spectra were remeasured manually. The particles were categorized into size groups based on their measurements. The analysis combined automated and manual processes to ensure accurate identification and optimize analysis time. The results revealed that microplastics were present in all types of bottled water, reusable glass bottles with plastic capping showing the highest contamination levels averaging 6292 ± 10521 particles per litre. Reusable PET bottles contained 4889 ± 5432 particles per litre and single-use PET bottles had the lowest levels, with 2649 ± 2857 particles per liter. The possible contamination was from the bottle cap. The size distribution showed that over 90% of detected microplastics were smaller than 5 micrometers, with many below 1.5 micrometers. High concentrations of pigmented microplastic particles were found in bottles with paper labels, particularly in reusable bottles. Pigment analysis indicated that contamination likely came from label pigments entering the washing process during bottle cleaning.

CHAPTER 3

METHODOLOGY

3.1 Materials

The samples used for this study were 18 bottles of bottled mineral water. For the materials, this study used a vacuum pump, a set of filter funnel, Leica MZ16 stereomicroscope, Motic dissecting microscope, 90 sets of petri dish with lid, a glass filter funnel, an oven, a water bath with shaker, and 90 pieces of nylon-66 membrane filter 0.45-micron pore size.

3.2 Sample Collection and Preparation

A total of 18 samples of 250 mL bottled mineral water were purchased from local market store. These samples were brought to the laboratory for microplastic detection. Then, the characteristics of the bottled mineral water were observed and recorded. The procedure of the sample preparation was carried out following the methodology by Oßmann et al. (2018). First, the labels on all samples were removed. Then, the outside of the bottles was washed using detergent. After that, the bottles were rinsed using distilled water and were dried using cotton tissue.

3.3 Screening Test

A set of filter funnel, petri dishes, and a glass filter funnel were rinsed with distilled water before use. A screening test was performed to detect the presence of any microplastics prior to the experiment. A sample of mineral water was poured into the filter funnel assembly, where the water was passed through the nylon membrane filter 0.45-micron pore size and the microplastics were captured. The bottle was rinsed with the filtrated mineral water, and the rinse was poured into the same filter funnel, passing through the nylon membrane filter 0.45-micron pore size and collected in the same filter funnel set to capture any residual microplastic. Then, the membrane filter was dried in an oven with 60°C until it reached constant weight. After that, the microplastics presented were analysed under a stereomicroscope and the results were recorded. The procedure was repeated until all 18 sample bottles had undergone the screening test.

3.4 Analysis of Microplastics

To analyse the factors that contribute to microplastic leaching from bottled mineral water, three key parameters had been implemented which were the temperature test, the shaking test, and the reusability test. The temperature test determined how varying temperatures influence the leaching of microplastics, while the shaking test was to assess whether movement affects microplastic release. The reusability test investigated whether repeated use of the same bottle impacts the amount of microplastic leaching over time.

3.4.1 Temperature Test

Three samples were labelled with room temperature 1 (RT1), room temperature 2 (RT2), and room temperature 3 (RT3). The samples for room temperature were maintained at 25°C ± 2°C. Conversely, the samples labelled peak temperature 1 (PT1), peak temperature 2 (PT2), and peak temperature 3 (PT3) were placed in a water bath and subjected to a peak temperature of 60°C ± 2°C. The samples remained static at their respective temperatures for 4 hours. After the 4-hour duration, the presence microplastic particles were determined using the same method as in section 3.3.

3.4.2 Shaking Test

Two different speeds were applied to the samples labelled as 30 and 60 RPM (revolutions per minute) in which the shaking influences was investigated about the leaching of microplastics from bottled mineral water.

For the shaking speed of 30RPM, three samples were labelled with shaking room temperature 1 (SRT1), shaking room temperature 2 (SRT2), and shaking room temperature 3 (SRT3). Another three samples were labelled as shaking peak temperature 1 (SPT1), shaking peak temperature 2 (SPT2), and shaking peak temperature 3 (SPT3). All six samples were placed in water bath at their respective temperature and were shaken at 30RPM for 4 hours. After the 4-hour period, the same procedure as outlined in Section 3.3 was performed.

As for the 60RPM shaking speed, three samples were labelled with shaking room temperature 4 (SRT4), shaking room temperature 5 (SRT5), and shaking room temperature 6 (RT6). Another three samples were labelled as shaking peak temperature 4 (SPT4), shaking peak temperature 5 (SPT5), and shaking peak temperature 6 (SPT6). All six samples were placed in water bath at their respective temperature and were shaken at 60RPM for 4 hours. After the 4-hour period, the same procedure as outlined in Section 3.3 was performed.

3.4.3 Reusability Test

This test was conducted after all samples from temperature test and shaking test were completed. The same samples from temperature test (section 3.4.1) and shaking test (3.4.2) underwent repeated use for three times and the presence of microplastic particles were determined.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Sample Preparation

The samples used in this study were bottled mineral water purchased from a local market. The characteristics observed from the bottled mineral water revealed that the bottle caps were made of polypropylene (PP), while the bottle packaging was composed of polyethylene terephthalate (PET). The caps were green, and the bottles were transparent. Additionally, the water was sourced from underground water in Kuantan, Pahang.

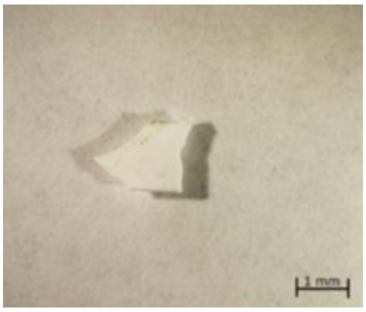
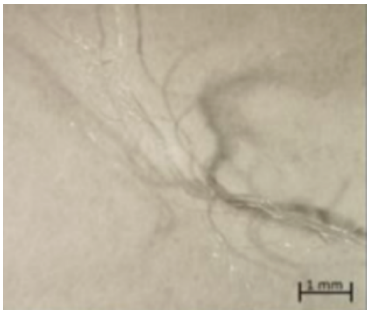
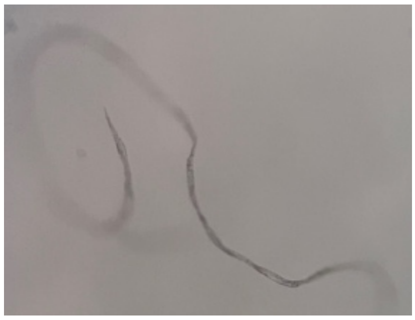
Prior to use, the outside of the bottles was washed with detergent to prevent potential contamination that could interfere with the screening results (Oßmann et al., 2018). By washing the bottles, any external contaminants were removed, ensuring they did not affect the accuracy of the results.

4.2 Screening Test

In the screening test method, nylon-66 membrane filters with a pore size of 0.45 μm and a diameter of 47 mm were used to filter microplastics presented in the mineral water. The membrane filter of 0.45 μm pore size was selected since the size of microplastic particles is in range of 1 μm to 5 mm (Dobaradaran et al., 2018). Once captured, the membrane filters were dried in an oven at 60°C until the membrane filters reached constant weight. This procedure was followed from the previous study by Praveena et al. (2022). Then, the dried membrane filters were examined using an MZ16 stereomicroscope.

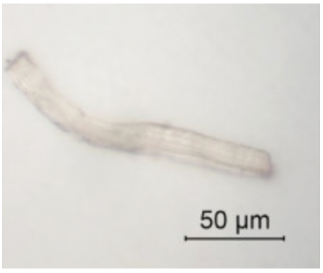
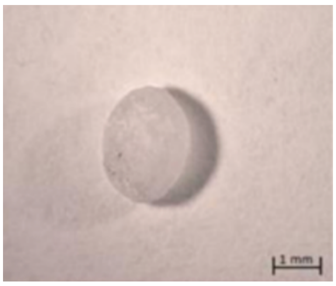
Photographs of the microplastics were taken using a smartphone camera through the 8.0 magnification of stereomicroscope lens. The shape of the microplastics observed in the samples were categorised by referring to the reference shape and the current study of microplastics as shown in Table 4.1.

Table 4.1 The reference shape of microplastics and the current study of microplastics.

Shape	Reference Shape of Microplastics	Current Study of Microplastics
Fragment	 (Marrone et al., 2021) Fragments are hard and jagged plastic particles (Singh et al., 2022)	
Fibre	 (Marrone et al., 2021) Fibres are very thin or straight or fibrous plastic particles (Singh et al., 2022)	

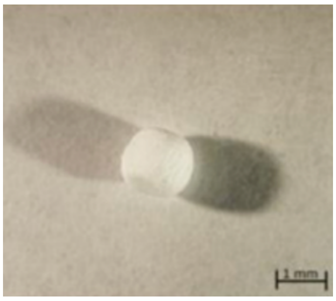
To be continued…

… continued

Shape	Reference Shape of Microplastics	Current Study of Microplastics
Film	 (Marrone et al., 2021) Films are thin plane of flimsy plastic particles (Singh et al., 2022)	
Filament	 (Sun et l., 2022) Filaments are hard and cylindrical shape plastic particles (Singh et al., 2022)	No microplastics observed for this shape
Pellet	 (Marrone et al., 2021) Pellets are sphere plastic particles (Singh et al., 2022)	No microplastics observed for this shape

To be continued…

…continued

Shape	Reference Shape of Microplastics	Current Study of Microplastics
Foam	 (Marrone et al., 2021) Foams are lightweight and sponge-like plastic particles (Singh et al., 2022)	No microplastics observed for this shape

Based on the theoretical shape of microplastics, the shape of microplastics observed in this study are comparable. The table includes the best images of microplastics captured. Figure 4.1 shows the total number of microplastic shapes in all samples.

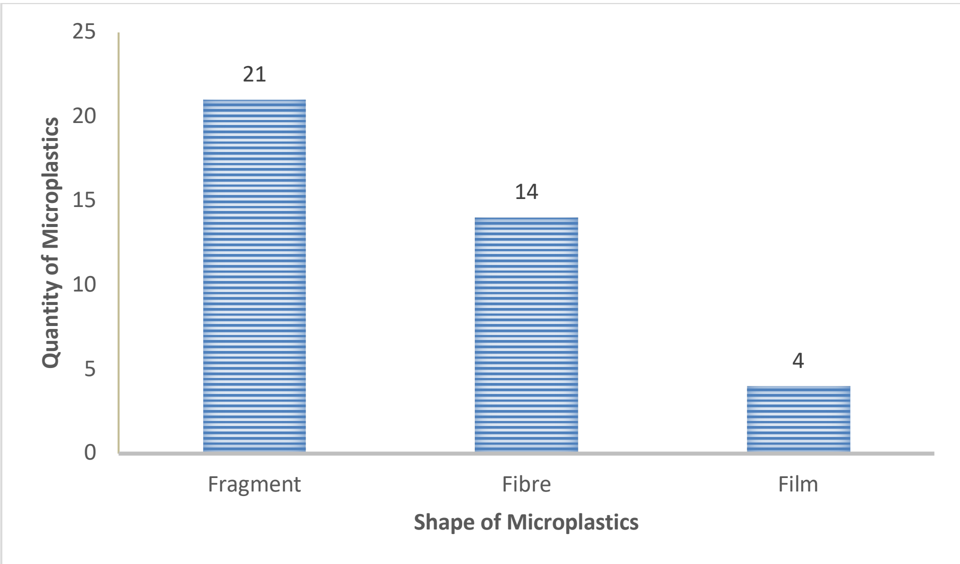


Figure 4.1 The total quantity of microplastics observed in 18 samples based on their shapes (n=3)

The screening test conducted on all samples revealed a total of 39 microplastic particles. Figure 4.1 presented that only three shapes of microplastics were observed which namely are fragments (21 particles), fibres (14 particles), and films (4 particles). As for the colour of the microplastics, they are varied. The colour of microplastic presented were transparent, dark grey, and green.

The colour variations indicated that transparent microplastics are likely a result of leaching from the bottles themselves, while green microplastics may originate from the leaching of bottle caps. In contrast, the dark grey colour microplastics may stem from degradation or contamination during the manufacturing process (Weisser et al., 2021). After the screening test, temperature test was conducted on room temperature (RT) and peak temperature (PT) samples.

4.3 Temperature Test

A temperature test was conducted on the samples, where they were exposed to two different conditions which are 25°C ± 2°C, representing the room temperature (RT), and 60°C ± 2°C, representing the peak temperature (PT). The purpose of this test was to investigate whether thermal stress influences the leaching of microplastics from bottled mineral water. The minimum temperature corresponds to room temperature, while the peak temperature was determined following the temperature in the study conducted by Bach et al. (2013). The samples were left for four hours at their respective temperature before filtration to mimic real-life conditions, typically the hottest time of the day. Figure 4.2 shows the quantity of microplastics observed in RT and PT samples.

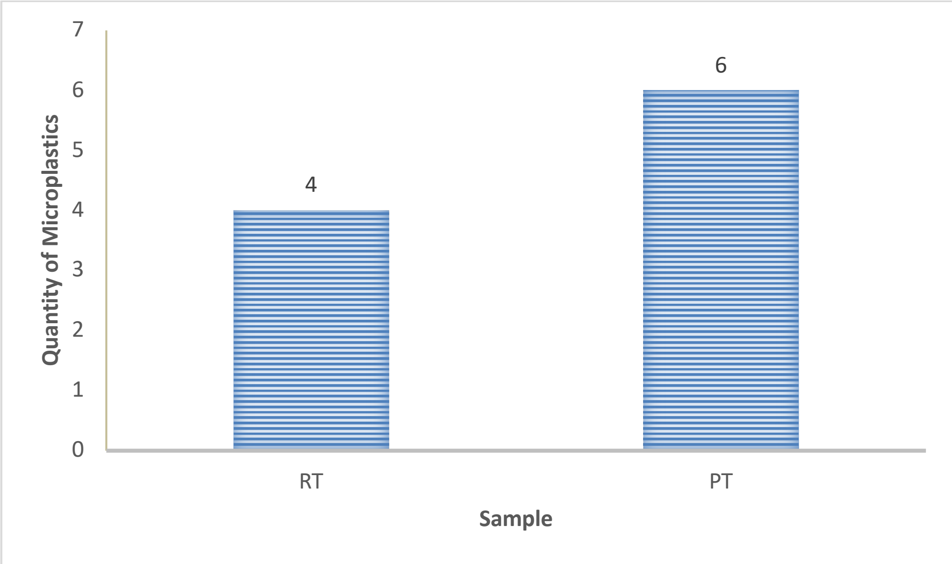


Figure 4.2 The quantity of microplastics observed in RT and PT samples (n=3)

The temperature test results showed that the RT samples contained 4 microplastic particles, while the PT samples had 6 particles. Figure 4.2 shows the difference in microplastic quantities, with the PT sample having a higher amount than the RT sample. According to Li et al. (2023), the leaching of microplastics at elevated temperatures is attributed to the breakdown of polymer materials. Higher temperatures accelerate the degradation of the polymer matrix, resulting in an increased release of microplastics. Therefore, elevated temperatures influenced the leaching of microplastics. Figure 4.3 shows the shape of microplastic observed in RT and PT samples.

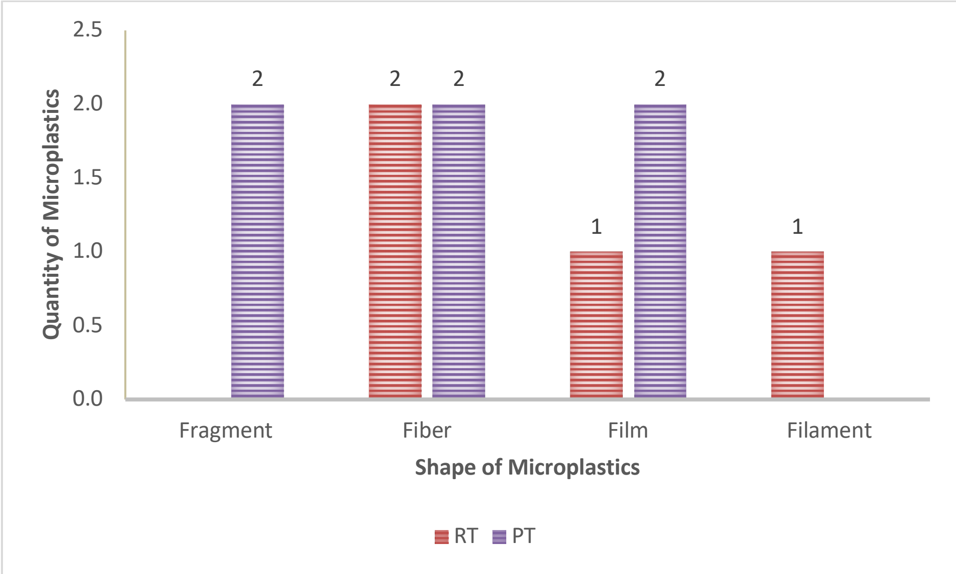
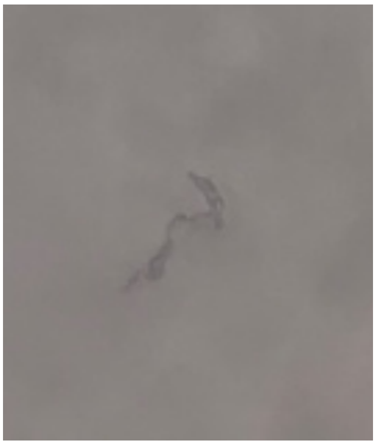


Figure 4.3 The shape of microplastics observed in RT and PT samples (n=3)

The shape of microplastics observed in RT samples included 2 fibres, 1 filament, and 1 film-shaped microplastics. As for PT samples, it contained 2 fragments, 2 fibres, and 2 film-shaped microplastics. The shape of the leached microplastics depends on the type of polymer used in the plastic material. According to Hossain et al. (2023), fibres and fragments are the most detected microplastic shapes in bottled mineral water. The presence of other shapes may be attributed to the abrasion of bottle caps and seals. In terms of colour, the RT samples had transparent and green-coloured particles while the PT samples had transparent and dark grey particles.

The microplastics observed for temperature testing are presented in Table 4.2, which includes the best images of microplastics observed in RT and PT samples. All images of microplastics were captured using the same method as the screening test.

Table 4.2 The microplastics presented in RT and PT samples.

Shape	Microplastics Observed	
	RT	**PT**
Fragment	No microplastics observed for this shape	
Fibre		
Filament		No microplastics observed for this shape

To be continued…

…continued

Shape	Microplastics Observed	
	RT	**PT**
Film		

After temperature test, shaking test was conducted on shaking room temperature at 30 RPM (SRT30), shaking peak temperature at 30 RPM (SPT30), shaking room temperature at 60 RPM (SRT60), and shaking peak temperature at 60 RPM (SPT60) samples.

4.4 Shaking Test

This test aimed to determine whether mechanical stress influences the leaching of microplastics into the water. Unlike the method used in a previous study by Winkler et al. (2019), which applied mechanical stress by squeezing or crushing, this study utilised a water bath equipped with a shaker to simulate mechanical stress.

The chosen shaking speeds of 30 RPM and 60 RPM were selected to represent real-life conditions, where 30 RPM mimics the motion of walking, and 60 RPM simulates the speed of vehicles during the transportation of bottled mineral water. Figure 4.4 shows the quantity of microplastic particles observed in SRT30, SPT30, SRT60, and SPT60 samples.