RECOMMENDED HARMONIZED PROTOCOL FOR SAMPLING, ANALYSIS, AND MONITORING OF MICROPLASTICS IN SEWAGE TREATMENT PLANTS AND RIVERINE ENVIRONMENTS IN ASEAN

In collaboration with

RECOMMENDED HARMONIZED PROTOCOL FOR SAMPLING, ANALYSIS, AND MONITORING OF MICROPLASTICS IN SEWAGE TREATMENT PLANTS AND RIVERINE ENVIRONMENTS IN ASEAN

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EXECUTIVE SUMMARY

Microplastics (MPs) are plastic fragments smaller than 5 mm in size that pose a significant threat to the environment and human health. They are generated from primary and secondary sources, forming a part of waste from commercial products and through the degradation of larger plastic pieces. An increasing number of scientific studies have shown that MPs have been detected in fish species, salts, and even human breast milk and placenta samples.

Sewage treatment plants (STPs) are major conduits for MPs to enter the environment, particularly in receiving water bodies. According to numerous recent studies, most MPs are eliminated during the primary treatment stages of STPs. Nevertheless, the disparity between the results of these studies demonstrates the requirement for standardized protocols and procedures for the treatment of MPs in sewage.

Why do we need a standard or Harmonized Protocol?

The issue of MPs is particularly pressing in member countries of the Association of Southeast Asian Nations (ASEAN) due to the following reasons: the local population is highly reliant on marine resources due to the presence of extensive coastlines in these countries—hence, the presence of MPs in fish species used for consumption negatively impacts people's health; additionally, due to the rapid urbanization and industrialization in these countries, large amounts of plastic waste are produced, which in turn degrades into MPs over time. Furthermore, monitoring MPs in STPs and receiving water bodies, particularly in rivers in member countries of ASEAN

(hereinafter referred to as “ASEAN countries”), lacks a standard or Harmonized Protocol, resulting in difficulties regarding comparing data across studies and regions.

Hence, in response to these concerns, the Japan-ASEAN Integration Fund (JAIF) has launched a new project titled “Strengthening Capacity Development for Local Governments in ASEAN to Tackle Microplastics and Water Pollution through Decentralized Domestic Wastewater Management Approach” with the aim of facilitating the process of developing and introducing a standardized and Harmonized Protocol for monitoring MPs in STPs and receiving water bodies in ASEAN countries.

This recommended Harmonized Protocol is developed by the Institute for Global Environmental Strategies (IGES), Japan, in collaboration with AMH Philippines, Inc. and the University of the Philippines Diliman. The implementation of this protocol is anticipated to contribute to improved data comparability, enhanced performance evaluation of wastewater treatment processes, more reliable and scientifically derived evidence for policymaking, and increased cooperation between ASEAN countries in a collective effort to combat pollution caused by MPs. This protocol is not intended to present general standards; instead, it has been prepared with the expectation that it will be helpful in implementing harmonized methods for generating comparable results.

Who will benefit from this Harmonized Protocol?

The Harmonized Protocol has been designed to be used by relevant units within either central or local governments (e.g. centers for environmental monitoring, research institutions), STP operators, academic institutions, and other relevant stakeholders. During instances of sampling from rivers, collecting wastewater, working alone, processing samples in the field and laboratory, characterizing samples in the riverine environment, and analyzing samples, users should adhere to appropriate health and safety provisions and adopt safe work practices.

ACKNOWLEDGMENTS

This Harmonized Protocol has been developed by IGES, Japan, in collaboration with AMH Philippines, Inc., and the University of the Philippines Diliman. It is part of the ASEAN Regional Program titled "Strengthening Capacity Development for Local Governments in ASEAN to Address Microplastics and Water Pollution through a Decentralized Domestic Wastewater Management Approach," spanning from March 2022 to March 2024. The project is led by a team of international experts from IGES, comprising Dr. Pham Ngoc Bao (Project Leader), Yukako Inamura (Deputy Project Leader), Sui Kanazawa (Policy Researcher), Shom Teoh W. C. (Program Manager), and Miyako Culshaw-Ishii (Project Administrative Staff Member). Operating under the auspices of the ASEAN Working Group on Water Resources Management, this initiative, also known as the PoDiWM-2 Project, is funded by the JAIF. Its primary objective is to assist ASEAN cities and municipalities in achieving inclusive, sustainable, resilient, and dynamic growth—aligning with the ASEAN Vision 2020, the ASEAN Framework of Action on Marine Debris, and the ASEAN Strategic Plan on the Environment, with a particular focus on the third, fourth, and fifth strategic priorities of this plan.

This publication was made possible by generous funding from the Government of Japan through the Japan-ASEAN Integration Fund (JAIF). The project team would also like to express our sincere gratitude to the ASEAN Secretariat for their tremendous support, coordination, and guidance, which were essential in ensuring the smooth and successful implementation of this significant regional initiative.

The final design of this Harmonized Protocol was completed with the strong support and dedicated efforts of all relevant members from AMH Philippines, Inc. and the University of the Philippines Diliman. Specifically, we extend our gratitude to the following individuals for their invaluable technical expertise and inputs: Dr. Maria Antonia Tanchuling, Asst. Prof. Ma. Brida Lea Diola, Engr. Ezra Osorio, Engr. Marian Jave Delos Santos, and Engr. Maria Deandra Andal. In addition, we wish to express our appreciation to the reviewers of this Harmonized Protocol: Ms. Rodessa Mae Ortega and Mr. Roger Evangelista Jr. of the Environmental Research and Laboratory Services Division at the Environmental Management Bureau of the Philippines, and the representatives of the ASEAN Secretariat.

Further acknowledgment and sincere appreciation are extended to the National Water Resources Board (NWRB) of the Philippines for their continuous support, guidance, and insightful advice during the implementation of the project; specifically, the contribution of Mr. Ricky A. Arzadon, Executive Director, along with Madam Susan Abano and Mrs. Snoofey Cabag-Iran is recognized.

Table of Content

List of Figures

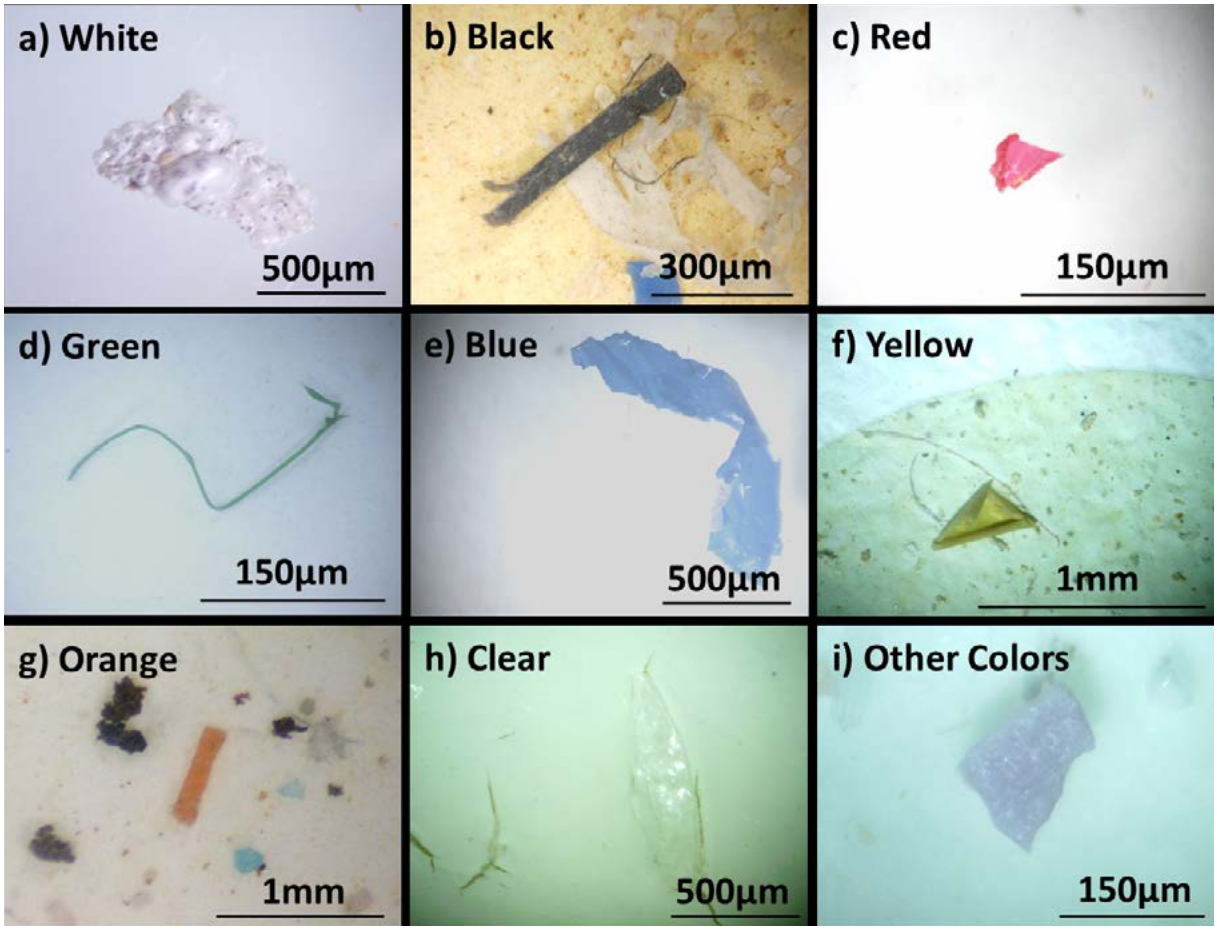
List of Tables

List of Abbreviations and Acronyms

°C	Degree Celsius
AMH	AMH Philippines, Inc.
ASEAN	Association of Southeast Asian Nations
ATR	Attenuated Total Reflectance
CAS	Conventional Activated Sludge
DENR	Department of Environment and Natural Resources
DWTs	Decentralized Wastewater Treatment Systems

EPA	Environmental Protection Agency
ERDB-CRERDEC	Ecosystems Research and Development Bureau-Coastal Resources and Ecotourism Research, Development, and Extension Center
FTIR	Fourier Transform Infrared Spectroscopy
g	Gram
GESAMP	Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
GIS	Geographic Information System
IGES	Institute for Global Environmental Strategies
JAIF	Japan-ASEAN Integration Fund
L	Liter
m^3	Cubic Meter
MPs	Microplastics
NOAA	National Oceanic and Atmospheric Administration
NWRB	National Water Resources Board
PE	Polyethylene
PET	Polyethylene Terephthalate
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl Chloride
PoDIWM	Policy Dialogue and Network Building of Multi-stakeholders on Integrated Decentralized Domestic Wastewater Management in ASEAN Countries
QA	Quality Assurance
QC	Quality Control
SBR	Sequencing Batch Reactor
STP	Sewage Treatment Plant
UNEP	United Nations Environment Programme
USD	US Dollar
WPO	Wet Peroxide Oxidation

Chapter 1
INTRODUCTION



1.1. Rationale

Microplastics (MPs) are plastic debris that are smaller than 5 mm in size. They are generated from primary and secondary sources. Primary sources include those produced for commercial use, such as microbeads for cosmetic products, whereas from secondary sources are products of the degradation of larger plastic pieces into smaller plastic fragments. Although MPs are not easily observed by the human eye, they have potential negative impacts on the environment. Cabansag et al. (2021) studied some fish species in Eastern Visayas, Philippines, and found MPs in the gut contents of marine and freshwater fishes. MPs have also been found in commercial table salts (Lee et al., 2019). Furthermore, MPs have been found in human breastmilk (Ragusa et al., 2022) and placenta (Ragusa et al., 2021).

Sewage treatment plants (STPs) are one of the ways through which MPs enter the environment. Microbeads in cosmetic products such as facial cleansers and toothpastes (Cheung & Fok, 2017), microfibers generated from washing synthetic clothing (Acharya et al., 2021), MPs in leachate due to microorganism decomposition, and MPs from plastics industries and vehicles via atmospheric deposition (Liu et al., 2021) are discharged into wastewater (Lares et al., 2018) that is eventually treated at STPs and subsequently released into recipient waters through the nearest outfall. Carr et al. (2016) studied the concentration of MPs at different stages in seven STPs. They found that most MPs were removed in the primary treatment zones because of solid skimming and sludge settling processes. Furthermore, they found that the contribution of the discharge of secondary and tertiary wastewater treatment plants to MP concentration in oceans and surface water environments was minimal. A similar study was conducted by Iyare et al. (2020) to examine and quantify the MP removal efficiency in wastewater treatment plants. They also found that most of the MPs were removed in the preliminary and primary treatments, especially via sedimentation and solids skimming.

Previous studies indicate that the high MP removal efficiency of STPs can be attributed to the retention of MPs in sewage sludge (Carr et al., 2016; Iyare et al., 2020). However, studies focusing on occurrence, transformation, and mobilization of MPs are limited (Sun et al., 2019). Although STPs show an MP removal efficiency above 99% (Carr et al., 2016), MPs that escape extraction in these facilities continuously and significantly contribute to the pollution in receiving water bodies. The abundance of MPs in sewage sludge, through disposal, reuse, and other applications, can harm the land-related environment.

The variability in the results of different studies regarding the occurrence of MPs in wastewater can be attributed to many factors. Time, day, year, and population size associated with the STP; speed and volume of effluent; treatment technology; and stages selected for sampling are among the variables that have affected the study results (Tagg et al., 2020). Furthermore, the type of wastewater, such as domestic, industrial, or agricultural wastewater, affects the behavior of the treatment process, subsequently causing variation in the obtained results (Kwon et al., 2022). These factors emphasize the need for using harmonized protocols and methods in future studies to ensure better comparability among the obtained results (Sun et al., 2019).

Over the last two decades, 10 ASEAN countries have made remarkable progress in improving domestic wastewater and sanitation management and reducing the

impacts of wastewater on the environment, including the coastal environment, by increasing the ratio of households with access to improved sanitation facilities such as septic tanks. However, sanitation is not limited to the provision of sanitation facilities to private homes alone. It is essential to consider the whole sanitation service chain, including how to safely manage, treat, dispose, and reuse treated wastewater and sludge, either on-site or off-site, using either a centralized or decentralized system (e.g., for nutrient/resource recovery through treated wastewater and sludge reuse in agriculture, as well as for other purposes locally to meet a portion of the increasing demand for water resources).

In the context of ASEAN countries, decentralized STPs or decentralized wastewater treatment systems (DWTs) have proven to be a long-term and cost-effective solution because they do not require high capital costs and show reduced operational and maintenance costs. These facilities can be constructed within communities, particularly those with limited space and small populations.

Sewage treatment facilities, such as DWTs, serve as a pathway through which MPs enter the environment, particularly receiving water bodies. Moreover, it has been proven that large amounts of MPs can bypass sewage treatment facilities if these facilities are not designed or optimized for the removal of MPs. Despite their small size, MPs have harmful effects on the ecosystem. Hence, reducing the amount of MPs in the environment presents exceptional challenges today.

Owing to the ASEAN region's extensive coastline, the local population is dependent on marine resources. Furthermore, because of rapid urbanization and industrialization, the issue of MPs is especially pressing in this region because wastewater comprises varying concentrations of MPs. Consequently, a better understanding of the physical, chemical, and compositional characteristics of MPs in STPs, as well as their removal efficiency throughout the various treatment processes, is crucial. There is also a lack of standard or harmonized sampling and analytical protocols for monitoring MPs in STPs and receiving water bodies among ASEAN countries. This lack of standardization makes it difficult to compare data across studies and regions, hindering stakeholders' ability to comprehend the scope and impact of pollution caused due to MPs. Factors affecting the difference in the MP monitoring data are attributed to the differences in the equipment used in sample collection, volumes of collected wastewater, laboratory processes, spectroscopy methods, seasons of collection, capacity and flow rate of the influent and effluent, treatment technologies, and wastewater types. Several studies have also used different units of MP concentration for conducting analysis.

The following project has been launched in response to the strong need and concerns of ASEAN countries regarding the above issues identified from the recently implemented project, "Policy Dialogue and Network Building of Multistakeholders on Integrated Decentralized Domestic Wastewater Management in ASEAN Countries" (PoDIWM) funded by the JAIF (2018-2020) and IGES, in cooperation with the ASEAN Secretariat and NWRB of the Philippines: "Strengthening Capacity Development for Local Governments in ASEAN to Tackle Microplastics and Water Pollution through Decentralized Domestic Wastewater Management Approach." This project is intended to be implemented for a period of 2 years (04/2022-03/2024) and is funded by the JAIF.

This project aims to enhance scientific knowledge and practical experience sharing among ASEAN countries. This will promote evidence-based policymaking for improved decentralized domestic wastewater treatment and management under climate change conditions and benchmarking of good practices regarding resilient decentralized domestic wastewater treatment solutions. This will also promote the expansion of the technical capacity of relevant local governments in all ASEAN countries, especially targeting vulnerable cities, for better and more resilient domestic wastewater planning and management through the dissemination and application of a regional guidebook and training modules on decentralized wastewater treatment systems developed as part of this project.

The present document aims to provide a Harmonized Protocol (hereinafter referred to as "Harmonized Protocol") for investigating the occurrences and removal efficiency of MPs in selected STPs and receiving water bodies in the context of ASEAN countries. This document reports one of the outcomes of this project, introducing a harmonized sampling and analytical protocol for monitoring MPs in STPs and receiving water bodies in ASEAN countries, which can contribute to: (i) improved data comparability, allowing for more accurate comparison of data across studies and regions, and enhancing the understanding of the scope and impact of pollution due to MPs; (ii) better assessment of MP removal efficiencies of wastewater treatment processes and the potential risks posed by MPs to aquatic ecosystems and human health; (iii) provision of more reliable and comparable data on MPs that can gradually inform the development of effective policies and regulations to manage MP pollution; and (iv) promotion of further cooperation among ASEAN countries in tackling the shared problem of MP pollution.

This Harmonized Protocol was developed after a comprehensive analysis of existing studies, a compilation of various methodologies, and a series of consultation meetings with relevant experts and stakeholders in the field. It adopted best practices based on previous research suitable for the geographical and economic conditions of ASEAN countries and offered alternatives by considering the capacities of national government agencies. It is expected that the release of this Harmonized Protocol will significantly facilitate regional efforts to address the emerging issue of microplastic pollution.

1.2. Objectives

The objectives of this Harmonized Protocol are as follows:

- It aims to provide a scientifically sound and harmonized protocol for sampling and analysis of microplastics in STPs and receiving water bodies. The harmonization of these protocols across ASEAN countries is crucial for generating reliable data, which is essential for informed policy-making and effective environmental management strategies.
- To discuss the challenges and gaps in the existing MP sampling and analysis procedures.
- To recommend an MP monitoring plan that can be adapted by relevant stakeholders such as STP operators.

1.3. Scope and Limitations

The Harmonized Protocol and the recommendations provided as part of this protocol are intended for use by relevant national and local government units, STP operators, academia, and other stakeholders. The presented procedure considers various constraints such as limited human and financial resources.

The Harmonized Protocol is not intended to present general standards; instead, it has been prepared with the expectation that it will be helpful in choosing harmonized methods that would derive comparable results.

1.4. Status of MP Monitoring in ASEAN Countries

MPs are an emerging contaminant that is part of the growing problem of global plastic pollution. These particles have been found in various environments, including water, air, and land, and in plants, animals, and other organisms. In addition, a significant amount of MPs have been found in wastewater released from treatment plants, particularly in ASEAN countries. Therefore, many studies have been conducted in this region to monitor MPs in wastewater treatment plants and evaluate their MP removal efficiencies before the treated water enters the environment. Table 1 shows the compilation of studies conducted in ASEAN countries to determine the baseline data regarding the occurrence and removal efficiencies of different wastewater treatment plants in selected sites.

TABLE 1. Studies on treatment of MPs in wastewater treatment plants in ASEAN countries (IGES, 2024)

Country	Capacity (m^3/day)	Treatment technology	Average MP concentration		Average MP removal efficiency	Reference
			Influent (particles/m^3)	Effluent (particles/m^3)		
Philippines	Not specified	Activated sludge system	4,370	1,100	74.8%	(World Bank, 2021) *Ongoing publication*
	Not specified	Activated sludge system	2,500	140	94.4%	

Country	Capacity (m^3/day)	Treatment technology	Average MP concentration		Average MP removal efficiency	Reference
			Influent (particles/m^3)	Effluent (particles/m^3)		
Philippines	10,400	Sequencing batch reactor (SBR)	1,000	200	80.0%	(World Bank, 2021) *Ongoing publication*
	15,400	Activated sludge system	510	400	21.6%	
	567	Sequencing batch reactor	3,860	760	80.3%	
	100,000	Sequencing batch reactor	1,750	315	82.0%	(IGES, 2022) *Unpublished*
	40	Anaerobic treatment	49,250	790	97.53%	(IGES, 2023) *Unpublished*
	10	Anaerobic treatment	2,920	828	71.62%	
	50	Anaerobic treatment	4,125	989	76.02%	
	60	Anaerobic treatment	1,334	430	65.13%	
	10,000	Conventional activated sludge process	3,900	235	92.66%	
	500	Advanced oxidation	470	213	55.15%	(IGES, 2023) *Unpublished*
	200	Conventional activated sludge process	1,666	402	75.82%	
	110	Anaerobic treatment	475	142	67.36%	

Country	Capacity (m^3/day)	Treatment technology	Average MP concentration		Average MP removal efficiency	Reference
			Influent (particles/m^3)	Effluent (particles/m^3)		
Thailand	200,000	Sequencing batch reactor	12,200	2000	83.6%	(Hongprasith, et al., 2020)
	350,000	Biological activated sludge process	16,550	3,520	78.73%	(Tadsuwan & Babel, 2022)
	120,000 (dry season)	Biological activated sludge process and a pilot-scale UF	77,000	2,330	96.97%	
	300,000 (rainy season)					
Vietnam	17,000	Activated sludge system	24,300	810	96.7%	(Le, et al., 2023)
	7,500	Aerobic treatment system	125,250	140	99.9%	

A lack of harmonization or standardization in the protocols or guidelines used for MP treatment may result in ineffective comparison of MP data. Table 2 summarizes the published documents as references for the methodologies used to assess MP removal efficiency. The use of different methodologies derived from various protocols and guidelines may result in different baseline and monitoring data. For example, during on-site filtration, the mesh pore size of the nets and sieves used may differ and affect the quantification of MPs removed during treatment. The use of wet peroxide oxidation (WPO) and enzymatic digestion for MP treatment may also have different effects on the chemical composition of MPs. Studies have also used different units of measurement to determine the abundance of MPs. In addition, the volume of water samples collected may vary, thus impacting the concentration of MPs observed in the samples.

The use of a bucket is a common tool for collecting wastewater samples in studies conducted in ASEAN countries. Le et al. (2023) collected wastewater samples at various sampling points in selected sites in Vietnam using a stainless steel bucket with a known volume. A similar study in Thailand employed a volume-reduced sampling method and grab sampling using a stainless steel bucket, following the method of Tadsuwan et al. (2022). Similarly, studies in the Philippines, conducted by Osorio et al. (2021) and the World Bank (2021), used buckets with known volumes to collect wastewater samples. The use of buckets in collecting samples is an alternative to using pumps and nets because of the availability of the equipment and the flow condition of the wastewater.

For water samples obtained from the riverine environment, Osorio et al. (2021) used a bucket as an alternative to a Manta net. This modification was due to the presence of macroplastics and other large debris along the sampling points of the rivers. Subsequently, the samples were sieved before transport to the laboratory. In Malaysia, glass bottles with a volume of 1 L were held at a certain depth underwater to collect water samples; subsequently, they were also used to store the collected samples for experimental analysis (Zaki, Ying, Zainuddin, Razak, & Zaharin, 2021). A Manta trawl was used in a study by Lestari et al. (2021) to collect samples from the surface, middle, and bottom of the river water column.

In various studies conducted in the Philippines (Osorio et al., 2021), Malaysia (Saipolbahri et al., 2020), and Thailand (Chanpiwat et al., 2021; Pradit et al., 2023), MP extraction has been performed using WPO and density separation methods. In Vietnam, enzymatic digestion was employed to eliminate organic matter (Le et al., 2023). The variation in laboratory techniques used for MP extraction can be attributed to the availability of materials and discoveries regarding the effects of strong chemicals on MP samples.

The optical microscope is a commonly used tool to characterize the shape, size, and color of extracted MPs. To validate and identify the polymer types of the samples,

spectroscopy techniques such as FTIR and/or Raman spectroscopy are used. These can also be used to determine the possible sources, passage, and fate of MPs; however, due to budget and resource limitations, few to no samples undergo spectroscopy.

Vietnam has taken a positive step toward reducing the amount of MPs released into the environment. Through collaboration among the government, private sector, and citizens, plans are being developed to establish monitoring systems for plastic waste and MPs. These plans include various policies aimed at controlling and reducing the release of MPs. Some of these policies are as follows: a ban on the use of primary MPs in products; measures to decrease the release of MPs from fisheries and aquaculture activities; managing the release of MPs from wastewater; and monitoring the level, accumulation, and impact of MPs. The Decision 1891/QD-BKHCN of the Ministry of Health approved the research and evaluation of the accumulation and impact of MPs on the riverine ecosystem in the South-Central Coast of Vietnam. The ministry also intends to develop a technical guide to determine the accumulation and ecotoxicity of MPs in estuary systems and use this information to assess the level of accumulation and ecotoxicity of MPs in some aquatic species in the study area (Strady et al., 2023). In Thailand, the Ministry of Public Health banned the import, production, and sale of cosmetic products containing microbeads (Aung et al., 2021).

Currently, there are no standardized methods or parameters for measuring the amount of MPs in the environment, particularly in ASEAN countries. Past studies have identified the presence of MPs in certain areas and emphasized the need for proper monitoring of MPs in land and water environments, focusing on the impact of treatment methods employed by STPs on MP pollution. Although some institutions and STP operators have expressed an interest in studying MPs, ASEAN countries do not have established parameters for monitoring MPs in wastewater effluent and ensuring the subsequent water quality standards.

Chapter 2
METHODOLOGY

2.1. Reference Guidebooks

Several guidelines regarding monitoring MPs in STPs and riverine environments in ASEAN countries have been published by organizations such as UNEP, EPA, Ministry of the Environment of Japan, GESAMP, and NOAA. Presently, these references offer best practices; however, they are limited in terms of not considering the geographic and economic conditions in ASEAN countries. Hence, there is a need for alternative and modified guidelines to suit the specific conditions in these countries.

Consequently, based on the following existing guidelines, the Harmonized Protocol has been drafted **(Table 2):**

TABLE 2. Guidelines for Monitoring and Assessment of MPs

Document	Publisher/ Author	Year Published	Type of Samples	Quantification Units	Collection	Organic Removal	Inorganic Removal	Characterization
Monitoring Plastics in Rivers and Lakes: Guidelines for the Harmonization of Methodologies	UN Environment Programme (UNEP)	2021	Wastewater, drinking water, freshwater biota, surface water, water column, and sediments	Particles per volume in International System of Units (SI) Mass per volume in SI units	Use of a pump, grab samples, or surface skimming device for wastewater sampling Use of a net, pump, or series of filters of decreasing mesh sizes for sampling the water surface and water column Use of a grab sampler or corers to collect the top 15 cm of sediment or use of metal spoons for collecting shore samples	Employment of enzymatic treatments to extract MPs from samples	Addition of dense salt solutions such as sodium chloride (NaCl), sodium iodide (NaI), and zinc chloride ($ZnCl_2$) to separate MPs from the samples	Performing a visual/microscopic inspection Touching the particles with a heated dissecting needle or staining the solution with dyes, such as Nile Red, in combination with fluorescence microscopy Use of mass-based analytical methods, such as thermoextraction, or particle-based analytical methods, such as FTIR and Raman spectroscopy
EPA's Microplastic Beach Protocol	United States' Environmental Protection Agency (EPA)	2021	Sand on the beaches or shorelines of oceans and bays, lakes, and rivers	Number of MP particles (pieces)	Scraping the surface of the sand using a metal cup or flat dustpan	Removal of any large pieces of natural debris and litter using a brush or placing the sample into a 1-mm sieve and performing the "sink test" and "squish test"	Removal of any large pieces of natural debris and litter using a brush or placing the sample into a 1-mm sieve and rinsing it with water	Use of a magnifying glass to visually identify MPs

Document	Publisher/ Author	Year Published	Type of Samples	Quantification Units	Collection	Organic Removal	Inorganic Removal	Characterization
Guidelines for Harmonizing Ocean Surface Microplastic Monitoring Methods	Ministry of the Environment, Japan	2020	Marine surface water	Number of particles per volume (pieces/m^3)	Filtering of a large mass of water using nets such as Neuston and Manta nets Collection of smaller MP particles; use of bottles, buckets, pumps, and other materials in sampling	Implementation of WPO to digest the organic matter In some cases, digestion is performed via hydrolysis or enzymatic reactions	Performing density separation by mixing the sample into a solution with higher specific gravity values such as sodium chloride (NaCl), sodium iodide (NaI), and zinc chloride ($ZnCl_2$)	Isolation of MPs and visually observing them under a stereoscopic microscope Identifying polymer types using spectral optical instruments such as IR/Raman spectroscopy
Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean	Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP)	2019	Beach sediments, marine surface water, water column, sea floor, and marine biota	Mass or number of particles per unit distance (pcs/m) (g/m) Mass or number of particles per unit area (pcs/m^2) (g/m^2) Mass or number of particles per volume (pcs/m^3) (g/m^3)	Sieving the surface beach sediments by using a range of sieve mesh sizes, such as, <5, <2, <1, <0.5, <0.25 mm Use of net tows such as Neuston or Manta nets for sampling surface waters	Addition of 30% hydrogen peroxide with ferrous iron (Fenton's reagent) to the samples to remove organic matter Controlling the temperature of the samples at 70°C or lower (it should be noted that the sample cannot below 15°C)	Performing density separation by agitating the sample mixed with a dense solution, such as NaCl, NaI, and $ZnCl_2$, and subsequently allowing the mixture to settle for 10 min up to 24 h	Use of an optical microscope to aid in visual identification

Document	Publisher/ Author	Year Published	Type of Samples	Quantification Units	Collection	Organic Removal	Inorganic Removal	Characterization
						Other digestion methods can be used, such as acid digestion, alkaline digestion, and enzymatic digestion.	Filtration or sieving of the supernatant by using a filter paper or fine mesh screen, respectively	Identification of polymer types by using spectral optical instruments such as Fourier transform mass spectroscopy, Raman spectroscopy, scanning electron microscopy, thermal analysis, and novel methods including automation
Laboratory Methods for the Analysis of Microplastics in the Marine Environment: Recommen-dations for Quantifying Synthetic Particles in Waters and Sediments	National Oceanic and Atmospheric Administrati-on (NOAA)	2015	Marine water, beach sand, and bed samples	Mass of MPs per total mass of dried material within a specific size range (gMP/gsolids) Mass of MPs per volume of samples (gMP/mL) Mass of MPs over a certain area (gMP/m^2)	Use of a 0.335-mm surface net for sampling surface water and subsequent filtration through 5.6 mm and/or 0.3 mm sieves Use of a shovel or spade to collect beach sands and sieving dry samples with a 5-mm sieve	Oven-drying the sieved material at 90°C for 24 h, subsequently subjecting to WPO at 75°C on a hotplate	Addition of 6 g of NaCl per 20 mL of the solution and use of a funnel as a density separator to allow the other solids to settle overnight	Use of a dissecting microscope with 40× magnification for visual identification Use of FTIR to identify polymer types

Document	Publisher/ Author	Year Published	Type of Samples	Quantification Units	Collection	Organic Removal	Inorganic Removal	Characterization
					Use of a corer or grab sampler to collect beach sands; subsequent disaggregation of the sediments by adding 400 mL potassium metaphosphate (5.5 g/L of water) and sieving using stacked 5 mm and 0.3 mm sieves		Use of forceps to remove MPs and collect floating solids in a 0.3-mm custom-made sieve Addition of 30 mL of aqueous lithium metatungstate solution to dried sediments for density separation before being subjected to WPO	
Studies in the Philippines *Microplastics in surface water of Laguna de Bay: First documented evidence on the largest lake in the Philippines*[1]	Arcadio et al.[1]	2022	Surface water	Total number of MPs per volume (pcs/m^3)	Use of a plankton net with a mesh size of 20 µm submerged at a depth of 20 cm	Soaking samples in KOH solution, and subsequently heating the mixture in an oven at 60°C for 24 h	Addition of NaCl at 30% concentration for density separation Performing vacuum filtration by using a Millipore set and Whatman glass filter	Use of a microscope with 40×magnification to determine MPs based on morphology Use of PerkinElmer Spectrum Two™ FT-IR spectrometer to identify polymer types

Document	Publisher/ Author	Year Published	Type of Samples	Quantification Units	Collection	Organic Removal	Inorganic Removal	Characterization
Unraveling Microplastic Pollution in Mangrove Sediments of Butuan Bay, Philippines[2]	Navarro et al.[2]	2022	Mangrove sediments	Number of particles per mass (pcs/kg)	Use of a metal spoon to collect 2 cm of the topmost sediment	Soaking samples with 300 mL of 10% KOH solution subsequently heating the mixture in an oven at 60°C for 24 h	Performing vacuum filtration by using a Millipore set and a 40-mm diameter GF/C glass filter Use of 30% NaCl solution to allow settled MPs to float	Use of an optical microscope with 40×magnification Use of Perkin-Elmer Spectrum Two FT-IR Spectrometer to identify polymer types
Microplastic Occurrence in Surface Waters and Sediments in Five River Mouths of Manila Bay[3]	Osorio et al.[3]	2021	Surface water and sediments	Number of particles per volume of water (pcs/m^3) Number of particles per mass of sediment (pcs/kg)	Use of a bucket for surface water sampling; subsequent use of the stacked stainless sieves with mesh sizes of 2.36 mm (No. 8), 1 mm (No. 18), 500 µm (No. 35), 250 µm (No. 60), 125 µm (No. 120), and 75 µm (No. 200) Use of an Ekman grab sampler (~0.1 m^2) to collect the top 5 cm of the sediments	Oven-dried samples for 72 h at 90°C, then subjected to WPO at 75°C and agitated at 200 rpm using magnetic stirring hot plates for at least 45 min	Addition of high-density NaCl to the mixtures for density separation Addition of $ZnCl_2$ to samples of sediment sized 75 µm Filtration of the supernatant by using a longer medium-high flow rate peristaltic pump with 47-mm Whatman® GF/C filters to separate MPs	Examination of MPs using a 3.1-Megapixel CMOS camera connected to an ST-7045 Stereomicroscope of 10× to 40× magnification Determination of the polymer type using FTIR spectroscopy (Nicolet 6700 FTIR ATR spectrometer with a diamond accessory)

2.2. Methodological Flowchart

The process for MP monitoring is simplified in **Figure 1**.

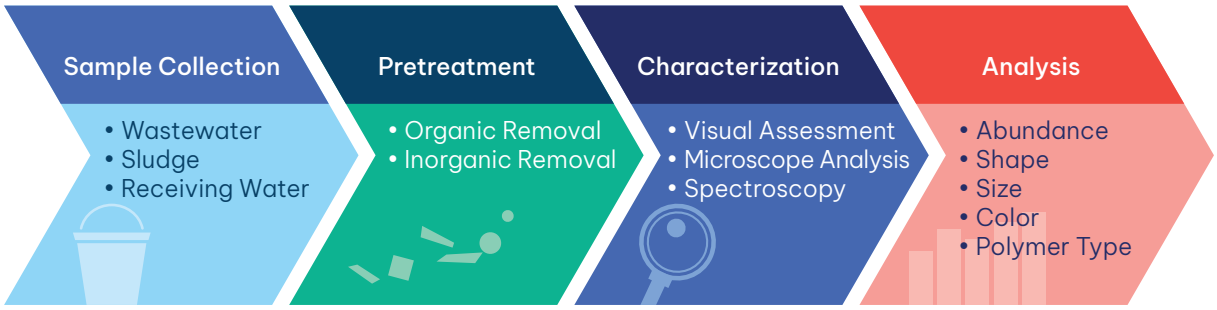


Figure 1. General Process for MP Monitoring

A decision flow diagram showing alternative pathways for conducting MP analysis at STPs depending on the desired output and available resources was developed (**Figure 2**). This diagram depicts a customized methodology tailored to the researchers' objectives and accessible materials and equipment.

Approximate costs are presented as a guide for customizing the methodology (**Table 2**). In case of unavailability of materials or funds, it is possible to opt out of sieving, organic removal, density separation, microscopic analysis, or spectroscopy. The most rudimentary method for MP analysis consists only of filtration and visual assessment. This will enable researchers with extremely limited resources to conduct MP monitoring.

Organic removal is highly recommended for wastewater and sludge samples. This can be achieved through oxidative, alkaline, or enzymatic digestion. Oxidative digestion is preferred over alkaline and enzymatic digestion because the latter two methods can damage MP particles. However, these continue to be acceptable methods for organic removal in cases where there is no supply of hydrogen peroxide, ferrous sulfate heptahydrate, or sulfuric acid to conduct WPO, a method commonly used in previous research and existing guidebooks (**Table 1**). Hence, the use of WPO in MP monitoring will enable more consistent data comparison.

The use of other methods for organic matter removal should not affect the sample in terms of the removal of MPs because this process is intended for organic materials. Loss and contamination of the sample may occur; however, these can be prevented with the quality control practices discussed in Section 6.

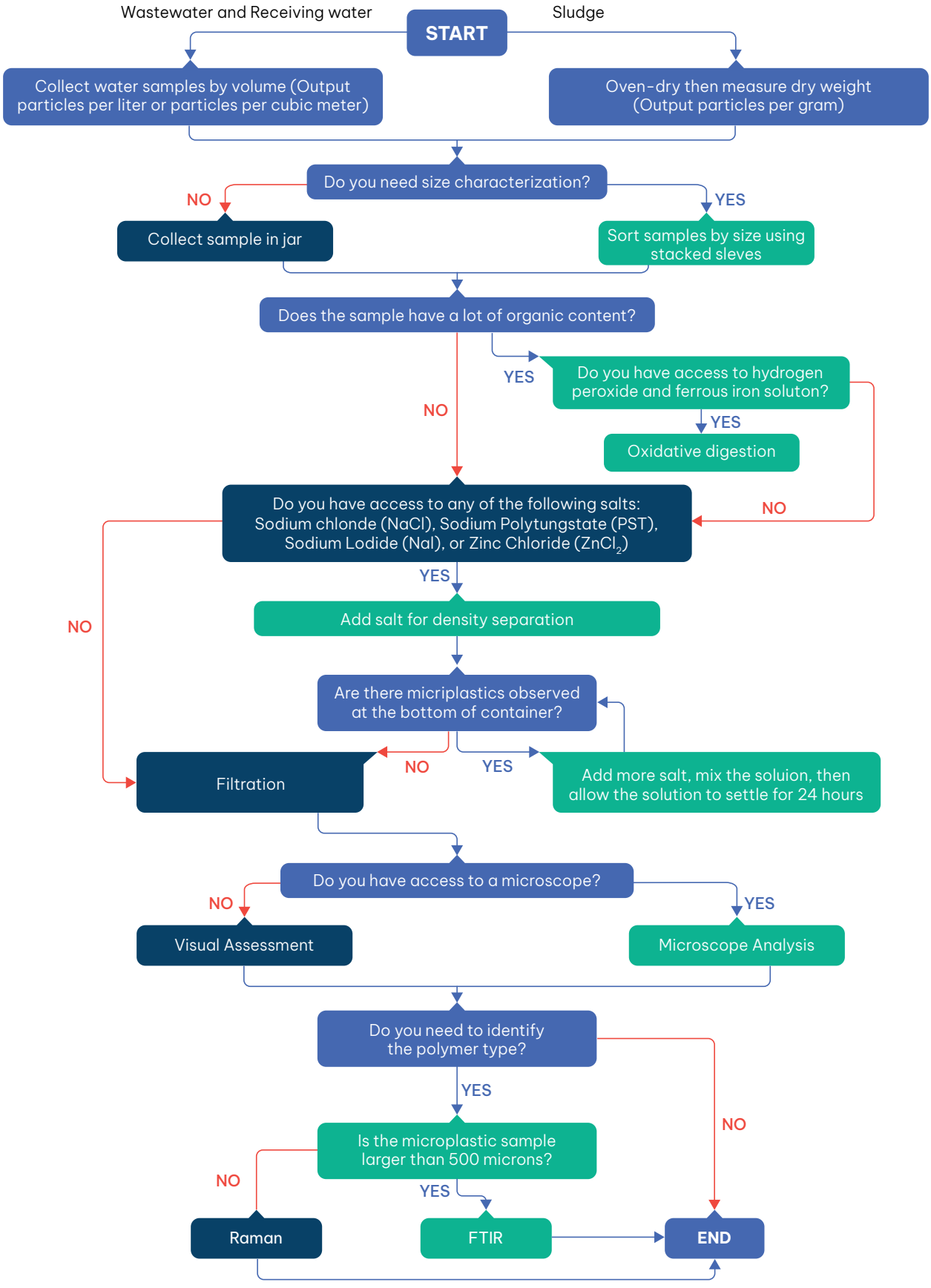


Figure 2. Flowchart of MP Sampling and Analysis at STPs

2.3. Approximate Costs

The cost per step can be approximated using the materials and equipment required. These estimates exclude labor and logistical costs for sampling such as transportation, accommodation, meals, and professional fees. Basic laboratory materials such as beakers, stirring rods, hot plates, and petri dishes are also excluded.

TABLE 3. Approximate Cost per Step

Step		Materials and Equipment	Quantity	Approximate Cost	Cost per Sample
Sample Collection	Size Distribution	W.S. Tyler Test Sieve U.S. Standard #4 mesh to #200 mesh	5	US$ 60-80 per sieve	N/A
Pretreatment	Organic Removal	30% Hydrogen Peroxide	1 L	US$ 50-55	US$ 6.5-7.0
		Iron (II) Sulfate Heptahydrate	500 g	US$ 20-25	US$ 0.015
		Concentrated Sulfuric Acid AR	2.5 L	US$ 65-70	US$ 0.005
	Density Separation	Sodium Chloride (NaCl)	500 g	US$ 10-15	US$ 1.5-2.0
	Filtration	Whatman Glass Microfiber Filters GF/A 47mm diameter	100 pcs per box	US$ 115-120 per box	US$ 1.15-1.20
		Rotary Vane Vacuum Pump ¼ hp VP115 Single Stage (1.8CFM)	1 unit	US$ 40-45	N/A
		500ml Filtration Apparatus Vacuum Lab Filtering Unit with Funnel and Clamp	1 set	US$ 45-50	N/A

Step		Materials and Equipment	Quantity	Approximate Cost	Cost per Sample
Characterization	Microscope	0.7X to 90X Zoom Magnification LED Trinocular Stereo Microscope	1 unit	US$ 1,000	N/A
		3 MP High Resolution Digital Microscope Camera (Live Video and Still Image Capture) with USB Computer Connectivity	1 unit	US$ 275-365	N/A
		0.5X C-mount Reduction Lens for Microscope Cameras	1 unit	US$ 50-55	N/A
	Spectroscopy	Raman	N/A	US$ 85-90 per hour	US$ 9.5-10.0
		Fourier Transform Infrared (FTIR)	N/A	US$ 90-95 per sample	US$ 90-95 per sample

Sample collection can cost up to USD 370 considering five sieves, similar to the study presented in this document. Organic removal using oxidative digestion approximately costs USD 7 per sample. The cost of density separation ranges from USD 10–15 per sample.

In the case of extremely limited funds, the cost for the most rudimentary MP extraction method consisting of filtration and visual assessment can be as low as USD 85 for the filtration setup and a minimum of USD 115 for a box of filter paper.

The cost of characterization using spectroscopy refers to the service fee and not the cost of the equipment. This varies significantly depending on the country and whether the laboratory has private or government ownership. The rates presented above are based on accredited government-run laboratories in the Philippines that offer spectroscopy services. The rate is presented on the basis of either per sample or per hour, depending on the laboratory. The total cost of spectroscopy per hour depends on the pace of the scientist conducting the analysis. Hence, the cost of spectroscopy can significantly vary per experiment and laboratory.

The following section discusses the full details of each process from sample collection to analysis.


USAID

Chapter 3
SAMPLE COLLECTION

3.1. Wastewater at Sewage Treatment Plant

MATERIALS

a. A metal or hard plastic bucket/container with a known volume, tied with a long rope
b. Wooden stake or stick
c. Sieves with mesh sizes of less than 5 mm
d. Data sheet
e. Marker and labels
f. Flow meter
g. Wash bottles
h. Glass jars
i. Steel tweezers
j. Steel brush
k. Ruler
l. Mobile with camera and GPS
m. Personal protective equipment (i.e., safety shoes, vest, gloves)
n. Large storage container

0. Before sampling in STPs, the points where the wastewater will be collected should be defined. For example, samples are generally collected at the influent and effluent points. Coordination with the STP operators or appropriate authority should be ensured to obtain the necessary permit for sampling.

> **NOTE:** The priority sampling points to determine the overall MP removal efficiency and the MP concentration of the water discharged to the environment at an STP are the influent and effluent points. However, if there exist no budget or resource constraints, it is recommended to collect samples at all stages of the STP to evaluate the efficiency of each process in removing MPs.

1. During sampling, record and obtain the following data:

- Time and date of sampling
- Coordinates of the sampling point
- Current weather
- Description of rain events, if any, for the last 3 days
- Coverage of the STP

> **NOTE:** The flow rate can be obtained during sampling using a flow meter. If applicable, it can be used in receiving waters; however, it is not recommended for STP sampling. If a flow meter is not available, an alternative method can be performed: a bucket should be filled with wastewater transported through pipes; the rate of filling the bucket should be timed to determine the actual flow rate. However, this can only be applied if the pipes are easily accessible. In the absence of actual data for flow rates, the design flow rate and/or average daily historical flow rate during a 6-month period can be obtained and used as a reference.

1. **Collection of wastewater samples directly from the STP using a bucket.** A wooden stake or stick should be used to push the bucket downward to enable the collection of samples (**Figure 3**).

Figure 3. Collection of wastewater samples using a bucket with the aid of a wooden stick

> **NOTE:** *Why use a bucket?* It is readily available and has a definite volume. The use of a net is not applicable in the chambers of the STP because in some points of the STP, the wastewater is stagnant.

2. The wastewater samples collected from each bucket should be passed through a stack of stainless sieves. Each wastewater sample must be at least 20 L.

> **NOTE:** The sizes of the sieves used depend on their availability. The number of sieves between the top and bottom sieves can also be adjusted if more size classes are being studied. In previous studies, the lowest mesh size available was only 75 microns (No. 200). The lowest mesh size is considered to be the lowest limit of the MP particle to be studied.

> **NOTE:** No classification of size is defined for MPs measuring 5 mm across. However, a similar study on quantifying MPs emphasized the importance of sieving in MP quantification (Prume et al., 2021). This technique allows samples to be subcategorized into smaller particles of similar sizes and reduces the quantity per subsample. Furthermore, it is more efficient to subsequently detect and identify nonoverlapping particles of similar sizes.

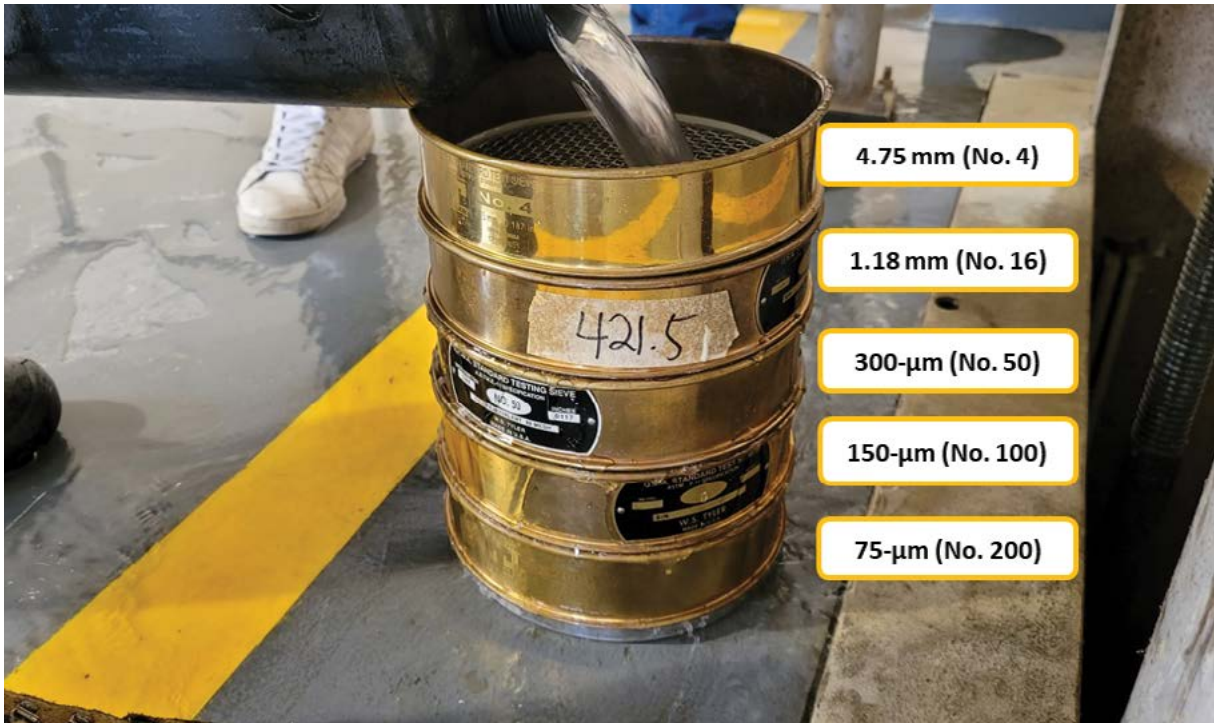


Figure 4. Collected wastewater passes through a stack of sieves with decreasing mesh sizes from top to bottom

NOTE: *How much wastewater should be collected at each sampling point?* This depends on the turbidity and the amount of suspended particles present in the collected wastewater, as well as the mesh size openings of the sieves and the amount of wastewater that the sieves can hold without the water overflowing. Based on previous experience, using the same number and size of sieves as in the photo above, approximately 20–200 L of influent samples can be collected. However, once 20 L of wastewater is collected at the influent point, the suspended solids and suspected MPs retained in the sieves accumulate and block most pores in the wire mesh, especially in the lower sieve sizes. This impedes the water flow through the sieves. If more water were to be poured into the topmost sieve, the water would overflow and leak from the joint between each sieve. Hence, the particles in the water samples could spill out. The volume of wastewater collected at the effluent point was slightly larger at 50–200 L because the treated water had significantly less suspended solids. Hence, the volume of collected wastewater varies depending on the threshold at which water overflows from the sieves. The amount of wastewater at each sampling point should be recorded.

3. The retained particles with sizes greater than 5 mm should be discarded.

NOTE: In most cases, plastics with sizes greater than 5 mm are not retained in the topmost sieve. The MPs in the wastewater are generally observed to be smaller than 5 mm.

4. Each sieve (**Figure 5**) should be air dried before transferring the samples into the sieves. The location used for conducting air-drying should not be exposed to strong winds, busy roads, or other human activities that may affect and contaminate the samples.

Figure 5. Air-drying of Samples

5. The retained particles should be carefully transferred into glass jars using a steel brush; further, the used sieves should be thoroughly rinsed with distilled water.

Figure 6. Transfer of samples to glass jars

6. It should be ensured that the glass jars are properly capped, labeled, and stored in a storage box to prevent loss and contamination of the stored samples during transportation to the laboratory.

Alternative Method	Advantage(s)	Disadvantage (s)	Materials
Plankton net-based approach for MP sampling	Collection of water samples by using a net is relatively time-saving.	Change in retained particle size spectrum toward smaller sizes due to filling and clogging of pores of sieves with larger mesh size openings (Lenz & Labrenz, 2018). The precision of volume counting relies on the flow measuring device connected to the net or installed at the influent and effluent points of the STP. This approach may be more applicable for use in sampling the receiving water bodies due to the area required to collect samples.	Nets Sieves Flow meter
Use of pumps in wastewater sampling	Collection of larger volumes can be achieved, especially for samples with low MP concentrations (UNEP, 2021).	Volume counting is dependent on the STP flow meter connected to the pump. There is a risk of removing and adding particles during sampling (UNEP, 2021).	Submersible pumps Flow meter Stack of sieves
Use of a surface skimming device	A larger fraction of the cross-sectional area of the channel can be sampled (UNEP, 2021).	Size fractionation of the sample is not achievable on-site (UNEP, 2021) because of the single filter size.	Surface filtering assembly Filter

3.2. Sludge at Sewage Treatment Plant

MATERIALS

a. Makeshift shovel
b. Glass jars
c. Metal or wooden spoon
d. Data sheet
e. Marker and labels
f. Wash bottles
g. Mobile with camera and GPS
h. Personal protective equipment (i.e., safety shoes, vest, gloves)
i. Large storage container

0. Prior to sample collection in STPs, coordination with the STP operator or appropriate authority should be established to obtain the necessary permit for sampling.

1. During sampling, the following data should be recorded:

- Time and date of sampling
- Coordinates of the sampling point
- Current weather
- Last record of desludging in the STP
- Process where the sludge is produced in the STP
- Amount of sludge generated per year (if applicable)

2. The sludge samples should be directly collected from the STP chambers by using a makeshift shovel to scrape the sludge from the bottom (**Figure 7**).

Figure 7. Collection of sludge samples using a makeshift shovel

3. A representative sample of the collected wet sludge should be transferred to glass jars using a metal or wooden spoon. The volume of sludge depends on the volume of the glass containers in which the sample is stored.

Figure 8. Transfer of sludge samples to glass jars

> **NOTE:** Since the MP concentration in the sludge is assumed to be uniform, the volume of sludge to be collected depends on the volume of the glass containers; however, it is recommended to collect a sample with a minimum volume of 0.0005m^3, equivalent to a 500-mL glass container.

4. The containers should be labeled properly, stored in a box, and finally transported to the laboratory within the same day, if possible, for processing.

Alternative Method	Advantage(s)	Disadvantage (s)	Materials
Use of manual grab samplers for collecting sludge samples	When using a manual grab sampler, sludge samples are less likely to be washed away to the surface during recovery (Audibert & Huang, 2005). This method is also capable of collecting relatively more intact sludge samples, thus preventing the loss of suspected MP particles	A manual grab sampler is more expensive than constructing a makeshift shovel.	Grab sampler

3.3. Surface Water/Receiving Water

MATERIALS

a. A metal or hard plastic bucket/container with a known volume, tied with a long rope
b. Wooden stake or stick
c. Sieves with mesh sizes of less than 5 mm
d. Data sheet
e. Marker and labels
f. Flow meter
g. Wash bottles
h. Glass jars
i. Steel tweezers
j. Steel brush
k. Ruler
l. Mobile with camera, timer, and GPS
m. Personal protective equipment (i.e., safety shoes, vest, gloves)
n. Large storage container

0. Before sample collection of the surface water/receiving water of the STP, the points in the river where the samples will be collected should be defined. Site visits and ocular inspections should be performed to determine the accessibility of the sampling points. Coordination with the local authority should be conducted to obtain the necessary permit for sampling.

> **NOTE:** Parts of the receiving water near residential, industrial, commercial, or areas with major activities can also be considered when selecting the sampling points of the receiving water.

> **NOTE:** In previous studies, the average depth at which samples were collected was 25 cm (Pasquier, et al., 2022). This could be considered as a standard for sampling. However, it also depends on the height of the available equipment/tool to be used, such as buckets, pails, or nets.

1. During sampling, the following data should be recorded:

- Time and date of sampling
- Coordinates of the sampling point
- Current weather
- Recorded rain events from the last 3 days
- Width of the river
- Depth of the river
- Flow of water

2. Surface water samples should be collected directly from the upstream, midstream (near the effluent outfall), and downstream sampling points using a bucket. It is recommended to collect the samples from the middle of the river. A bridge or banca can be used to gain access to this location in the river. If the sample collection is conducted on a bridge, the bucket should be pushed downward using a wooden stake or stick to collect water samples.

Figure 9. Collection of surface water samples from a bridge

Figure 10. Collection of surface water samples by using a banca

NOTE: *Why use a bucket?* A bucket is readily available and has a definite volume. The use of a net is not applicable in study areas with the presence of macroplastics and large debris along the rivers.

NOTE: Sampling of the receiving water should be performed by the national and local governments because the receiving waters are being used for other purposes; hence, it is important to monitor MP concentration in these waters. STP operators can perform sampling at the receiving water body in addition to sampling at the effluent to generate MP emission scenarios from sources to rivers.

NOTE: There is no prescribed distance between the upstream, STP effluent, and downstream sampling points. However, it should be noted that sample collection at the upstream point of the river should be at an accessible area before the STP outfall, whereas collection from the downstream point of the river should be after the STP outfall.

3. The collected surface water samples from each bucket are passed through a stack of stainless sieves. The total volume of the collected surface water samples should be approximately 200 L.

Figure 11. Collected surface water passes through a stack of sieves with decreasing mesh sizes from top to bottom

> **NOTE:** *What should be the volume of surface water collected at each sampling point?* This depends on the turbidity and the amount of suspended particles present in the collected surface water, as well as the mesh size openings and the amount of wastewater the sieves can hold without the water overflowing. On the basis of previous experience of using the same sets of sieves from wastewater sampling, approximately 80–200 L of surface water samples can be collected. However, similar to wastewater sampling, the total suspended solids and suspected MPs retained in the sieves accumulate and block most pores in the wire mesh, especially in the lower sieve sizes. This impedes the flow of water through the sieves. If more water were to be poured into the topmost sieve, the water would overflow and leak from the joints between each sieve, and the particles in the water samples could spill out. Hence, the volume of the collected wastewater varies depending on the threshold at which water overflows from the sieves. The amount of surface water collected at each sampling point should be recorded.

4. The particles with a size greater than 5 mm retained at the topmost sieve should be discarded (**Figure 12**).

Figure 12. Particles retained in the topmost sieve

5. Each sieve (**Figure 13**) should be air dried before transferring the samples into the sieves. Furthermore, the location used for air-drying should not be exposed to strong winds, busy roads, or other human activities to prevent the contamination of samples.

Figure 13. Air-drying of samples

6. The retained particles should be carefully transferred into glass jars using a steel brush; the sieves should be thoroughly rinsed with distilled water.

7. It should be ensured that the glass jars (**Figure 14**) are properly capped, labeled, and stored in a storage container to prevent loss and contamination of samples during transportation to the laboratory.

Figure 14. Labeled glass jars containing samples

Alternative Method	Advantage(s)	Disadvantage (s)	Materials
Plankton net-based approach for MP sampling	Collection of water samples using a net is relatively time-saving.	Change in retained particle size spectrum toward smaller sizes due to the filling and clogging of pores of sieves with larger mesh size openings (Lenz & Labrenz, 2018). The precision of volume counting relies on the flow meter available during sampling.	Nets Flow meter
Use of pumping technology for sampling	Larger sample volumes can be achieved. Deeper parts of a water body can be sampled using submersible pumps (UNEP, 2021).	The cost of the pump and its operational expenses are high. The equipment requires electricity provided by a diesel generator (UNEP, 2021).	Submersible pump Flow meter Stack of sieves Diesel generator Diesel Boat

Chapter 4
SAMPLE LABORATORY PROCESSING

4.1. Oven-drying

MATERIALS

a. Laboratory oven with a capacity to heat to 100°C
b. Samples in glass jars
c. Silicon oven mitts
d. Glass jars
e. Laboratory gowns
f. Safety masks
g. Safety goggles
h. Nitrile gloves

0. Prior to oven-drying of samples, it should be ensured that the glass jars containing the collected samples are properly capped and labeled.

> **NOTE:** Since the parameter of weight is not used in this protocol and considering the small amount of solids present in these samples, determination of the moisture content of wastewater and surface water samples is not a priority. The purpose of oven-drying these samples is to kill bacteria and other microorganisms. For sludge samples, to facilitate drying, glass containers may be covered with aluminum foil with punched holes.

1. All samples, including wastewater, sludge, and surface water samples, should be dried at 90°C for at least 72 h (**Figure 15**). The sludge samples will take the longest, up to 5 days or more, to reduce down to their dry weight; it should be noted that the air-drying period of sludge samples depends on the volume of the collected sludge sample.

Figure 15. Oven-drying of samples

> **NOTE:** Moisture content can be used as a quantitative measure to determine whether the sludge is sufficiently dry for sieving. Sludge with a moisture content of no more than 10% can be formed into powder or granules (Sludge treatment properties of drying sludge, 2020), thus allowing the particles to pass through a stack of sieves. It should be ensured that the process of transforming the sludge sample into powder or granules is gentle to avoid further fragmentation of MPs mixed with organic and other inorganic matter.

> **NOTE:** While it takes more than 1 day to dry the samples at 60°C, using high temperatures of 100°C and above for oven-drying can alter the properties of MP particles, C. To achieve a balance between time and sensitivity of the material to heat, oven-drying the samples at 90°C is considered appropriate (Rodrigues et al., 2019).

Alternative Method	Advantage(s)	Disadvantage (s)	Materials
Air-drying the samples before transferring them into containers or glass jars	There is no need to use an oven to dry the samples. This is applicable to studies that do not require the abundance of MPs by mass.	Air-drying may take a longer time and needs to be carefully monitored to avoid particle loss or contamination.	Sieves

4.2. Sieving (for Sludge Samples Only)

MATERIALS

a. Sieves with mesh sizes of less than 5 mm
b. Marker and labels
c. Wash bottles
d. Glass jars
e. Steel tweezers
f. Steel brush
g. Laboratory gowns
h. Safety masks
i. Nitrile gloves
j. Safety goggles
k. Analytical mass balance

0. This process is only applicable to sludge samples because the wastewater and surface water samples are sieved on-site.

1. A minimum aliquot of 15 g should be taken from each oven-dried sludge sample per sampling point; it should be subsequently poured through the stacked stainless mesh sieves used in processing wastewater and surface water samples (**Figure 16**).

Figure 16. Sieving of the sludge samples

Figure 17. Sludge samples of different sizes

> **NOTE:** The sizes of the sieves used depend on their availability. The number of sieves between the top and bottom sieves can also be adjusted if more size classes are being studied. In previous studies, the lowest mesh size available was only 75 microns (No. 200). The lowest mesh size is considered to be the lowest limit of the MP particle to be studied.

> **NOTE:** No classification of size is defined for MPs measuring 5 mm across. However, a similar study on quantifying MPs emphasized the importance of sieving in MP quantification (Prume et al., 2021). This technique allows the samples to be subcategorized into smaller particles of similar sizes and reduces the quantity of MPs per subsample. Furthermore, it is more efficient to subsequently detect and identify nonoverlapping particles of similar sizes.

4.3. Organic Matter Removal

The WPO method is employed to remove organic materials mixed into the solids of the samples. This step is highly recommended for treating wastewater samples considering the relatively larger amount of organic content in wastewater compared with that in freshwater and saltwater.

MATERIALS

a. 7.5-g Iron (II) sulfate heptahydrate
b. 500 mL of distilled water
c. 3 mL of concentrated sulfuric acid
d. 30% hydrogen peroxide
e. Wash bottle
f. Watch glass
g. Magnetic stir bars
h. Magnetic stirring hot plate
i. Analytical mass balance
j. Beaker
k. Graduated cylinder
l. Pipette
m. Glass rods
n. Stainless steel laboratory spatula
o. Laboratory gown
p. Safety mask
q. Safety goggles
r. Nitrile gloves

0. Prior to the removal of organic materials, all equipment and laboratory materials should be cleaned and rinsed with distilled water. Glassware should be preferred over plasticware to avoid sample contamination.

1. At room temperature, an aqueous 0.05 M Fe (II) solution should be prepared by mixing 7.5 g of $FeSO_4 \cdot 7H_2O$ (**Figure 18**) with 500 mL of water and 3 mL of concentrated sulfuric acid (**Figure 19**). The solution should be stirred until $FeSO_4 \cdot 7H_2O$ is fully dissolved in water (**Figure 20**).

Figure 18. Weighing of $FeSO_4 \cdot 7H_2O$

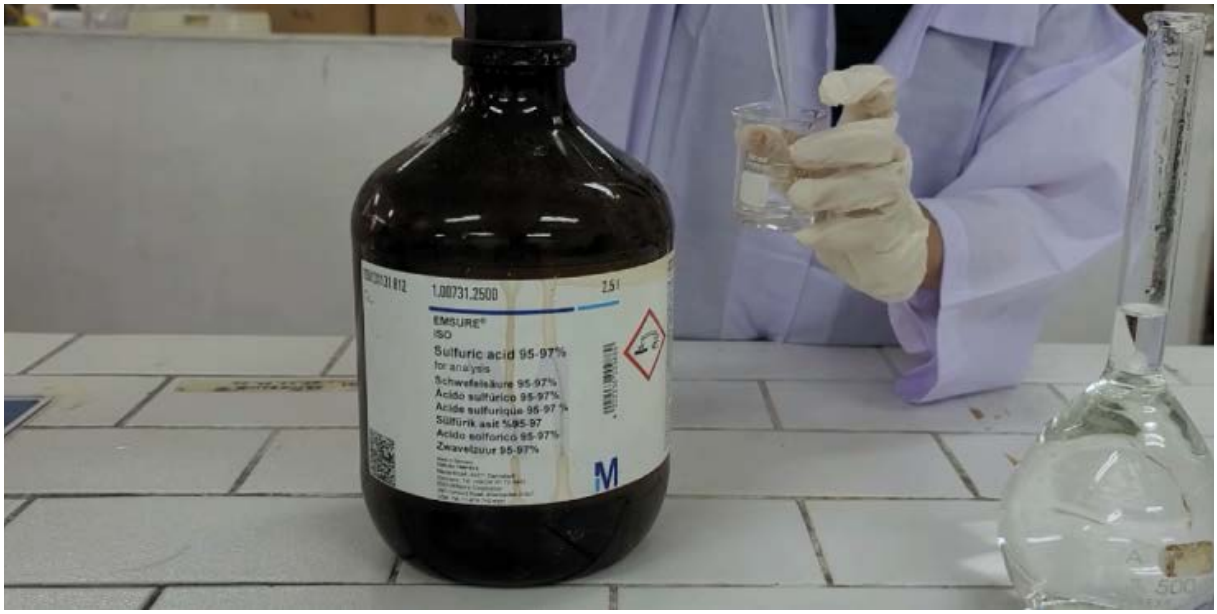


Figure 19. Addition of sulfuric acid to the solution

Figure 20. Addition of aqueous 0.05 M Fe (II) solution after $FeSO_4 \cdot 7H_2O$

2. A 20 mL aqueous 0.05 M Fe (II) solution should be added to the glassware containing the sample; this should be allowed to sit for 5 min.

Figure 21. Addition of 0.05 M Fe (II) solution to the sample

> **NOTE:** Glass jars are used to store the collected samples to avoid contamination during transportation to the laboratory. In addition, it is difficult to transfer samples containing MPs with smaller sizes that are not visible to the naked eye. The glass jars can also withstand the temperature required for digestion. Beakers having a higher volume than these glass jars can also be used, especially for storing sludge samples, because violent reactions may occur during the digestion process. It is important to carefully transfer the samples from glass jars to beakers.

3. A 20 mL 30% H_2O_2 solution should be added to the sample; the mixture should be heated to 75°C and agitated at 200 rpm on a magnetic stirring hotplate for at least 45 min, at which point all biological material appears to be visibly bleached (**Figure 22**).

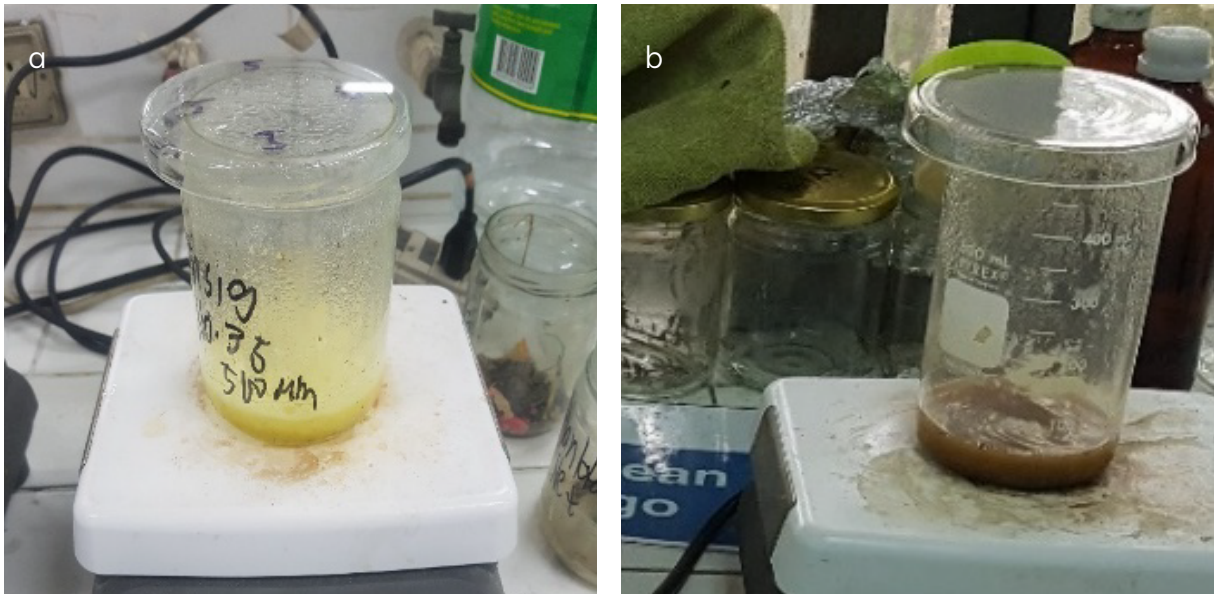


Figure 22. Digestion of (a) Surface Water Samples and (b) Sludge Samples

> **NOTE:** A high concentration of organic matter can be visually assessed if leaves, worms, grease, and other contaminants are present in the wastewater samples. Turbidity of the samples can also be an indicator of the presence of contaminants.

> **NOTE:** Magnetic stirring hotplates used in the laboratory have a digital interface that can be used to set the desired temperature. They also have a magnetic stirring function to constantly stir the heated samples. To compensate for possible temperature fluctuations, a thermometer can be used to monitor the required temperature.

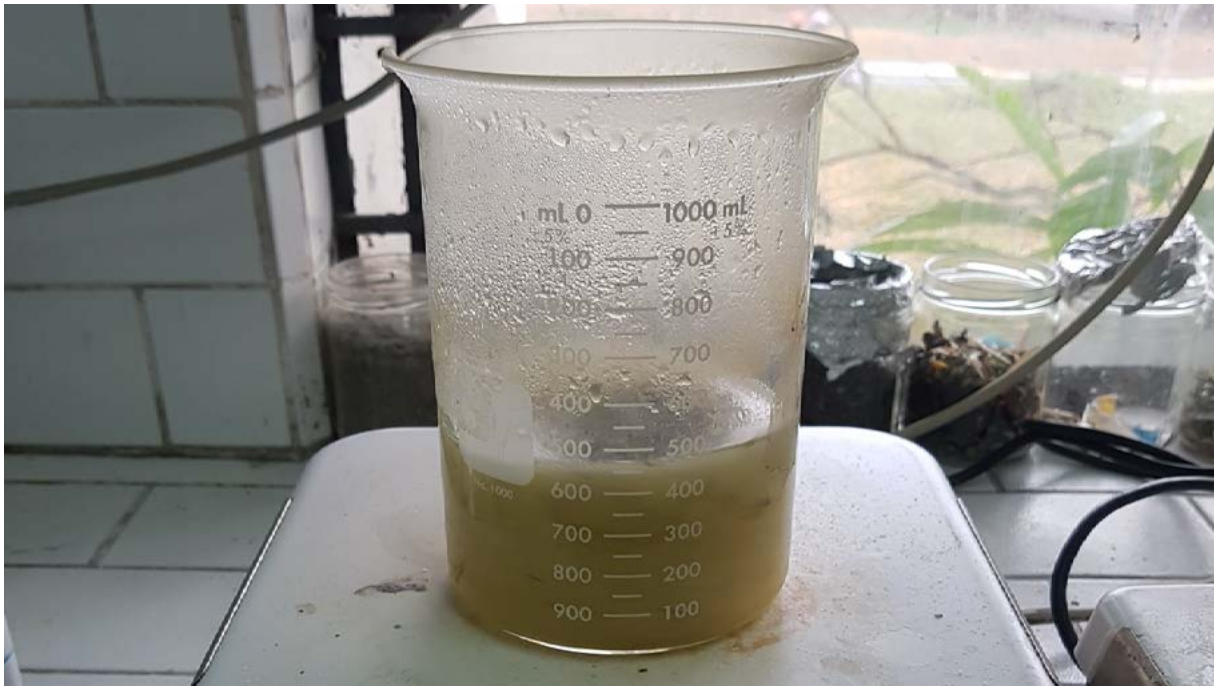


Figure 23. Presence of gas bubbles as an indicator of a violent reaction and digestion process

> **NOTE:** During the digestion process, the mixture may turn yellowish and affect the color of MPs in the mixture. On the basis of previous experiences, some MPs have faded colors, especially MPs with a smaller particle size; however, their true colors can be perceived using a microscope.

> **NOTE:** Water is added to the solution to prevent it from spilling during a violent reaction. However, the amount of added water should be minimal. Larger heat-resistant glassware should be used to handle this risk.

5. In instances where natural organic materials continue to be visible, 20 mL of 30% H_2O_2 should be continually added until no reaction or presence of gas bubbles is observed.

> **NOTE:** Throughout the WPO process, digestion of the sludge samples takes longer and requires higher quantities of 30% H_2O_2 compared with that of the surface water and wastewater samples, indicating that the sludge contains more organic matter than the collected solids in surface waters and wastewater samples.

Alternative Method	Advantage(s)	Disadvantage (s)	Materials
Use of a septic tank cleaner to remove organic matter in addition to Fenton's reagent (Lavoy & Crossman, 2021)	Septic tank cleaner, which is a premixed combination of four enzymes and bacteria, is inexpensive and widely available in hardware stores. Treatment with a septic tank cleaner reduces organic content more effectively than treatment using other materials.	There are insufficient number of studies that show that all septic tank cleaners are equally effective. The digestion process using septic tank cleaners takes longer than that performed with other materials.	Septic tank cleaner 30% hydrogen peroxide 95% sulfuric acid Iron catalyst ($FeSO_4$)
Use of enzymatic treatment	The contribution of the enzymatic approach in the further degradation of MPs is negligible (Löder et al., 2017). Technical grade enzymes are comparably inexpensive.	Use of enzymes in sample processing requires longer time of up to 16 days (Löder et al., 2017). Samples are filtered in between each step which may lead to particle loss/contamination (Schrank et al., 2022).	Sodium dodecyl sulfate Protease Phosphate-buffered saline Sodium Hydroxide Cellulase Hydrochloric acid (HClO) 35% hydrogen peroxide Chitinase
Use of acid digestion	Destruction of organic matter with acids is very effective (Schrank et al., 2022). It also has higher digestion efficiency than oxidation (Claessens et al., 2013).	Use of strong acids may lead to false-positive results and acidic degradation of certain polymers (Löder et al., 2017; Schrank et al., 2022).	Nitric acid (HNO_3) Hydrogen peroxide Sodium hydroxide
Use of alkaline digestion such as KOH and NaOH	Many polymers resist 10% KOH (UNEP, 2021). Utilizing a strong base is effective especially for samples with animal tissues such as fish and invertebrates (GESAMP, 2019).	Use of strong bases may lead to false-positive results and further degradation of some polymer types (Löder et al., 2017; Schrank et al., 2022).	Potassium hydroxide (KOH) Hydrochloric acid (HCl)

4.4. Inorganic Matter Removal

The density separation method is applied to change the density of water, thus enabling polymers with higher density to float on the water surface.

MATERIALS

a. Analytical-grade sodium chloride (NaCl)
b. Analytical mass balance
c. Watch glass
d. Glass stirring rods
e. Distilled water
f. Stainless steel laboratory spatula
g. Laboratory gown
h. Safety mask
i. Safety goggles
j. Nitrile gloves

0. All equipment and laboratory materials should be cleaned and rinsed with distilled water before conducting the removal of inorganic materials. Glassware should be preferred over plasticware to avoid sample contamination.

1. A total of 6 g of salt per 20 mL of the mixture should be added to the solution to increase the density of the aqueous solution (**Figure 24**).

Figure 24. Addition of laboratory-grade sodium chloride salt to the mixture

2. The sample should be stirred using a glass stirring rod until the salt completely dissolves. The glass stirring rod should be washed with distilled water while simultaneously ensuring that the postwash water falls directly into the solution to avoid loss of sample.

3. The glassware containing samples should be covered with a nonplastic cap or watch glass for at least 48 h to observe the level at which the suspected MPs float and the other heavier particles settle down (**Figure 25**).

Figure 25. Settling down of particles for at least 48 h

> **NOTE:** In many studies, high-density sodium chloride (NaCl; 1.202 g/mL) is added to the mixtures because NaCl is an inexpensive, accessible, and ecofriendly salt (Galgani et al., 2013). In addition, NaCl is an efficient broad-spectrum method of extracting plastics ranging from 0.91–0.97 g/mL for PE, 0.94 g/mL for PP, 1.05 g/mL for PS, and 1.14 g/mL for PVC (Van Cauwenberghe et al., 2015).

Alternative Method	Advantage(s)	Disadvantage (s)	Materials
Use of sodium polytungstate (PST) (1.4 g/cm^3), NaI (1.6 g/cm^3), and $ZnCl_2$ (1.7 g/cm^3) for density separation (GESAMP, 2019)	These salt solutions are denser than sodium chloride (1.2 g/cm^3), which results in high recovery of MPs during density separation.	These salt solutions are more expensive than sodium chloride. Procurement of the materials may be difficult due to uncertain product availability.	PST, Sodium Iodide (NaI), or Zinc Chloride ($ZnCl_2$)

4.5. Filtration

MATERIALS

a. Vacuum peristaltic pump
b. Vacuum filtration setup
c. 47-mm Whatman® GF/C filters with 1 µm pore size
d. Petri dish
e. Distilled water
f. Stainless steel tweezers
g. Marker and labels
h. Laboratory gown
i. Safety mask
j. Safety goggles
k. Nitrile gloves

0. All equipment and laboratory materials should be cleaned and rinsed with distilled water before filtration. Glassware should be preferred over plasticware to avoid sample contamination.

1. The vacuum pump should be connected to the vacuum filtration setup using silicon tubing (**Figure 26**).

Figure 26. Filtration setup using a pump

2. The filter paper should be placed in the setup (**Figure 27**), and the supernatant should be slowly poured from the glass jars containing the samples (**Figure 28**).

Figure 27. Placement of the filter paper in the filtration setup

Figure 28. Transfer of supernatant from the mixture to the filtration setup

3. The pump should be turned on until the supernatant water is filtered (**Figure 29**).

Figure 29. Filtration of the supernatant containing MP particles

4. The filter paper containing the suspected MP particles should be carefully removed (**Figure 30**).

Figure 30. Filter paper containing the suspected MP particles

5. The filters used should be placed individually in their respective labeled petri dishes; further, they should be covered (**Figure 31**).

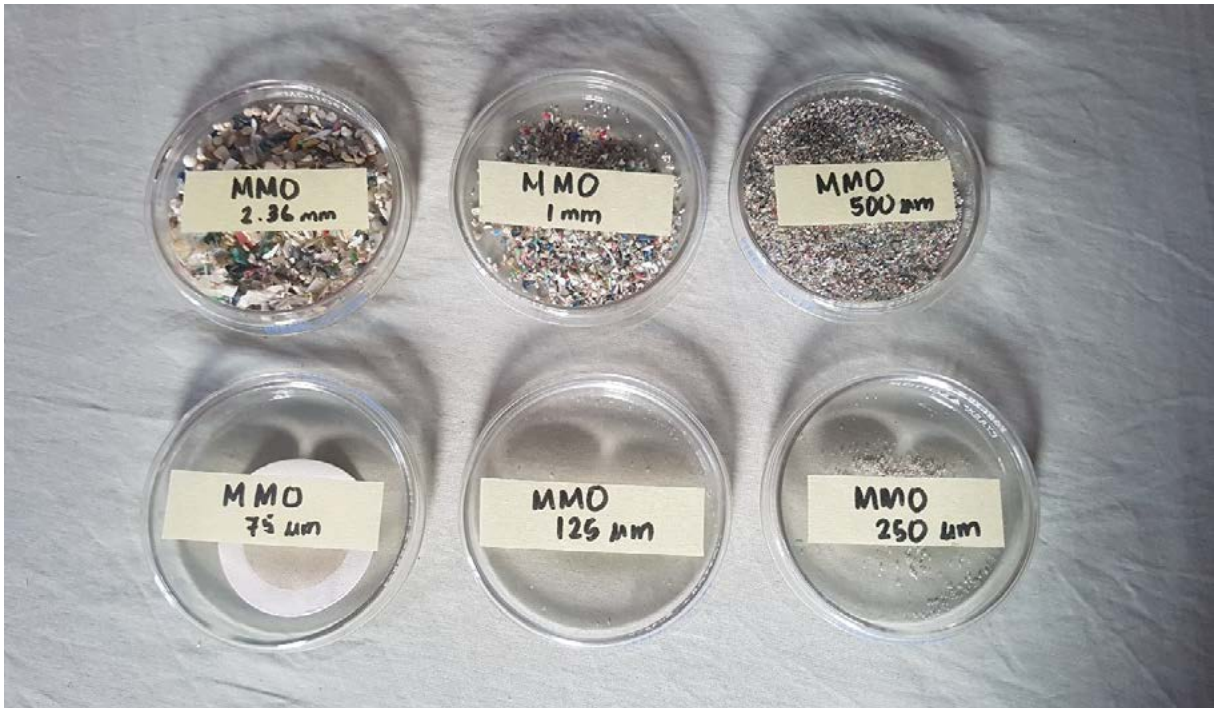


Figure 31. Filtered samples in labeled petri dishes

6. The filtered liquid should be disposed into the hazardous waste storage container. The collected liquid should be sent to a hazardous liquid waste treatment specialist for proper treatment.

7. The transfer apparatus should be washed with distilled water multiple times to minimize any sample loss due to the adhesion of MPs to the walls of the filter apparatus and beaker/glass jars.

8. The covered filters/samples should be air dried for further quantitative and qualitative analyses.

Method	Advantage(s)	Disadvantage (s)	Materials
Filtration without using a vacuum filtration setup	If there are any budget concerns, then there is no need to buy additional equipment.	Filtration and air-drying of samples takes longer. The supernatant may not be able to pass through filter papers with smaller pore sizes.	Filter paper Funnel
Use of a fine mesh screen	Fine mesh screens are inexpensive compared to other filtration methods. No vacuum filtration setup is required.	The pores of fine mesh screens are relatively larger than those of glass fiber filters; this limits the analysis of smaller MP particles.	Stainless steel fine wire mesh Funnel

Chapter 5
SAMPLE CHARACTERIZATION

5.1. Visual Assessment

The simplest method for the identification of MPs is visual identification. This assessment can be performed on MP particles that are visible to the naked eye only (>500 microns). Although this technique is more time consuming than other identification methods, it is often the most appropriate for studies characterized by high-volume samples, limited resources, and restricted access to expensive analytical instruments.

MATERIALS

a. Petri dishes containing samples
b. Steel tweezers
c. Nitrile gloves
d. Laboratory gown

0. Sealed samples in their respective containers should be properly labeled before visual analysis to avoid mixing up the samples from one sampling point to another. Performing the visual analysis should be avoided in windy and busy environments to avoid contaminating the samples.

1. The MP particles should be counted (**Figure 32**) per size class (**Table 4**) (i.e., 2.56 mm–4.75 mm sampling size class), according to their shape (**Table 5**), and color (**Table 6**) by visual observations.

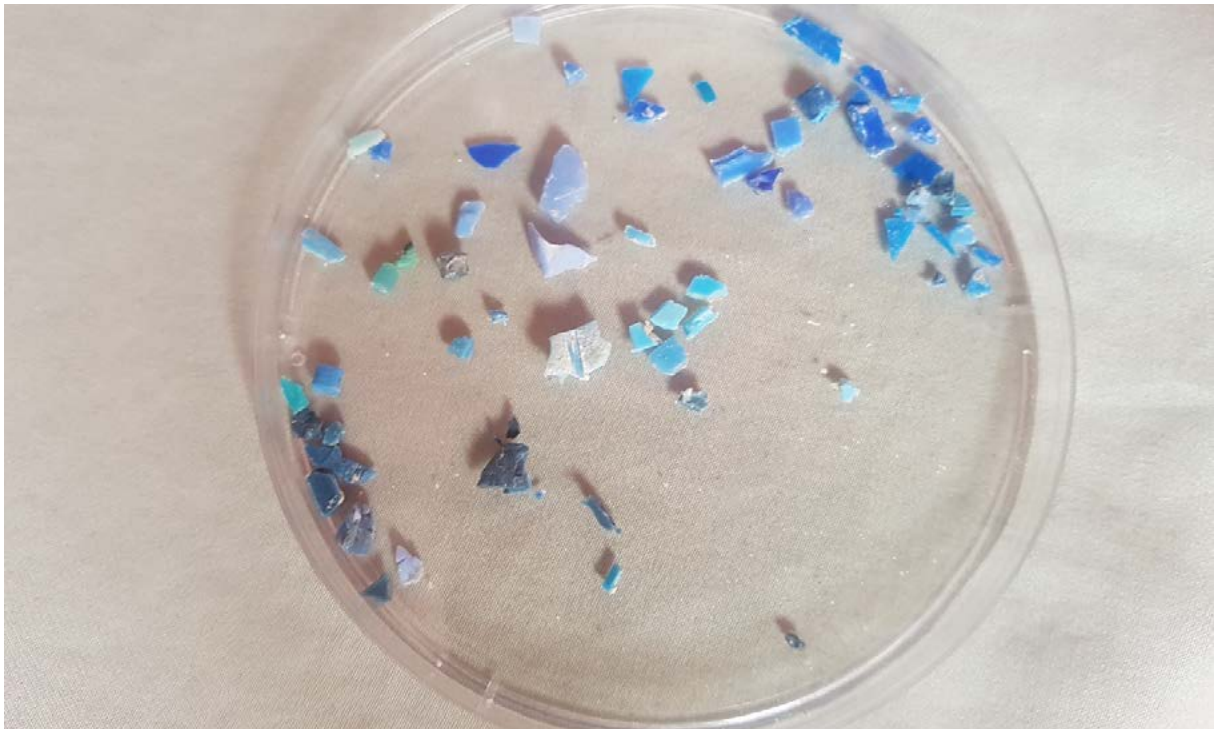


Figure 32. Identification of blue fragments as MP particles

TABLE 4. MP size classification

Sieve No.	Size Range
4	2.36 – <5mm
16	1 – <2.36mm
50	500 – <1000µm
100	250 – <500µm
200	125 – <250µm
> 200	75 – <125µm

NOTE: Past studies have used different sets of mesh sieves because the size distribution is not standardized. Similar to the methodology used by IGES (2023), size classification depends on the sieves used during sample collection. Table 4 can be modified by the user. Furthermore, some smaller particles may be mixed with larger particles. The size of these particles can be directly measured using a ruler or through a microscope equipped with a camera. The count of these particles should be added to the correct size range.

TABLE 5. MP shape classification

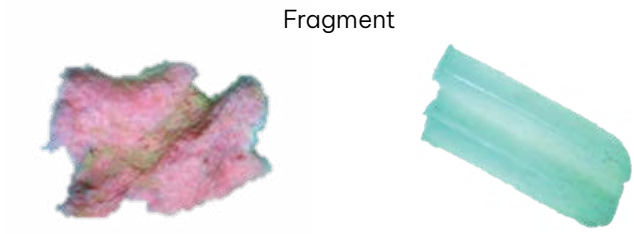
Shapes	Description
Fiber / Filament 	Elongated Thin Fibrous
Film 	Thin Soft Transparent Flat
Fragment 	Hard Jagged Angular/Flat
Foam 	Lightweight Porous Sponge-like plastic Can be round
Pellet 	Hard Spherical Ovoid/Round

TABLE 6. MP Color Classification

Categories	Color
White	
Transparent	
Red	
Orange	
Yellow	
Green	
Blue	
Black	
Other colors	

NOTE: The classification of colors may also be simplified to white, colored, and black particles. However, it is recommended to categorize MPs into more colors. The colors of plastics play key roles in MP formation and their environmental effects. Different color systems have different color wavelengths, which may affect plastic photoaging. Zhao et al. (2022) hypothesized that the longer the color wavelength, the stronger the light absorbance, the lower the UV resistance, and thus, the lower the photoaging rate. Blue plastics cannot absorb UV light efficiently, age faster in the sun, and exhibit more intense photoaging than both red and yellow plastics. This explains the abundance of bluish MPs in the environment with smaller particle sizes. On the other hand, larger MPs are usually observed to be reddish in color.

2. The count of the MPs should be charted into a spreadsheet on the basis of sampling point, size, shape, and color (**Table 7**).

TABLE 7. Sample sheet for the input of data

			Clear	White	Black	Red	Orange	Yellow	Green	Blue	Others	Total
Influent	No. 4	Filament		1	34	5				3		43
		Film	22									22
		Foam		2	3	1			4			10
		Fragment		2	6	2			4	3		17
		Pellet										0
	No. 16	Filament				3			1	2		6
		Film	4	2				5				10
		Foam										0
		Fragment	3			40		10	7	76	1	140
		Pellet										0
	No. 50	Filament		1	1	3				3		8
		Film										0
		Foam		1								1
		Fragment	8		2	14	1	1	36	64		126
		Pellet										0
	No. 100	Filament		1	1	3				3		8
		Film										0
		Foam		1								1
		Fragment	8		2	14	1	1	36	64		126
		Pellet										0
	No. 200	Filament								1		1
		Film										0
		Foam										0
		Fragment				5		2	2	11		20
		Pellet										0

3. After the counting of MPs, petri dishes containing the samples should be sealed with appropriate labels.

5.2. Microscope Analysis

MATERIALS

a. Stereomicroscope equipped with a camera
b. Lighting system
c. Computer
d. Digital camera control software
e. Image processing software
f. Steel tweezers
g. Laboratory gown
h. Safety mask
i. Nitrile gloves
j. Wipes, preferably Kimtech Science™ Kimwipes®

0. The watch glass of the microscope should be cleaned with wipes before analysis. Check the watch glass for any unwanted particles.

1. The microscope should be focused on the particles on the petri dish.

> **NOTE:** An optical microscope with 4000x magnification is highly recommended to capture images of MPs with sizes of up to 1 m.

2. The extracted MPs were examined and photographed using a camera connected to the microscope (**Figure 33**). The observed MPs were classified based on size (**Figure 34**), shape (**Figure 35**), and color (**Figure 36**) using the same classification standards used in the visual assessment (**Table 4, Table 5**, and **Table 6**).

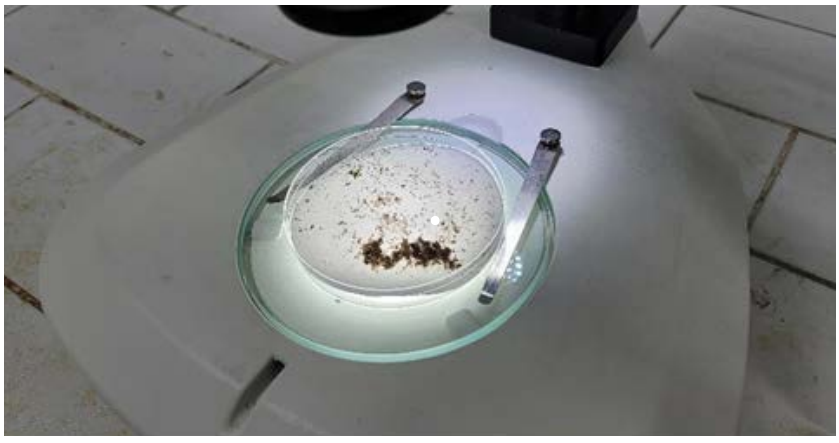


Figure 33. Microscopic analysis of MPs

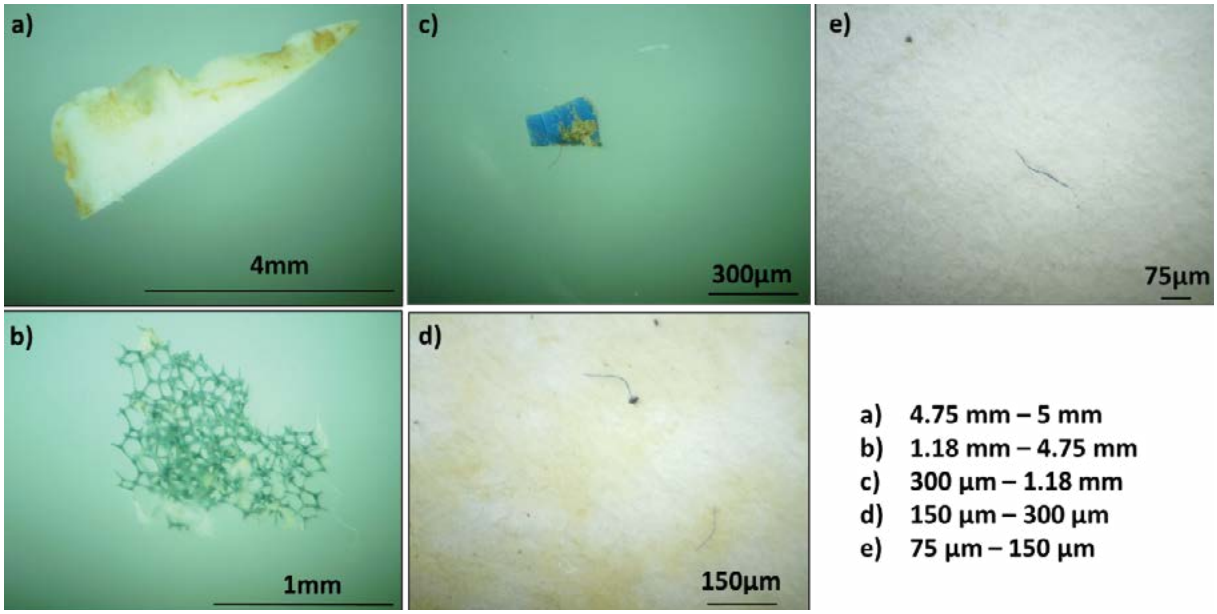


Figure 34. Sample photographs of MPs characterized by size by using a microscope

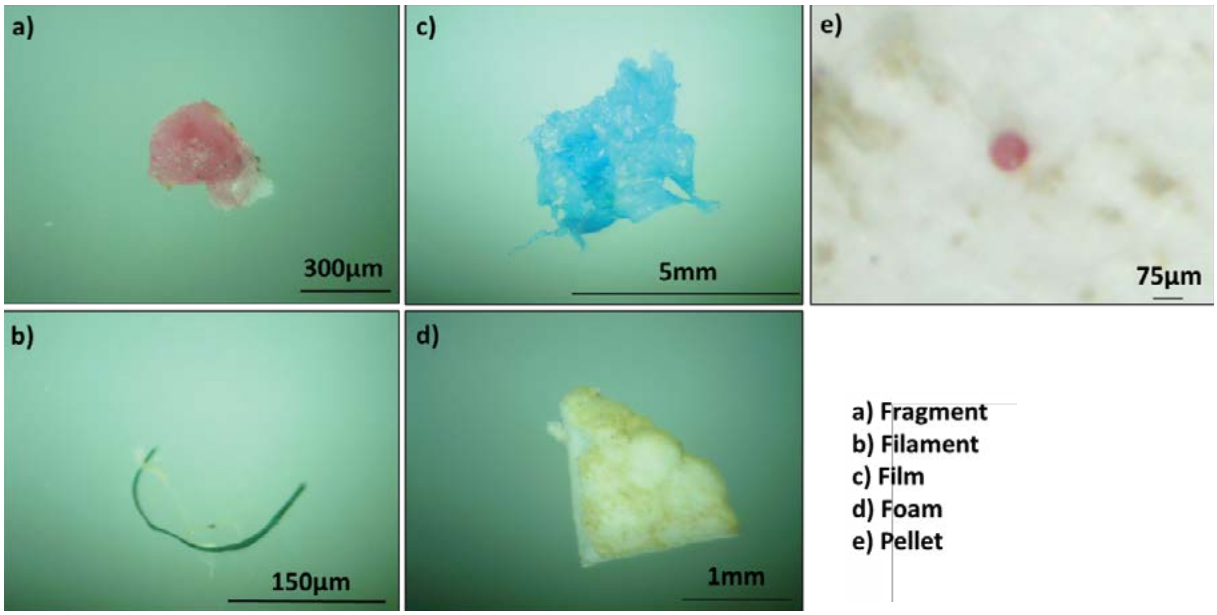


Figure 35. Sample photographs of MPs characterized by shape using a microscope

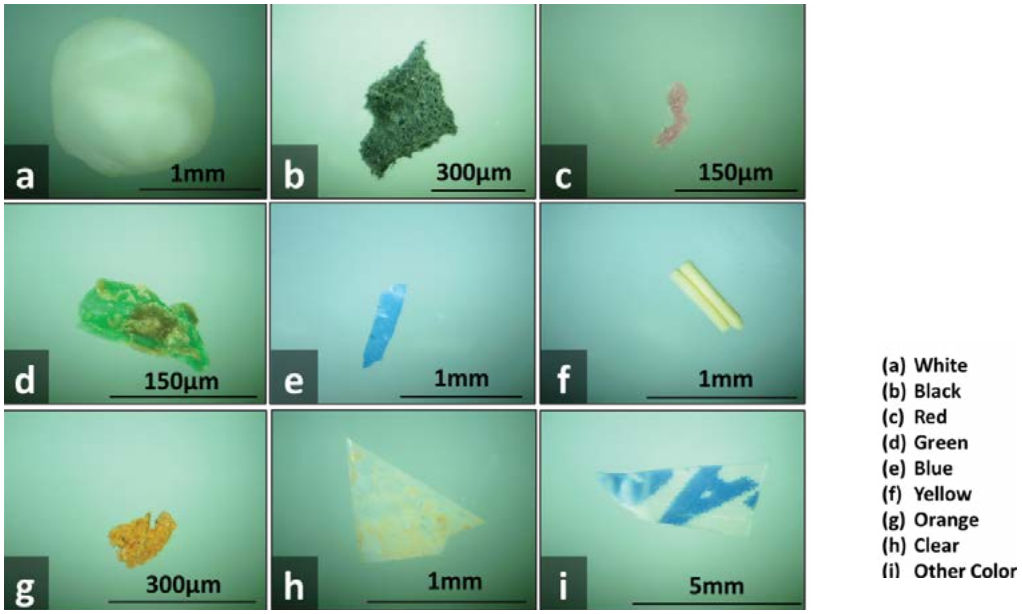


Figure 36. Sample photographs of MPs characterized by color using a microscope

> **NOTE:** To avoid counting some microscopic biological materials that may be wrongly classified as plastics (Ling et al., 2017) and to assess the identity of some particles that lost color due to peroxide digestion, the following criteria should be employed to visually identify MPs: (1) larger particles that cannot be easily fragmented using tweezers and (2) particles that do not exhibit tissue and cell structures should be considered as MPs (Cole et al., 2011; Hidalgo-Ruz et al., 2012).

3. The particles retained in the filter paper should be observed in a Z-shaped pattern of movement from left to right (**Figure 37**).

Figure 37. Observation of MP particles on filter paper using a Z-shaped pattern

4. In case of stray particles (especially smaller particles) that do not belong to the correct size range, the length and area of MPs should be measured by using image processing software after calibration with a standard (**Figure 38**). The count of these particles should be added to their correct size range.

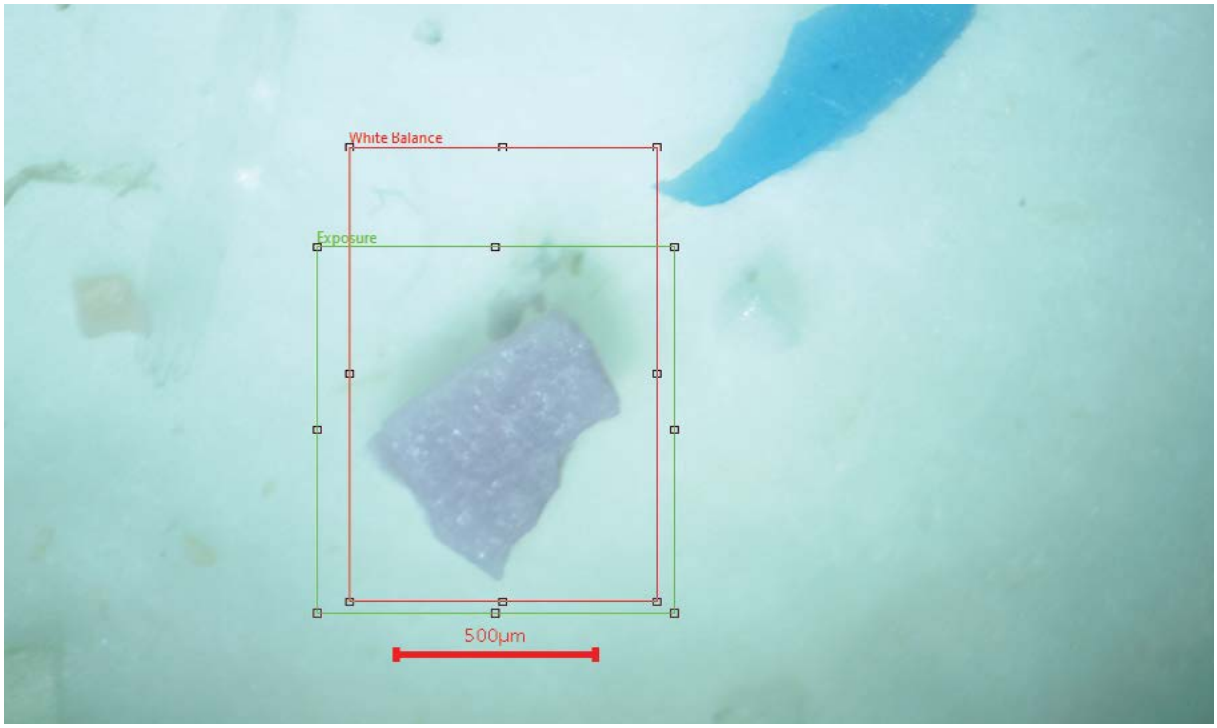


Figure 38. Measurement of length of MPs using image processing software

> **NOTE:** A microscope camera control software is used for image processing. This software can correct and adjust the luminance and color of the images to determine the colors of the MPs more accurately, especially those of MP fibers. The scale was calibrated with a standard ruler and subsequently used to measure the largest dimension of the MPs. In the case of unavailability of camera and imaging software, the particle sizes can be assumed to be within the range of the sieves used during sampling.

5. The count of MPs should be charted into the spreadsheet based on the sampling point, size, shape, and color.

TABLE 8. Sample sheet for input of data

			Clear	White	Black	Red	Orange	Yellow	Green	Blue	Others	Total
Effluent	No. 4	Filament										0
		Film										0
		Foam										0
		Fragment										0
		Pellet										0
	No. 16	Filament		2		1			2	3		8
		Film										0
		Foam		1								1
		Fragment		2	1				2			5
		Pellet										0
	No. 50	Filament		3		3		2	2	2		12
		Film	1	4								5
		Foam										0
		Fragment	1	6	1	2			1	2		13
		Pellet										0
	No. 100	Filament	4							2		6
		Film	3					1				4
		Foam										0
		Fragment	3			5				4		12
		Pellet										0
	No. 200	Filament	1		1	2		5	1	3		13
		Film		6								6
		Foam										0
		Fragment								5		5
		Pellet										0

6. The watch glass should be cleaned regularly with wipes, and the petri dishes containing the samples should be sealed with appropriate labels.

Alternative Method	Advantage(s)	Disadvantage (s)	Materials
Use of fluorescent dyes such as Nile Red in the identification of MPs	Identifying MPs using the staining method is efficient, less expensive, and time-saving compared with other MP identification methods. The staining method minimally contributes to the degradation of MPs in samples (Gao et al., 2022). Nile Red staining can help identify smaller MPs with sizes < 20 µm using automated image processing (Shruti et al., 2022).	There is lack of a standardized procedure for using staining dyes to identify MPs. As the remaining organic matter in treated samples would be stained and fluoresced, this could affect the quantification of MPs (Shruti et al., 2022).	Nile red dye Methanol Fluorescence microscope

5.3. Spectroscopy

False-positive MPs have been identified in numerous studies; hence, a subset of samples should be selected for spectroscopy to verify the accuracy of visual identification by determining the polymer composition of the selected particles..

The extracted particles were grouped (**Figure 39**), and representative MPs in each group were chosen to test the polymer types. This is implemented to strike a compromise between labor and cost.

Figure 39. Particles with similar shapes and colors are grouped for spectroscopy analysis

5.3.1. Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared (FTIR) spectroscopy has been widely used to identify the polymer composition of MPs because of its high reliability. Attenuated total reflectance FTIR (ATR-FTIR) can determine the polymer type of selected particles with sizes >500 microns, whereas micro-FTIR (μ-FTIR) can identify the polymer type of particles with sizes <500 microns.

> **NOTE:** The authors of a previous study used ATR-FTIR because μ-FTIR was not available during the study period. Therefore, the procedure for ATR-FTIR is discussed in this section. However, if μ-FTIR is available, the authors recommend this method for the identification of polymer-type MP particles (<500 microns).

MATERIALS

a. Nicolet 6700 FTIR ATR spectrometer equipped with a Smart Orbit diamond ATR accessory
b. Spectroscopy software (i.e., BioRad's KnowItAll® Informatics System)
c. Computer
d. Precision steel tweezers
e. Wipes, preferably Kimtech Science™ Kimwipes®

0. The diamond ATR accessory should be regularly cleaned with wipes and distilled water to prevent contamination of the samples during analysis. A blank test should always be performed before the analysis of each particle.

1. The particle to be analyzed should be inserted into the diamond ATR (**Figure 40**). It should be ensured that the particle covers the diamond area.

Figure 40. Setting up the analyzed particle for spectroscopy

2. A total of 32 scans at a resolution of 4 cm−1 should be obtained; further, each spectrum generated in the spectral range of 4000–400 cm−1 should be recorded (**Figure 41**).

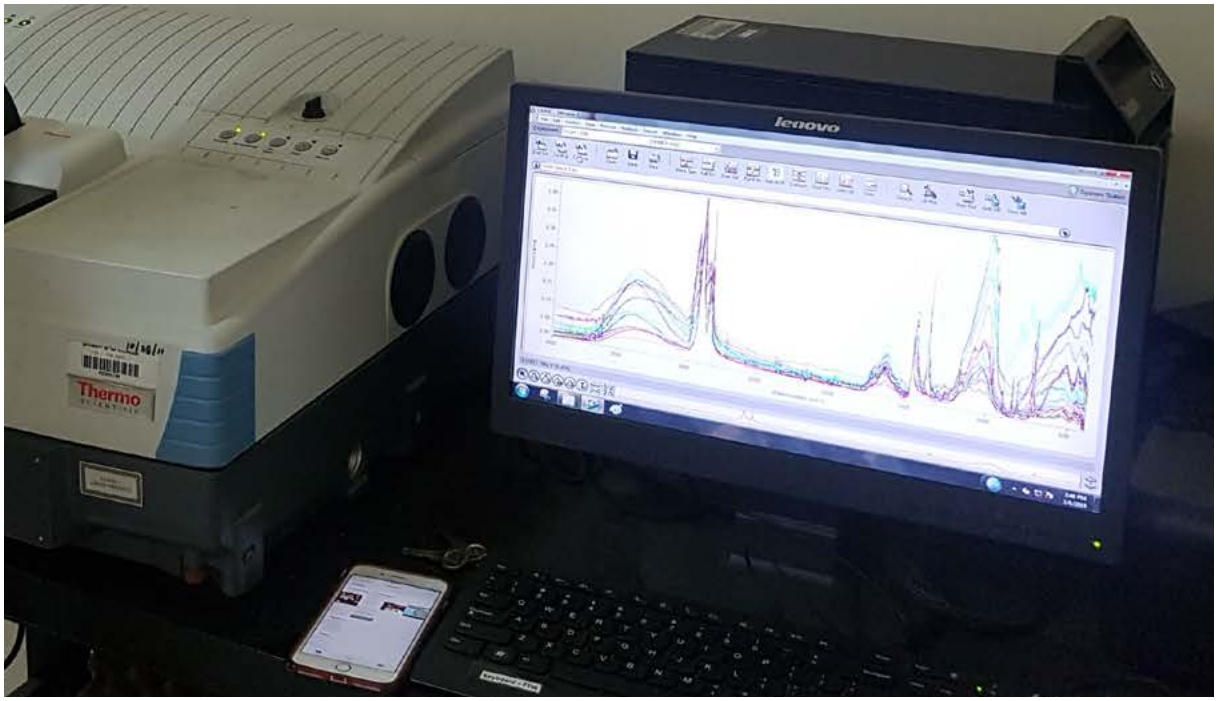


Figure 41. Generated spectrum for the analyzed particle

3. Each obtained spectra should be analyzed by using the spectroscopy software (i.e., BioRad's KnowItAll® Informatics System software) based on the FTR spectral library that contains an intensive database of known compounds. Each sample spectrum should be matched to several potential reference spectra, and the most appropriate match should be selected on the basis of matching peak wavenumber positions (**Figure 42**).

> **NOTE:** Spectroscopy software (i.e., BioRad's KnowItAll® Informatics System) performs optimized corrections for spectral matching, including intensity distortion, interdependent corrections of the baseline, and even axis shift, with further manual correction possible for correcting noise and baseline (Horton, et. al., 2016).

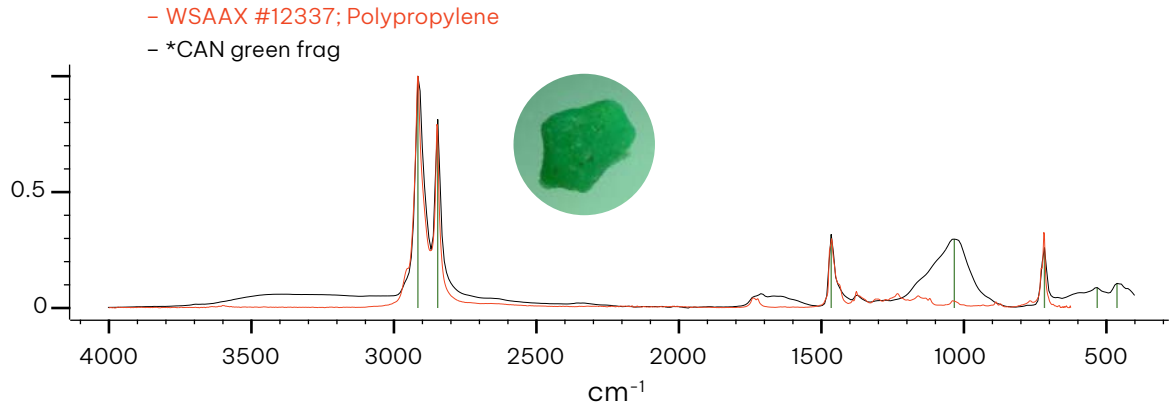


Figure 42. Sample–analyzed green fragment particle identified as polypropylene

> **NOTE:** Only those spectra that matched over 70% with the standard database were acceptable, indicating that the verification of MPs was reliable (Zhao, et. al., 2018).

5.3.2. Raman Spectroscopy

Raman spectroscopy is considered to be an efficient method for analyzing polymer morphology and providing valuable information about orientation effects and the crystalline structure of the polymer under investigation. FTIR is preferred over Raman spectroscopy for polymer identification because of the latter's issues with sample fluorescence. Most plastic materials are rarely pure polymers and are typically made of composite polymers. This may interfere with the Raman signal and induce thermal degradation of the sample.

> **NOTE:** If μ-FTIR is not available, μ-Raman spectroscopy can be used to identify the polymer type of smaller particles (<500 microns).

MATERIALS

a. μ-Raman spectroscopy setup
b. Spectroscopy software (i.e., BioRad's KnowItAll® Informatics System and OriginLab® softwares)
c. 532-nm continuous wave laser (Ventus, Laser Quantum)
d. Optical microscope
e. Imaging spectrometer (Shamrock 303i, Andor)
f. Computer
g. Precision steel tweezers
g. Nitrile gloves
h. Wipes, preferably Kimtech Science™ Kimwipes®

0. The watch glass of the microscope should be regularly cleaned with wipes and distilled water to prevent contamination of samples during analysis. A blank test should always be performed before the analysis of each particle.

1. The particles to be analyzed should be placed under an optical microscope (**Figure 43**).

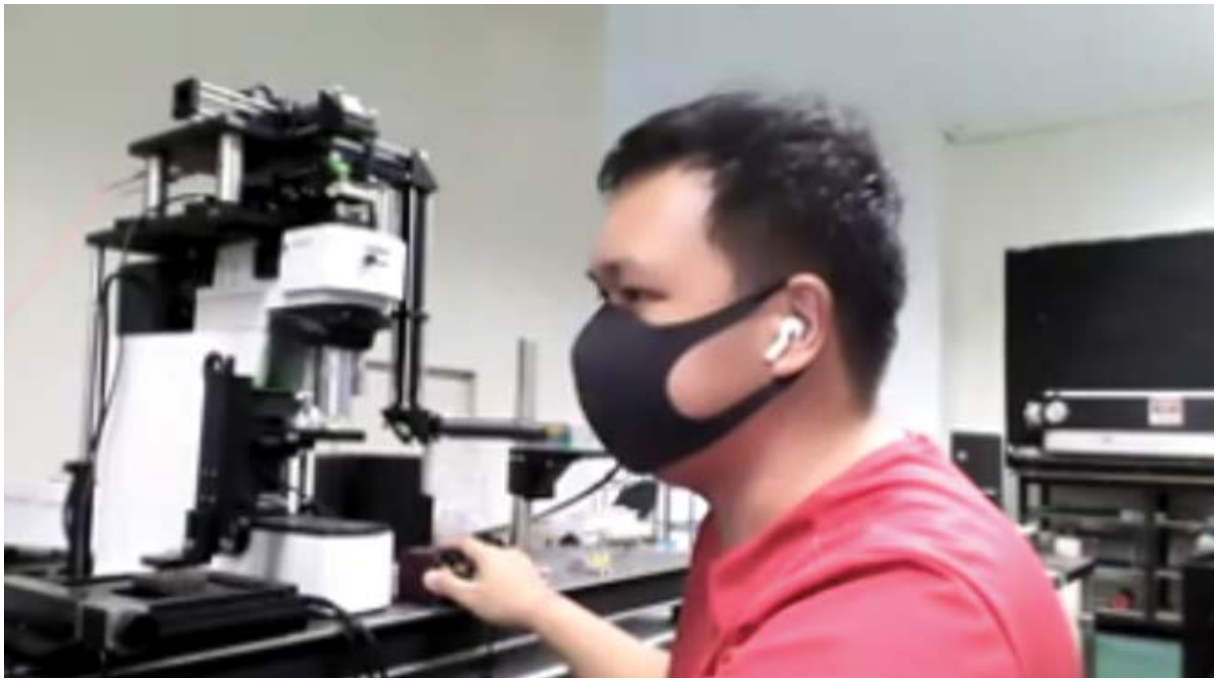


Figure 43. Analysis of particles using Raman spectroscopy

> **NOTE:** The samples were optically excited using a 532-nm continuous wave laser (Ventus, Laser Quantum) with a laser output power of 10 mW. The laser beam is directed to an optical microscope (Olympus BX51M) through a set of optical components. The 50× objective lens (Mitutoyo) of the microscope is used to focus the beam onto each sample. The backscattered optical emission of each sample is collected using the same set of optics and is subsequently fiber-fed to an imaging spectrometer (Shamrock 303i, Andor) with a grating density of 1200 lines/mm. The spectrometer disperses the optical emission and is projected onto a charge-coupled device (CCD) camera (iDus 401, Andor). The scan range was set from 200–3000 cm-1 and the acquisition time was set to 10 s (IGES , 2022).

2. Raw spectral data should be obtained.

3. A graph should be plotted using the obtained data and baseline correction should be performed using spectroscopy software (**Figure 44**).

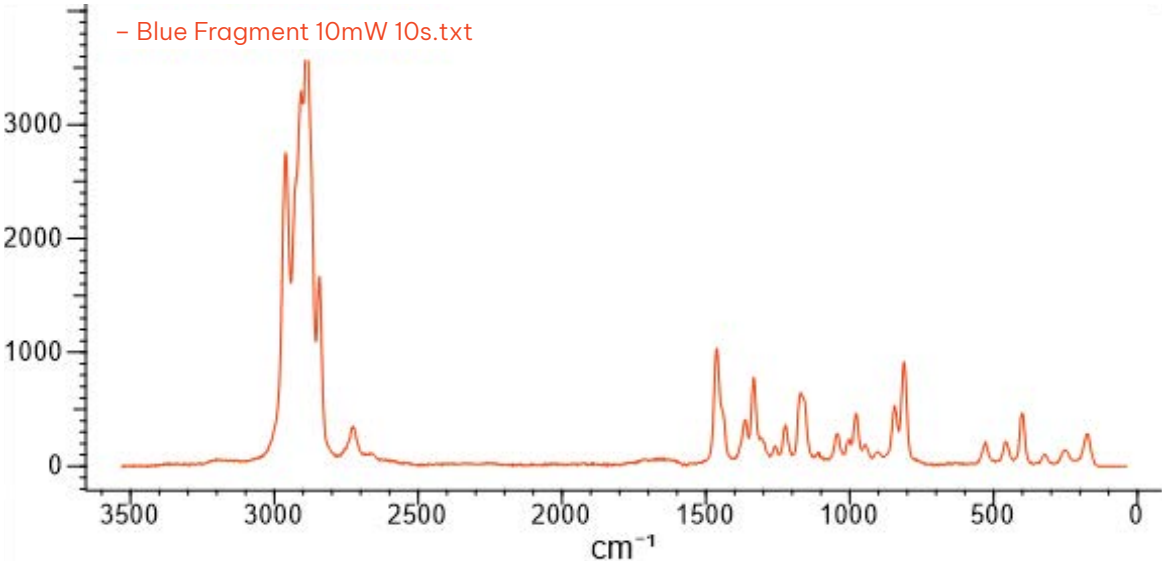


Figure 44. Sample baseline corrected spectral data using BioRad's KnowItAll® Informatics System

4. Each sample spectrum should be matched to several potential spectra from the related literature. The most appropriate match should be selected on the basis of the matching peak wavenumber positions (**Figure 45**).

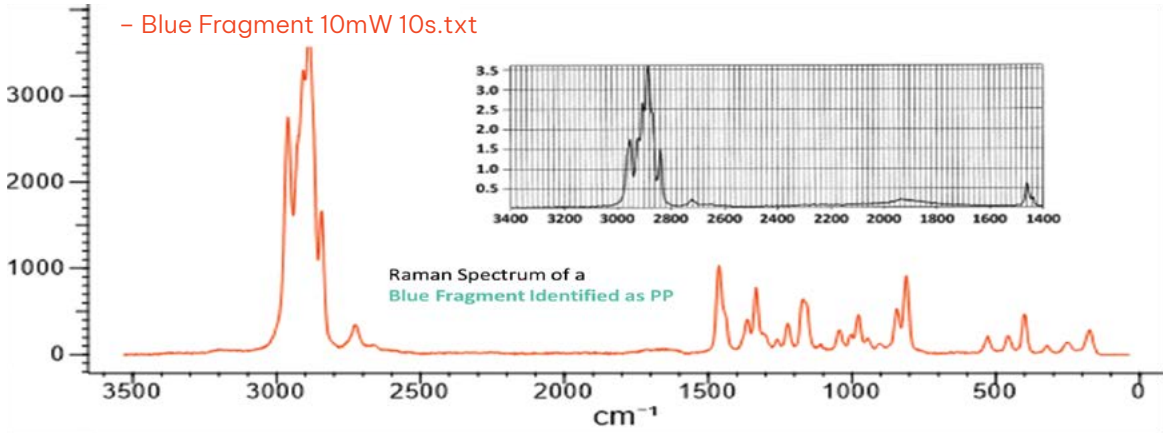


Figure 45. Sample Raman spectrum of a blue fragment identified as PP by using a database from related literature

> **NOTE:** Matching the spectra of the analyzed particles can be challenging since the identification of polymer type relies solely on the available related literature.

Chapter 6
QUALITY ASSURANCE AND QUALITY CONTROL

The following quality assurance and quality control (QA/QC) measures should be performed during the collection, pretreatment, and characterization of samples to ensure the accuracy and prevention of contamination, accumulation, and loss of MP particles (**Table 9**).

TABLE 9. Quality assurance and quality control

Aspect	Quality Assurance and Quality Control
General	All equipment, including laboratory equipment used for sampling and analysis of MPs, should not contain plastic materials because these may cause the addition of MPs to the collected samples, especially those that are not visible to the naked eye, thus skewing the results. Unpainted metal, glass, and wood are acceptable materials for MP sampling and analysis.
	The personnel performing the experiment and analysis should wear personal protective equipment such as laboratory gowns, safety masks, and nitrile gloves. It is also recommended to use 100% cotton laboratory gowns during sample processing to avoid contamination.
Sample Collection	It is recommended to have a negative control or blank test during air-drying; however, this entails additional costs for materials such as sieves; hence, to ensure that loss and contamination of MPs are prevented, air-drying should be performed in a controlled environment near the sampling point. The location for air-drying should not be exposed to strong winds, busy roads, or other human activities that may affect and contaminate the samples.
	Before storing the collected wastewater and stormwater samples, the containers should be washed and rinsed with distilled water.
	The extracted samples should be carefully transferred from the sieves to their respective glass containers using minimum cleaning via distilled water from a squirt bottle; subsequently, these containers should be covered with aluminum foil to prevent spillage and contamination.
Laboratory Analysis	The entire sample processing should be conducted in a laboratory with a controlled environment.
	Laboratory ware should be thoroughly washed with distilled water and dried; after sample collection, they should be covered with aluminum foil and subsequently used for analysis.

Aspect	Quality Assurance and Quality Control
Laboratory Analysis	Collected solids in the glass containers are transferred to a clean beaker using minimal rinsing performed via a squirt bottle containing distilled water, and the beaker is immediately covered with a watch glass to avoid spillage and contamination.
	The samples stored in glass petri dishes should always be covered to reduce the period of exposure.
	The beaker and all the transfer apparatus should be washed with distilled water multiple times to minimize any sample loss owing to the adhesion of MPs on the walls of the filter apparatus; further, all washing solutions should be filtered through the same glass fiber filter.
	Subsequently, the extracted MP particles should be individually sealed in covered glass petri dishes. The MPs can be retained in the petri dish where the sample was stored; therefore, the petri dish should be occasionally analyzed under a microscope.
	All filter nets used for MP analysis should be thoroughly cleaned in distilled water, dried, and analyzed under a microscope with a magnification of 40–45× for assessing the presence of external contamination. Clean filter nets should be stored with a cover of aluminum foil.
Blank Test	The distilled water used to rinse the laboratory apparatus used for detecting MP particles should be assessed for contamination using density separation and microscopic analysis.
	The Whatman® filter papers and petri dishes should be subjected to microscopic analysis before the filtration process of the samples to assess whether they are contaminated with MPs.
Spectroscopy Analysis	The crystal of the ATR-FTIR should be cleansed using Kimtech Science™ Kimwipes® and ethyl alcohol (96%) before and after analyzing each particle. Before the start of every FTIR analysis, a blank background scan should be performed.

Chapter 7
GAPS AND CHALLENGES

The gaps and challenges experienced during MP sample collection, pretreatment, and characterization are discussed below (**Table 10**).

TABLE 10. Identified gaps and challenges in MP-related studies

Aspect	Current Methodology	Gaps/Challenges	Proposed Recommendations
Sample collection	Only MP particles of sizes >75 microns are extracted because of the nonavailability of sieves with lower mesh sizes.	Smaller-sized MPs (<75 microns) were not investigated. It is possible that wastewater samples may contain MP particles with sizes less than 75 microns (such as microbeads).	A representative sample/ volume should be collected. It should be passed through the sieve with the lowest mesh opening. The collected sample should undergo density separation and microscopy analysis. However, a microscope with higher magnification is required to identify the smaller MPs in the samples.
	Containers are used for the collection of samples in receiving waters instead of nets.	Some guidelines and published studies usually use nets instead of containers for sample collection in surface waters.	It is recommended to use cheaper and readily available materials for sample collection, such as buckets. The use of nets is not applicable in study areas with the presence of macroplastics and large debris along the chambers of the STPs and the receiving waters.

Aspect	Current Methodology	Gaps/Challenges	Proposed Recommendations
Sample Pretreatment	NaCl is an efficient broad-spectrum means of extracting plastics from common polymers such as PP, PS, and PE. NaCl is used as a salt for density separation because it is easily accessible, ecofriendly, and inexpensive.	The density of NaCl is lower than that of other polymers such as PET, PVC, and acetate.	Other salts, such as zinc chloride, may be used to extract heavier polymers. However, this incurs additional costs due to its higher price compared with sodium chloride. These laboratory-grade salts are also not readily available and may require importation.
	Samples continue to be recommended for organic matter removal.	There is an unavailability of hydrogen peroxide and Fe solutions.	Samples can be treated without organic matter removal; however, organic matter will continue to be present in the mixtures; hence, extracting MPs will be more challenging. The use of a salt solution can facilitate the separation of organic and inorganic matter from MPs.
Sample Characterization	Raman spectroscopy is currently used to identify polymer type MP particles with sizes of >75 microns. With regard to FTIR, only ATR-FTIR is available in the Philippines that can only test particles of sizes >500 microns.	Some test particles, especially fibers and lines, are prone to thermal degradation because of issues with sample fluorescence in Raman spectroscopy.	In the absence of µ-FTIR to identify the polymer types, µ -Raman spectroscopy is a viable alternative. However, when using µ -Raman spectroscopy, the laboratory should be provided with extra particles of the same shape in case of thermal degradation. However, it is recommended to use ATR-FTIR for easily identifying the polymer types of larger particles (>500 microns).

Chapter 8
RESULTS PROCESSING AND DATA INTERPRETATION

Different studies have employed different methods to process and present the results. In this section, the characterization and analysis of MPs are patterned on the basis of most commonly used methods. The suggested Harmonized Protocol allows an effective comparison of MP abundance across the region.

8.1.1. Abundance Analysis

Currently, there is no uniform standard for quantifying the abundance of MPs in surface water, wastewater, and sludge worldwide. Several studies in Southeast Asia (**Figure 46**) have used the number of particles per volume (pieces/L or pieces/m^3) for quantifying the abundance of MPs in surface water and wastewater, whereas the number of particles per mass (pieces/kg) has been used for sediments, which can be applied to quantifying the abundance of MPs in sludge.

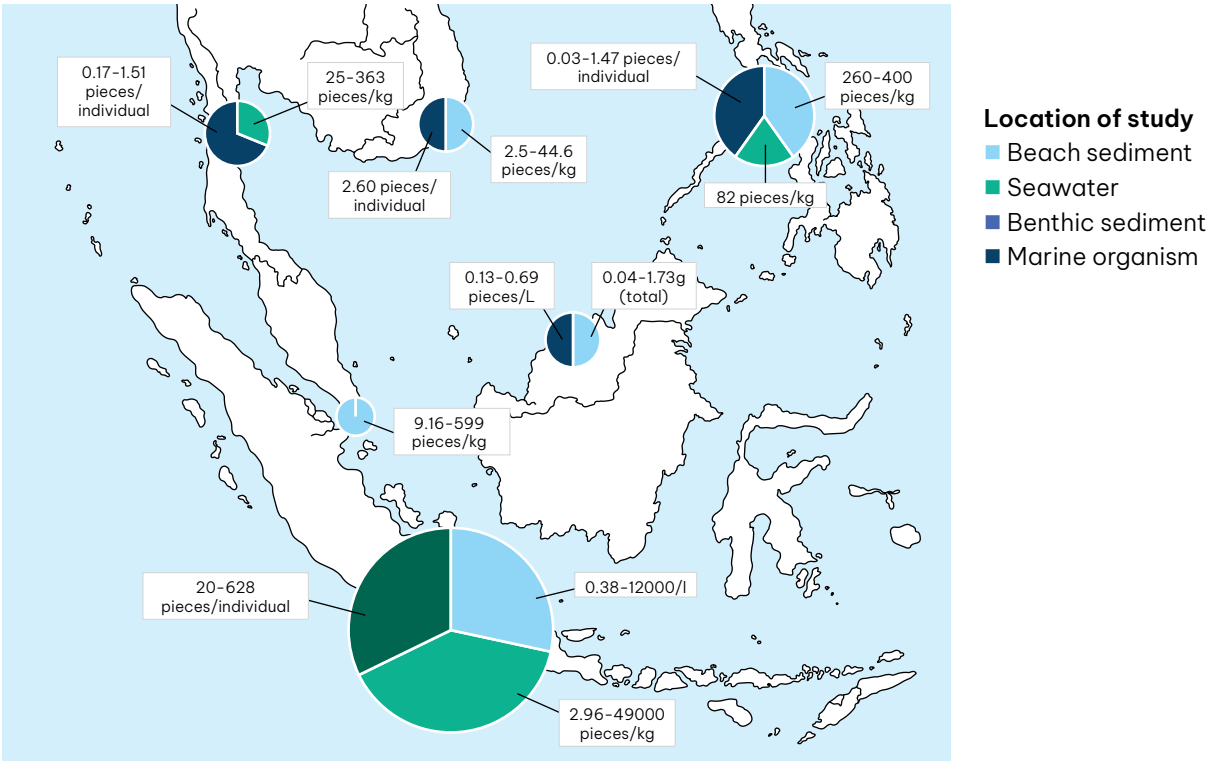


Figure 46. MP studies in the ASEAN region (Curren et al., 2021)

For this Harmonized Protocol, the abundance of MPs is quantified as particles/L, particles/m^3, and particles/kg for wastewater/surface water and sludge samples, respectively. Using the sample worksheet (**Table 7, Table 8**), the abundance can be computed using the equation below.

TABLE 11. Sample MP count in the influent sample of an STP

			Clear	White	Black	Red	Orange	Yellow	Green	Blue	Others	Total
Influent	No. 4	Filament		1	34	5				3		43
		Film	22									22
		Foam		2	3	1			4			10
		Fragment		2	6	2			4	3		17
		Pellet										0
	No. 16	Filament				3			1	2		6
		Film	4	1				5				10
		Foam							2	1		3
		Fragment				5			5	8	2	20
		Pellet										0
	No. 50	Filament			1	7		3	1	7	1	20
		Film						2				2
		Foam										0
		Fragment	3			40		10	7	78	1	140
		Pellet										0
	No. 100	Filament		1	1	3				3		8
		Film										0
		Foam		1								1
		Fragment	8		2	14	1	1	36	64		126
		Pellet										0
	No. 200	Filament								1		1
		Film										0
		Foam										0
		Fragment				5		2	2	11		20
		Pellet										0
Total			**37**	**8**	**47**	**85**	**1**	**23**	**62**	**181**	**4**	**449**

Abundance [particles/m^3] = **total count of particles** [particles] / **volume of sample collected** [m^3]

= 449 particles / 0.05 m^3 (or 50 L)

= **8,890 particles / m^3**

The abundance of the sampling points can also be mapped by using the Geographic information system (GIS) mapping software such as QGIS and ArcGIS.

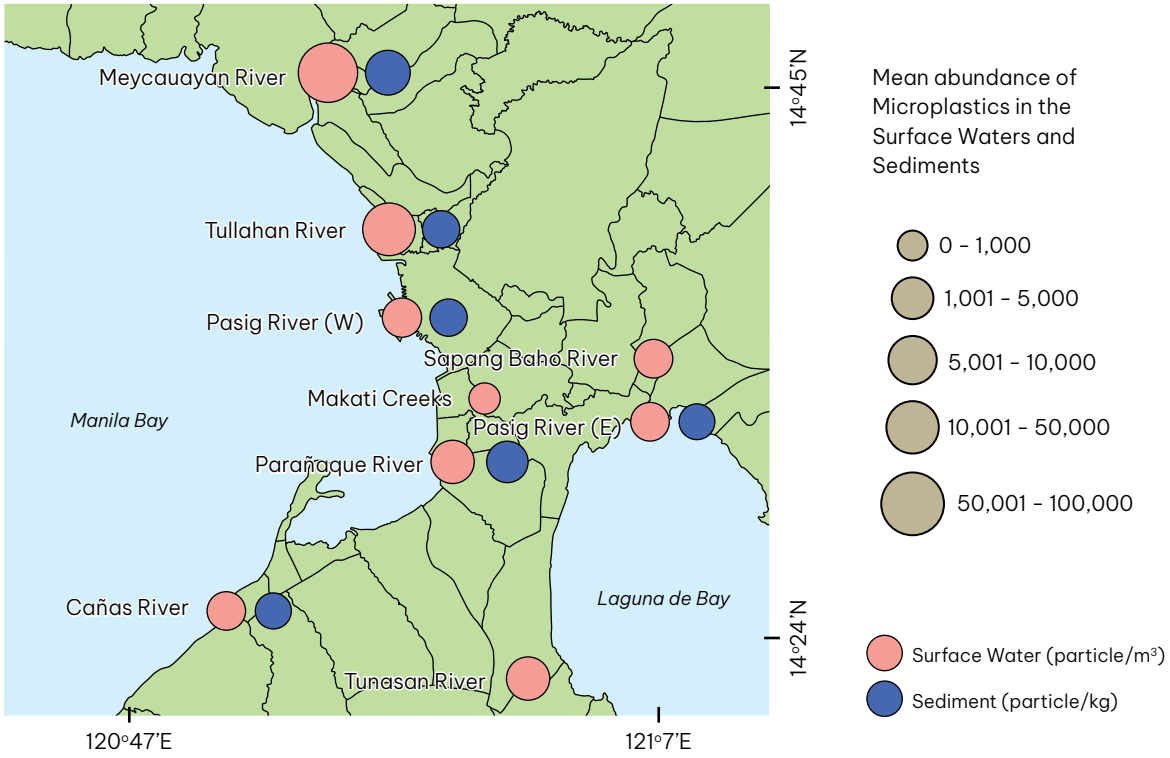


Figure 47. MP sample abundance map (Tanchuling & Osorio, 2020)

Comparison of the abundance of MPs found in samples with that in other studies can be performed if the studies have used the same units of measurement. However, it should be noted that these comparisons may be inconclusive because of differences in the methodology utilized, particle size under study, time and date of sampling, type of STP, influent and effluent rates, and river flow characteristics. Nevertheless, these studies provide an overview of the general conditions of MP contamination at each site (Osorio et al., 2021).

TABLE 12. Comparison of MP concentration of samples obtained from STPs

Facility (Technology)	Flowrate at Intake (m^3/day) [1]	Influent MP Concentration (particle/m^3) [2]	Effluent MP Concentration (particle/m^3) [3]	Removal Efficiency (%) [4] = |[2] x [3]| / [2] x 100	MP Concentration Discharged (x106 particle/ day) [5] = [1] x [3]
STP A (SBR – Combined)	56,865	1,753	315	82	18
STP B (CAS – Combined)	6,524	4,370	1,100	75	7.18
STP C (CAS – Pure sewage)	330	2,500	140	94	0.05

8.1.2. Analysis of Sample Characteristics

The characteristics of MPs, including shape, size, color, and polymer type, can be plotted and visually generated using the data from the sample worksheet (**Table 7 & Table 8**).

The following pertinent details should be included in the reports.

- Number/percentage of MPs per shape
- Number/percentage of MPs per size
- Number/percentage of MPs per color
- Number/percentage of MPs per suspected polymer type
- Quantification units used in the analysis

Doughnut diagrams prepared using Microsoft Excel can be used to provide the distribution of the MPs at a quick glance (**Figure 48**).

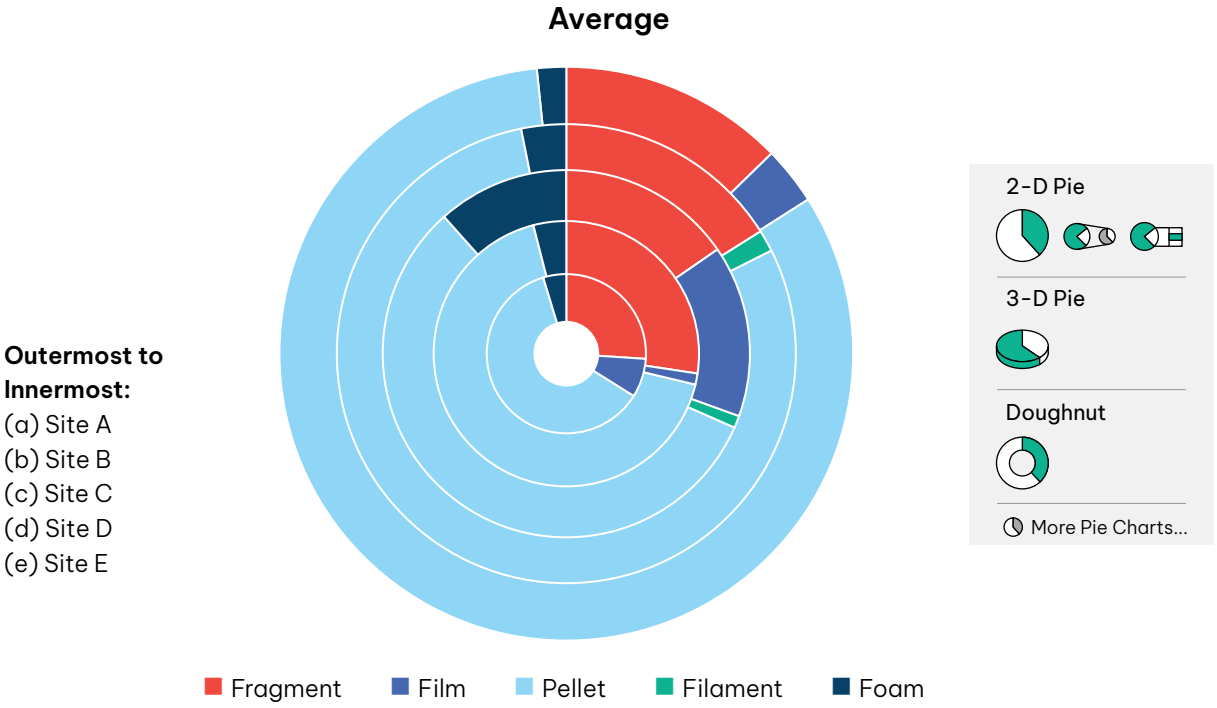


Figure 48. Sample doughnut diagram showing the shape distribution of MPs across sampling sites

Other data visualization applications such as OriginPro and Tableau can also be used to show the distribution of two characteristics of MPs in one graph (**Figure 49**).

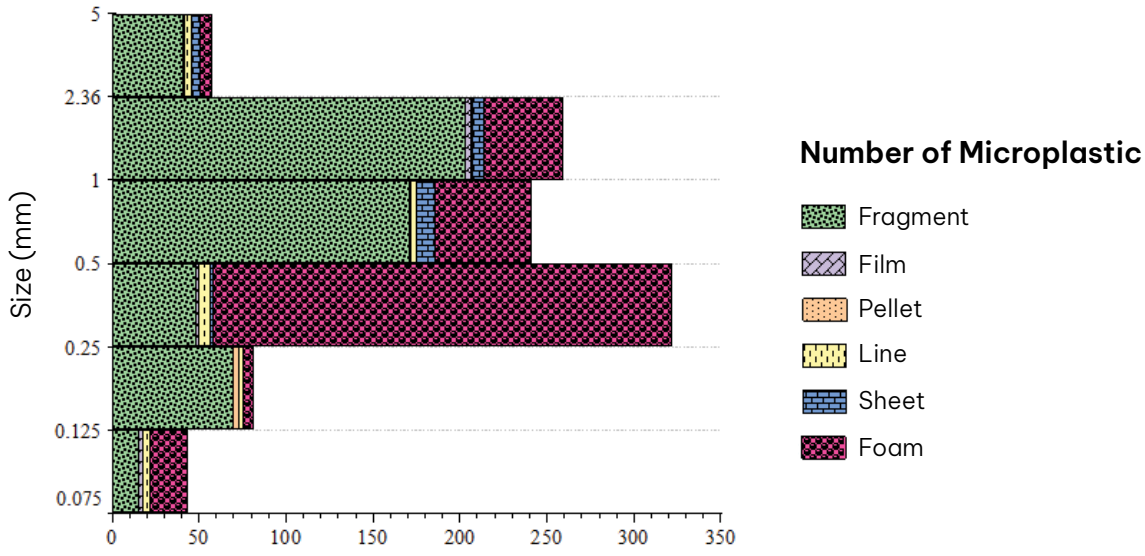


Figure 49. Sample distribution of MPs in terms of size and shape

Chapter 9

REPORTING AND COMMUNICATION

Effective reporting and communication of the results of MP monitoring is pivotal in informing relevant stakeholders, including national and local government units, STP operators, academia, etc. The necessity of this communication underscores the need for a baseline and a continuous monitoring plan to track MP levels and assess the effectiveness of implemented interventions to reduce the release of MPs into receiving waters.

The purpose of the monitoring plan is to document the monitoring programs essential for tracking the potential contribution of STPs to pollution of the receiving environment caused by MPs (**Table 13**) and to mitigate leakage by improving the STP system.

It is recommended that the operator of an STP, whether it is a public or a private entity, to be the lead person in conducting the baselining and monitoring activity. The frequency of MP monitoring in STPs should be tailored to the seasonal variations unique to each country in ASEAN. For instance, in the Philippines, with distinct dry and wet seasons, a biannual monitoring frequency is recommended. Seasonal variation could be a factor in the varying MP concentration in STPs. Earlier studies have found that COD concentrations were higher during the dry season than during the wet season, possibly due to dilution caused by high flow rates enhanced by rainfall (Makuwa et al., 2022; Osuolale et al., 2015). Accordingly, the removal of MPs and COD was proportional, indicating that COD and MPs are further removed as the wastewater goes through the treatment processes (Kwon et al., 2022; Donoso et al., 2020). The results of Uogintė et al. (2022) also supported their hypothesis that the amount of precipitation affects the MP concentration in the influent. In Qingdao, China, people's lifestyles and tourism activities may have increased the amount of rayon in the environment, which may explain the seasonal variation in MP concentrations (Jiang et al., 2022).

It is recommended to collect samples during weekdays and weekends every 6 months. Coordinating MP monitoring schedules with routine wastewater quality assessments conducted by local government units or private operators ensures a comprehensive understanding of the relationship between MP concentration and other monitoring parameters. The collection of samples at influent and effluent points in an STP should be prioritized to calculate the STP's MP removal efficiency. Monitoring MP concentrations in intermediate processes, such as at points between influent and effluent points and sludge, can be optional, depending on budget constraints. Additionally, regarding monitoring receiving water, conducting biannual monitoring of samples collected from the upstream, midstream, and downstream is crucial to determine MP abundance.

According to the guidelines provided in this Harmonized Protocol, it is estimated that the cost for each sampling is approximately USD 350 . An STP operator requires a minimum of 1,800 USD to conduct a complete MP monitoring process in the STP and the receiving water. This amount is considered high in many ASEAN countries; hence, reducing the size ranges of the particles to be studied may reduce the costs incurred for laboratory analysis.

TABLE 13. Sample MP Monitoring Plan

Parameter to be Monitored	Sampling Point		Sampling and Measurement Plan		Lead Person	Estimated Cost[1] in USD
			Method	Frequency		
MPs	STP	Influent	Bucket collection	Biannual	STP operator	350
		Effluent	Bucket collection	Biannual	STP operator	350
		In between influent and effluent points	Bucket Collec-tion	Biannual	STP operator	350/process
		Sludge	Grab collection	Biannual	STP operator	350
	Receiving water	Upstream	Bucket collection	Biannual	STP operator Local Government Unit	350
		Midstream (near the effluent outfall)	Bucket collection	Biannual	STP operator Local government unit	350
		Downstream	Bucket collection	Biannual	STP operator Local government unit	350

[1] The estimated cost is limited to sample collection and laboratory analysis with only 1–2 particle types to be analyzed for spectroscopy using the methodology described in this document. It is assumed that the lead person conducting MP sampling will have access to a microscope for conducting analysis.

The monitoring report must encompass the findings outlined in Section 8, with a specific emphasis on MP abundance and characteristics (**Table 14**). To facilitate data comparisons regarding abundance, it is advisable to include suggested units of measurement in the report.

TABLE 14. Sample reporting of MP data to the monitoring report

Date	Sampling Point	MP Abundance	Description
January 2, 2024	Influent	150 particles/m^3	Mostly colored fragments with sizes of <500 microns were observed.
	Effluent	20 particles/m^3	Few filaments with sizes >100 microns were detected.

It is recommended to submit this report to prominent national environmental agencies, such as the Department of Environment and Natural Resources (DENR) in the Philippines. Alternatively, incorporating the MP level as a tested parameter into customary compliance reports should be considered; further, effluent water quality test reports should be routinely submitted to these agencies. This practice of reporting data to leading agencies is pivotal in the collective efforts aimed at addressing the challenges associated with pollution caused due to MPs.

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