Marine Pollution Bulletin 210 (2025) 117342

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Aquatic microplastics research in the ASEAN region: Analysis of challenges and priorities

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A R T I C L E I N F O

Keywords:
ASEAN
Microplastics
Digestion
Identification
FTIR

A B S T R A C T

Research on microplastics must be harmonized. Therefore, we thoroughly evaluated in the Association of Southeast Asian Nations (ASEAN) region, addressing challenges and priorities in protocol harmonization and microplastics research promotion. Of the 615 papers searched by the Web of Science, 164 were used for this systematic review. The number of ASEAN research articles has increased over time. Examination of research protocols in various sampling environments revealed several challenges: 1) Disparities in access to sampling locations affect the research extent; 2) Outdated protocols and limited access to technologies such as FTIR (Fourier-transform infrared) spectroscopy result in less harmonized and potentially lower-quality data; and 3) Insufficiently detailed methods and QA/QC information hampers comparability. We offer procedure updates to overcome these limitations and cover environmental microplastic study gaps. Other countries in the Global South may encounter similar challenges, making this review a valuable contribution to advancing global microplastics research and fostering international collaboration.

1. Introduction

Since the initial global study in 2015, estimates have been made regarding the volume of plastic waste entering the oceans from land-based and sea-based sources (Jambeck et al., 2015; Lebreton et al., 2017; Schmidt et al., 2017). This research has ignited worldwide efforts to decrease plastic waste, especially in the ASEAN (Association of Southeast Asian Nations) region, where plastics emissions have been estimated to be very high (e.g., Jambeck et al., 2015; Meijer et al., 2021). In this regard, evidence-based precautionary approaches are likely to be crucial in mitigating plastics pollution and averting its adverse environmental effects. In particular, primary and secondary microplastics, which are plastic particles measuring <5 mm that result from manufacturing and the fragmentation of plastic waste (e.g., Andrady, 2011), are serious pollutants in the world's oceans. Secondary microplastics are known to have greater emissions than primary microplastics (e.g., Nakano et al., 2021a). This microplastic pollution poses a threat to various marine organisms, such as zooplankton (e.g., copepods, Alfonso et al., 2023, 2024), fishes (e.g., anchovy, Tanaka and Takada, 2016), invertebrates (e.g., crabs, Tanoiri et al., 2024), and

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https://doi.org/10.1016/j.marpolbul.2024.117342
Received 5 August 2024; Received in revised form 22 October 2024; Accepted 22 November 2024

corals (e.g., Jandang et al., 2024). In addition, microplastics have been found in terrestrial areas (e.g., in agricultural fields by Surendran et al., 2023). The potential effects of microplastics on human health are of great importance (e.g., Li et al., 2023; Winiarska et al., 2024). Consequently, global awareness and concern regarding the escalating issue of microplastics pollution have increased substantially in recent decades. As the amounts of plastic waste emission should be reduced (Higuchi and Isobe, 2024) to reach the goal of Osaka Blue Ocean Vision, there is no doubt about the importance of elucidating the plastics pollution in the environment to prepare evidence-based mitigation plans.

Currently, many nations have published comprehensive road maps and policies aimed at addressing the issue of plastics pollution. These initiatives often encompass a range of socioeconomic and environmental objectives, including the development and promotion of biodegradable plastic alternatives, the enhancement of public awareness and education campaigns, and the implementation of robust monitoring systems to track plastics pollution levels. For example, in the ASEAN region, several countries have incorporated the collection of empirical evidence on plastics pollution as a key target within their roadmaps and policy frameworks, recognizing the importance of data-driven decision-making to tackle this pressing environmental challenge (e.g., the Action Plan on Plastic Waste Management Phase II (2023–2027), Pollution Control Department, Ministry of Natural Resources and Environment, Thailand, 2023; Table S1). This means that, to meet both global and national goals, it is essential that we elucidate the current status of plastics pollution.

The ASEAN region stands out as one of the primary contributors to the global plastic waste problem, with six ASEAN countries (Philippines, India, Malaysia, Myanmar, Vietnam, and Thailand) ranking among the top 10 worldwide contributors to plastics pollution (Borrelle et al., 2020; Meijer et al., 2021). With a population exceeding 400 million, the region's environmental management and waste disposal infrastructure still need optimization (United Nations Economic and Social Commission for Asia and the Pacific (UN. ESCAP), 2020). Given its extensive coastline, large population, and considerable volume of improperly managed waste, Southeast Asia represents a large source of land-derived debris that ultimately finds its way into the oceans (Omeyer et al., 2022). Also, Southeast Asia has become a major hub for plastic waste imports, exacerbating local waste management challenges and contributing to the region's environmental pollution problems (Brooks et al., 2018). In fact, of the 50 largest plastics-emitting rivers in the world, 28 are found in ASEAN countries, making the region the top plastics polluter from riverine sources (Meijer et al., 2021). Effectively addressing the plastics pollution crisis in Southeast Asia is imperative for minimizing the global impact of this environmental challenge.

However, a bibliographic review in 2019 found that there has been a severe shortage of plastics pollution research in the ASEAN region, with the exception of Indonesia (Lyons, 2019), suggesting the importance of more comprehensive research. Although several worthy narrative reviews have well documented the current state of knowledge in each ASEAN country (Ali et al., 2021; Choong et al., 2021; Curren et al., 2021; Raja Sulaiman et al., 2023; Sin et al., 2022; Veettil et al., 2022), the challenges and research priorities still need to be clarified. A systematic review, along with an evaluation that used an index of current pollution in Southeast Asia, was provided by Ali et al. (2024). However, their review paper focuses on the abundance of microplastics in each sampling field, and the challenges and priorities in plastics pollution research remain to be clarified.

Southeast Asia is a crucial global biodiversity hotspot, featuring various plant and animal species across its coastal, marine, and terrestrial ecosystems (Cárdenas, 2007; Hughes, 2017). Despite its relatively small size (3 % of the Earth's surface), this region houses an unusually large portion of the world's biodiversity, including one-fifth of all plant and animal species, one-third of the world's coral reef species, and substantial portions of global coastal habitats and tropical peatlands (Burke et al., 2002; Sodhi et al., 2010). This ecologically valuable area faces substantial threats from extinction and ecosystem degradation, with plastics pollution posing a notable risk to its delicate natural environment. However, such threats have not been comprehensively examined because of the lack of harmonized protocols.

Furthermore, from a global perspective, there is an urgent need for more field surveys on marine microplastics in the ASEAN region. The distribution maps proposed by Eriksen et al. (2014) and Isobe et al. (2021) demonstrate a lack of in-situ abundance data for microplastics in the seas surrounding the ASEAN region (e.g., Michida et al., 2019). Also, previous studies have struggled with data comparability because of a lack of research results produced by the harmonized techniques proposed by, for example, Michida et al. (2019) (e.g., Nakano et al., 2024). This gap in comprehensive and harmonized monitoring data could lead researchers from nations in the ASEAN region to fall behind in addressing this global issue, but the challenges producing the gap have not yet been documented.

Therefore, here, we review the challenges and priorities in microplastics research in the ASEAN region. For this purpose, the research protocols for aquatic environments, including river and ocean surface waters, river and seafloor sediments, beach sediments, and marine biota, in each country were compared. As outlined in Section 3, this review paper also determines the current status of microplastics surveys and checks whether subsequent analyses are likely to be harmonized. In Section 4, we discuss recommendations for achieving potential best practice in microplastics studies in the ASEAN region. Although this review paper was based on papers concerning the ASEAN region, shortcomings and areas for improvement in sampling and subsequent analyses are found not only in the ASEAN region but also in microplastics studies conducted worldwide.

2. Material and methods

2.1. Literature survey

A bibliographic search using the terms "microplastic" and "country name" (Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand, Vietnam) for all the category fields (Topic, Title, Author, Publication Titles, Affiliation Funding Agency, Publisher, Publication Date, Abstract, Accession Number, Address, Author Identifiers, Author Keywords, Conference, Document Type, DOI, Editor, Grant Number, Group Author, Keyword Plus ®, Language, PubMed ID) was conducted through the Web of Science on 2 February 2024, yielding 615 papers. The range of publication dates was set from 1st January 2014 to the searched date. Each paper was screened by the authors on the basis of its title, abstract, and main text, and only those specifically addressing microplastics research in aquatic environments were selected. Inaccessible papers, conference proceedings, review articles, or duplicates were manually excluded. After screening, 164 papers were included in our literature review (Supplemental List S1). The documents for each country-based list were checked individually, and scientific papers on environmental microplastics in the sampling fields—surface waters, riverine/seafloor sediments, beach sediments, and biota—were retrieved. Sampling methods, sample processing (e.g., chemicals used and temperature setting during digestion, and density of chemicals used for density separation), identification techniques (microscopy and spectrometry, including Fourier transform infrared spectroscopy (FTIR) and its function), and particle shape categories were summarized after the screening process (Table S2a–d) and after review (Tables S3 to S6).

2.2. Statistical analysis

To confirm the differences in the numbers of papers between sampling fields, one-way ANOVA and the Tukey-Kramer test were used. The statistical analysis was conducted by using BellCurve for Excel (version 4.02; Social Survey Research Information Co., Ltd., Japan).

3. Results and discussion

3.1. Overviews of microplastics research

The number of microplastic surveys in aquatic environments increased mainly from 2020 onward in the ASEAN region (Fig. 1, Table 1), yielding 49 microplastics research papers from Thailand and 45 from Indonesia, followed by 27 papers from Malaysia, 21 from Vietnam, and 15 from the Philippines. The number of research papers published exhibited an increase over time (Fig. 1), with a notable exception in 2022 because of the substantial impact of COVID-19 on research activities in ASEAN countries during 2020 and 2021. The small number in 2024 is attributable to the fact that the literature survey was conducted early in that year (2 February). Notably, the high-income country of Singapore contributed relatively few research papers despite having sufficient scientific resources, likely because its coastal area is limited. Conversely, researchers in middle-income countries such as Thailand, Indonesia, Malaysia, the Philippines, and Vietnam produced relatively large numbers of scientific papers.

Among all aquatic environments (surface waters, riverine/seafloor sediments, beach sediments, and biota), research on surface waters was the most frequently conducted in Indonesia (21 research papers), Malaysia (20), Thailand (19), and Vietnam (13) (Fig. 2b and Fig. S1). In riverine/seafloor sediment research, 21 studies were conducted in Indonesia and 11 in Thailand. In the field of beach sediments, 9 studies were conducted in Thailand. In the case of biota samples, 22 studies were published in Thailand and 19 in Indonesia. If we consider only the top five countries contributing papers on microplastics—Indonesia, Thailand, Malaysia, Vietnam, and the Philippines—the analysis revealed that, in each of these countries, the number of research papers focusing on beach sediments differed significantly from the numbers focusing on the other environmental compartments (Fig. 2b–e; $p < 0.05$ by one-way ANOVA and Tukey-Kramer test). This might have been due to the likelihood that the surface water, riverine/seafloor sediments, and biota research was conducted together with surface water and riverine/seafloor sediment sampling to reveal the interactions among targeted species and the environments they inhabited (see Table S2), whereas the beach samplings may have been conducted independently of the biota research.

In our further analysis in the succeeding sections, the results represent mainly the situation in the abovementioned top five nations, given the small numbers of scientific papers from the other countries.

Table 1
Number of papers from each country 1st January 2014 until 2 February 2024.

Country	Number of papers
Thailand	49
Indonesia	45
Malaysia	27
Vietnam	21
Philippines	15
Singapore	3
Myanmar	1
Brunei	1
Cambodia	1
Laos	1

3.2. Monitoring methods

In the ASEAN region, surface water sampling was conducted predominantly in rivers and coastal areas (Fig. S2). Notably, the open ocean, including the South China Sea, has not been extensively targeted for research to date. Among the reviewed papers, only four papers focused on open ocean investigation (Curren and Leong, 2023; Khalik et al., 2018; Md Amin et al., 2020; Yusof et al., 2023); moreover, the sampling stations in these four studies were very close to the coast.

Of the reviewed papers that had sampled surface waters, 46.3 % used grab sampling (a single sample taken in a certain spot) by water samplers such as a Van Dorn bottle, whereas and 45.0 % used net towing, such as a Neuston net survey, to collect surface waters ("All" in Table S3a). In the case of surface water sampling of rivers (Table S3a), net sampling was dominant in Thailand, Indonesia, Vietnam, and the Philippines. The literature indicates that researchers commonly utilize net samplers with mesh sizes of 300 or 333 μm, although some studies have employed smaller mesh dimensions (e.g., 200-μm-mesh manta net by Cordova et al., 2022; 80-μm-mesh plankton net by Strady et al., 2020). In Malaysia, however, net sampling was not used to collect surface microplastics samples. Likewise, in coastal waters, both towing nets (45.9 %) and water samplers (grab sampling: 43.2 %) were the predominant tools used to collect surface microplastics. Overall, the use of water samplers as often as nets in the past decade is characteristic of microplastics surveys in ASEAN surface waters (Fig. 3a, "All" in Table S3a). The amount of water sampled by water samplers (~100 L) would have been less than that sampled by nets (Table S2a). Given the histogram of the size distribution of microplastics, there are relatively few large microplastics and relatively large numbers of smaller microplastics (e.g., Isobe et al., 2015). Therefore, to verify the abundance of larger microplastics, a large amount of water (around 100 m^3, e.g., 20-

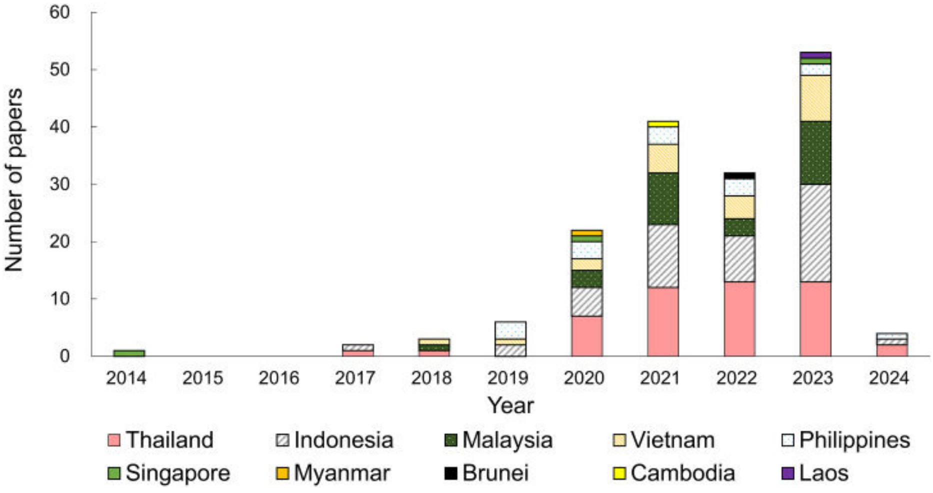


Fig. 1. Numbers of publications per country per year. The survey was conducted in February 2024, so the results for that year are incomplete.

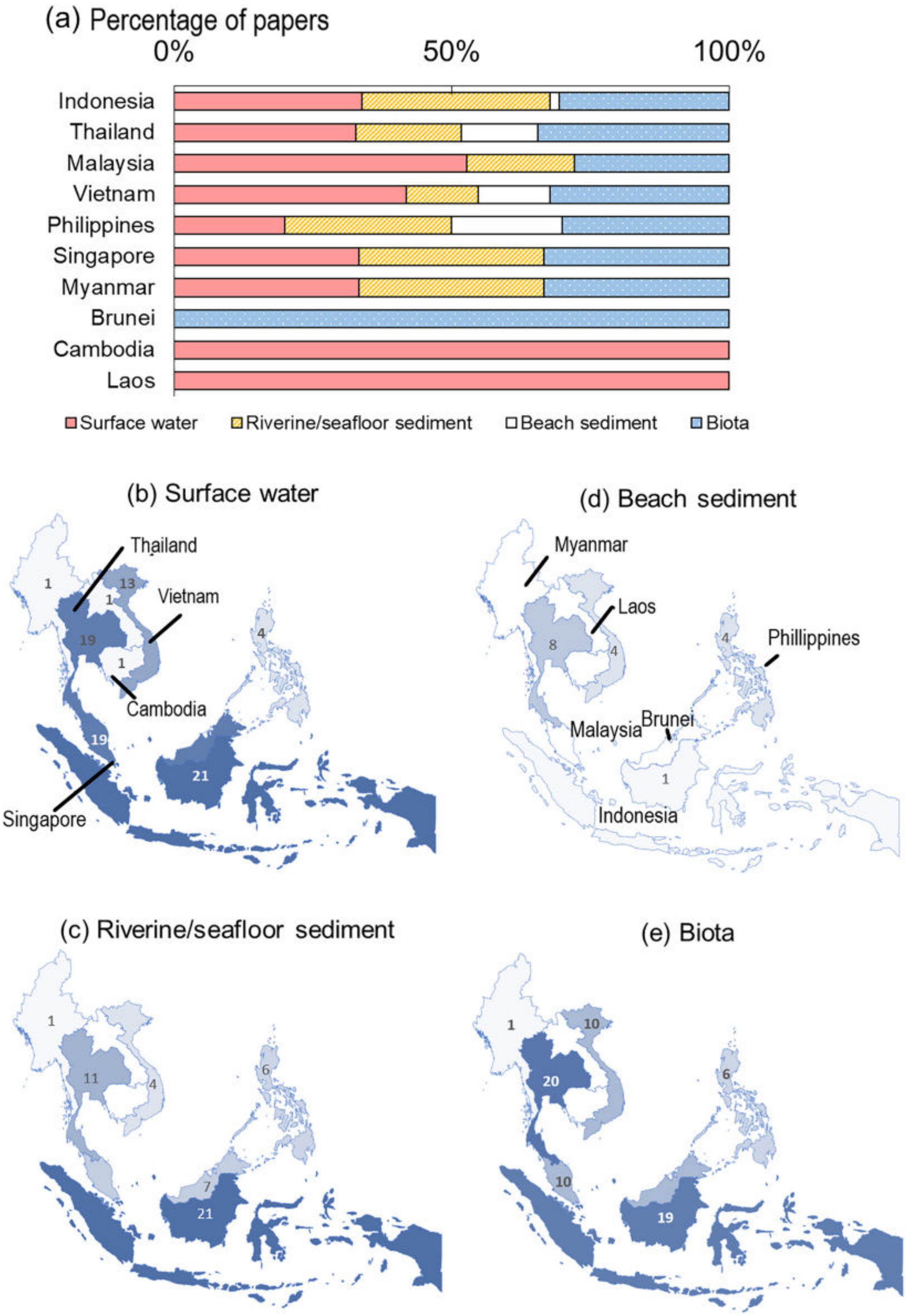


Fig. 2. Percentages and numbers of research papers examining microplastics in each sampling environment. Percentages of research papers (a); and heat maps of (b) surface waters, (c) riverine/seafloor sediments, (d) beach sediments, and (e) biota. To make these figures, papers published regarding surveys conducted in several sampling fields were redundantly counted in each field. The numbers shown in (b), (c), (d) and (e) represent the numbers of published papers. Darker colors indicate that more papers have been published.

min net towing at 2 knots is equivalent to 200 m^3 to 500 m^3 by Michida et al., 2019) should be collected. Targeting larger microplastics by using a relatively small sampling volume could cause the microplastics pollution results to be over- or underestimated. A comparison of sampling methods has revealed that the abundance of microplastics taken by grab sampling methods using water samplers is of the order of four times that of microplastics collected by net sampling, although grab sampling has a risk of collecting non-representative water volumes if the research focused on large MPs (e.g., Poli et al., 2024). We propose conducting multiple grab sampling replicates to address the limitations posed by the small sampling volume and ensure a more comprehensive assessment of the prevalent microplastics pollution. Monitoring methods should be selected to suit the study goals.

Unlike the case with surface water sampling, the methodology used to collect microplastics in riverine/seafloor sediments tended to be standardized across the ASEAN region (Fig. 3b and Table S4). Grab sampling was used in 32 papers (62.7 %, Table S4a), followed by core sampling in 12 (23.5 %). For beach sediment sampling, quadrat sampling was the main method adopted in 15 papers (88.2 %), followed by core sampling (1 paper; 5.9 %) (Fig. 3c and Table S5a). A smaller number of branches in Fig. 3 represents a smaller number of methods used, so the small number of branches in Fig. 3c suggests that the methodology used in beach sediment research in ASEAN regions is relatively limited.

In the case of biota samples, purchase from the market (16 papers, 22.9 %) or aquaculture farms, and catching by using fishing gear (32 papers, 45.7 %), are commonly used to collect samples (31.4 % of papers (22 papers) have yet to show the sample collection methodologies) (Table S2d). The original method of collecting biota samples has yet to be discovered in the case of market purchase. Therefore, we did not examine biota sampling methods here. However, fish purchased at markets could be contaminated because of Styrofoam boxes usage and using plastic packaging for sale in the market. It is therefore better to explore the potential contamination that happened to the fish on sale. Also, the samples were biased against commercial fish (see Table S2d). As in-situ survey-based studies can help to reveal microplastic pollution in comprehensive ecosystems, so such a campaign should be planned.

3.3. *Digestion*

For sample processing, oxidative digestion by H_2O_2 (31.3 %) or the Fenton reaction (30.0 %) was mainly used to remove impurities from surface water samples (Fig. 3a), except in Vietnam, where a combination of sodium dodecyl sulfate (SDS), Biozym SE (protease and amylase), Biozym F (lipase), and H_2O_2 was used (Table S3b; e.g., Strady et al., 2020). Nineteen papers (24 %) did not use digestion or did not mention it (Table S3b). Similar to the case with surface water samples, for riverine/seafloor sediment samples (Fig. 3b), the Fenton reaction was predominant among digestion methods (19 papers, 37.3 %), whereas oxidization by using H_2O_2 accounted for 27.5 % (Table S4b). Fifteen papers (29.4 %) did not use any digestion (Table S4b). For beach sediment samples (Fig. 3c and Table S5b), oxidative digestion with either H_2O_2 (29.4 %) or the Fenton reaction (29.4 %) was mainly used, although six papers (35.3 %) did not use any digestion. For the digestion of samples to quantify microplastics in biota samples (Fig. 3d and Table S6a), KOH digestion was the method most commonly used during the digestion step (48.6 %), followed by H_2O_2 (17.1 %); two-step digestion was adopted only for biota samples (8.6 %).

The use of oxidative digestion (using H_2O_2 and the Fenton reaction) is recommended under moderate temperature conditions because high temperatures (>60 °C) can damage plastics (e.g., Hurley et al., 2018; Munno et al., 2018). Prior studies have highlighted the importance of maintaining proper temperature control in laboratory analyses of microplastics. In their study, Alfonso et al. (2021) explored the impact of various digestion methods on the weight, size, and polymer composition of microplastics. They discovered that maintaining a temperature of 40 °C for 72 h effectively prevented substantial alterations in polymer properties. Furthermore, they cautioned against using high-temperature (>50 °C) techniques, which could potentially harm microplastics samples. However, some of the reviewed papers used temperatures ranging from 70 to 80 °C. For instance, 30.6 % of studies employed oxidative digestion in surface water research (Fig. S3), in accordance with the NOAA (National Oceanic and Atmospheric Administration) guidelines (Masura et al., 2015), which recommend heating the sample to a high temperature (70 °C) for digestion (e.g., Munno et al., 2018). Additionally, two-step digestion (e.g., Alfonso et al., 2021; Tanoiri et al., 2023) is a recently developed technique that could be more effective in the presence of a high content of organic matter, but it is not widely applied, possibly because it is more time consuming. These findings indicate that the need to update digestion methodologies is a key issue for microplastics research in the ASEAN region.

3.4. *Density separation*

In most nations, NaCl is commonly used to extract suspected microplastics from samples in surface waters by density difference (43.8 % in all papers, 43.6 % of papers for river waters and 48.6 % for coastal waters, Table S3c, Fig. 3a). However, 41.3 % of papers ("All" in Table S3c) did not use density separation of surface water samples.

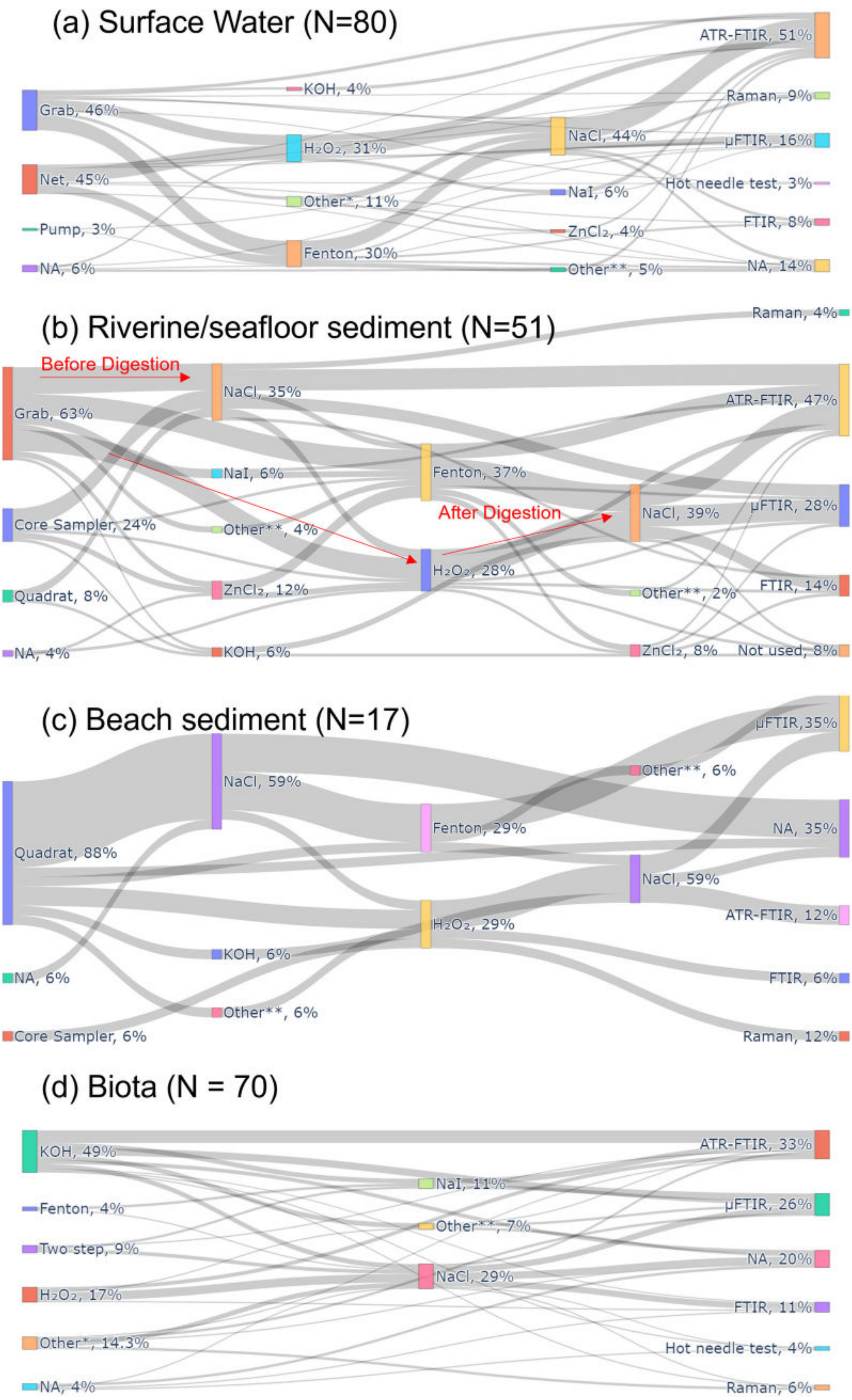
In the case of riverine/seafloor sediment samples, there were two main methods used (Fig. 3b), namely density separation before digestion (group A, 29 papers) and after digestion (group B, 26 papers) (Table S4c) The difference in methods probably occurred because the recommended order of digestion has fluctuated in the latest guidelines that are widely used worldwide: for instance, some guidelines (GESAMP, 2019) belong to group A, whereas Michida et al. (2019) and United Nations Environment Programme (UNEP) (2020) fall into group B. Also, a few papers have adopted density separation both before and after digestion. In terms of the chemicals used during the density separation step, NaCl was mainly used (62.1 % of papers in group A and 76.9 % of papers in group B, Table S4c). In a similar fashion, two main separation streams were used for beach sediment samples (Fig. 3c), with a high proportion of NaCl use (90.9 % of papers in group A and 83.3 % of papers in group B in a total of 17 papers) (Table S5c1 and S5c2, respectively). Digestion can remove biofilms and reduce particle density. Therefore, the sequence of density separation after digestion would be recommended.

Unlike in the case of surface waters, riverine/seafloor sediments, and beach sediments, density separation was not applied to biota samples (52.9 %; Table S6b). However, it was applied to sandfish samples, as their gut contents consisted partially of sediment (Riani and Cordova, 2022). When density separation was used, NaCl was the dominant agent employed (28.6 %; Fig. 3d).

The above results indicate that saturated NaCl solution ($\sim$1.2 g/cm^3) has been predominantly used in ASEAN regions owing to its eco-friendly nature and cost-effectiveness. However, other chemicals (e.g., NaI) can effectively collect plastic polymers that have a heavier density than NaCl solutions (e.g., Kye et al., 2023). Therefore, in situations where a diverse range of plastics is expected to be collected (e.g., sediments in rivers or coastal areas), it would be advisable to consider using alternative solvents, such as saturated solutions of NaI or $ZnCl_2$, both of which have a high density (> 1.6 g/cm^3), thus enabling the denser plastics to float (see Table 1 in Lusher et al., 2020). To balance cost and data quality, cost-effective density separation methods, such as the chemical reuse proposed by Rodrigues et al. (2019), should be introduced. Also, to increase the effectiveness of microplastics extraction from natural samples, there is another method that repeats the density separation process using NaCl solutions of varying concentrations (Cordova et al., 2023, 2024b; Riani and Cordova, 2022). This iterative technique facilitates the recovery of microplastics particles, enabling a more comprehensive assessment of microplastics contamination levels in the natural environment.

3.5. *Identification*

Among plastics identification methods, attenuated total reflectance FTIR (ATR-FTIR) was the main method used to measure the infrared spectra of suspected microplastics in surface water samples (51.3 %, Fig. 3a). Raman spectroscopy (8.8 %) and micro-FTIR (μFTIR) (16.3 %) were less commonly used (Fig. 3a and "All" in Table S3d). Some researchers used hot needle tests (2.5 %) to check whether the particles in surface waters were plastic. Some papers did not mention the specifications of the FTIR instruments used during identification (7.5 %, Table S3d). In the case of riverine/seafloor sediment samples, ATR-FTIR



(a) Surface Water (N=80)

(b) Riverine/seafloor sediment (N=51)

(c) Beach sediment (N=17)

(d) Biota (N = 70)

(caption on next page)

Fig. 3. Sankey diagrams showing the relationships between sampling/treatment methods and the corresponding analytical techniques for various environmental matrices: (a) surface waters, (b) riverine/seafloor sediment, (c) beach sediments, and (d) biota. The width of the connections reflects the proportion of samples processed by using each method. Dominant method-to-technique pairings are visible, such as grab and net sampling in surface water, paired with NaCl or Fenton treatments and ATR-FTIR analysis. Other* includes 1) a combination of sodium dodecyl sulfate (SDS), Biozym SE (protease and amylase), Biozym F (lipase), and H_2O_2, and 2) HNO_3. Other** includes a mixture of NaCl and $ZnCl_2$. Red arrows mean two streams of density separation. NA: details not mentioned in the paper. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was the most commonly used identification method (47.1 %), although μFTIR accounted for 27.5 % of the total (Table S4d). Because microplastics smaller than several hundreds of micrometers were targeted in the sediment surveys, μFTIR use would have been in higher demand than in the case of surface water samples. Moreover, μFTIR was the method most commonly applied to beach sediment samples (35.3 %), although 35.3 % of papers did not adopt spectroscopy to identify particles (Table S5d). In the case of biota samples, ATR-FTIR was used in 32.9 % of research papers, and μFTIR was used in 25.7 % (Table S6c), although 20.0 % of research papers did not use any identification method during analysis.

Throughout the review process, we found four major topics that should be clarified during non-spectroscopic research in the microplastics area. These dealt with the detectable size of microplastics, operational challenges, inadequate provision of methodology information, and failure to mention the percentage of particles measured.

The first topic was the detectable size of microplastics and spectra optimization. In ATR-FTIR, when particles are placed on the stage with tweezers or a needle, those that are too small have the potential to be lost before the spectral acquisition. A practical detectable size and optimization of the spectral acquisition can be introduced to obtain high-quality spectra. Optimization of spectral acquisition is time-consuming and requires a spectroscopist. Also, μFTIR equipped with ATR can simultaneously measure the filter paper spectrum if the prism is larger than the targeted particle (Fig. S4a). This means that the filter paper can interrupt the infrared absorbance spectra.

The second topic deals with μFTIR, which is a rapid method of determining the mean size and heterogeneity of microplastics samples. It is therefore useful for overcoming operational and time losses in ATR-FTIR measurements. However, μFTIR is not widely used and is available only in high-throughput laboratories. Even among those researchers who had used μFTIR, the instrumentally determined detectable size was not explained, leading to uncertainty regarding the minimum detectable size of particles.

The third topic is that the reviewed papers provided inadequate information about the measurement mode used. For example, the mode adopted for measurement (micro-ATR, transmission mode, refraction mode, or other techniques) may not have been described. In the case of reflection mode, there is no interruption by the filter paper; however, rough surfaces produce random reflections, making infrared radiation not return to the detector and thus bringing a weak infrared signal and producing low-quality data (Fig. S4c). To ensure spectral quality and integrity, the measurement mode should be described and examples of the acquired spectra should be given.

The last topic is that, although the percentage of particles measured in the samples affects the data quality and is important to the results, most of the papers did not supply these data. This measurement percentage should be described in papers because it provides useful information about the acquired samples. In addition, the libraries used for comparison and the acceptance threshold values indicating the matching rate or correlation coefficients were not mentioned. This lack of information leads to uncertainty in data quality and difficulties in data comparison.

3.6. *QA/QC and LOD/LOQ in reviewed papers*

In general, six processes of quality assessment (QA) and quality control (QC) were conducted in the reviewed papers (Fig. 4). Eighty-six studies (52.4 %) used negative controls, whereas 61 (37.2 %) used non-plastic equipment for as long as possible during the study process. Prefiltration was adopted in 50 studies (30.5 %). Positive controls were used in 22 reviewed papers (27.4 %); however, no details of the method used for these positive controls were given in almost all papers. Some of the reviewed papers did not perform QA/QC, leading to data uncertainty.

Contamination during sampling and laboratory experiments makes the results uncertain; therefore, QA/QC is mandatory to ensure data quality and reliability. Nonetheless, few papers to date have described the limit of quantification (LOQ) (e.g., Horton et al., 2020), which is usually defined as the lower limit of the concentration detectable in the facilities used by researchers. Detectable particle size (limit of detection; LOD) depends on several factors, including the monitoring method, the mesh opening size (e.g., Tokai et al., 2021), and the laboratory facilities, as well as the model and method of FTIR adopted by researchers. Authors should describe the detectable size. Otherwise, data on abundance—especially in the case of microplastics with sizes close to the actual minimum detectable size—become less reliable.

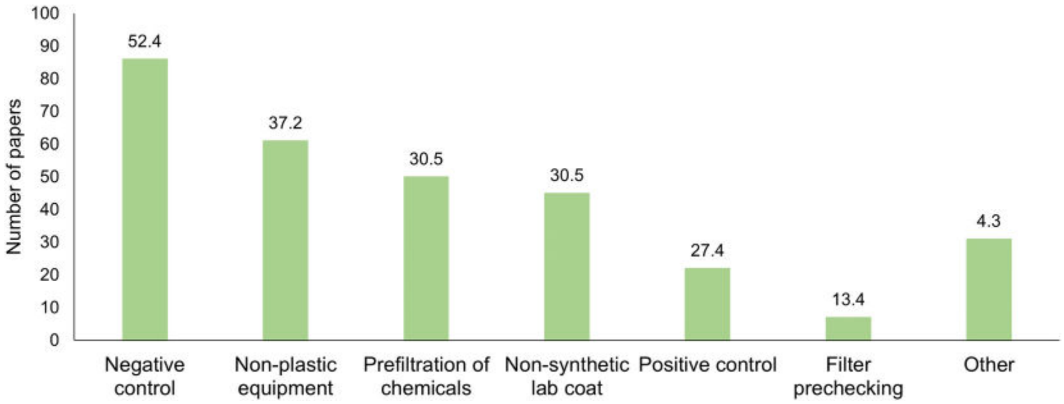


Fig. 4. Quality assessment and quality control methods used in the reviewed papers. Other activities included wiping the work table with ethanol and regular cleanup. Numbers above the bars indicates the percentages of the 164 papers.

3.7. *Particle shape categories*

Particle shape categories are likely to be confusing in microplastics studies conducted both worldwide and in ASEAN regions. In the reviewed papers, irregularly shaped particles were categorized mainly as "fragments" (Table 2). "Film" was a dominant shape category in the case of flattened microplastics. Many researchers debate the classification of microplastic fragments and films, as it is seen as somewhat subjective. Some argue that fragments and films can be discerned by their flat, transparent appearance. However, according to our analysis results, a few ASEAN researchers rely only on microscopic examination, without utilizing chemical characterization techniques such as FTIR or Raman spectroscopy. This approach may lead to subjective and potentially inaccurate identifications. A more thorough and comprehensive methodology is crucial for classifying different types of microplastics accurately. To describe long microplastics, "fiber" was mainly used, although "line" and "filament" were also used in the papers.

4. Recommendations and strategies for future studies to address microplastics pollution

The results of our review revealed several challenges in microplastics research in the ASEAN region. To address these challenges, the following subsections summarize recommendations and minimum requirements for microplastics research.

4.1. *Improve the sampling strategy: equipment and accessibility*

In the case of surface water sampling, both net sampling and grab sampling were used; however, as mentioned above, more water is required for larger microplastics sampling. Therefore, the sampling method and the targeted microplastic size should be linked to each other in the research planning stage. For riverine/seafloor sediment collection, grab sampling was the dominant method used, and for beach sediments the quadrat method dominated. It seems that these sampling methods have already been harmonized. In terms of biota sampling, because purchased samples are used, the absence of microplastics contamination during transportation to markets needs to be verified.

Because the accessibility of sampling fields varies, strategic research plans need to be based on both the socioeconomic demands and the research demands in the targeted research area. Although a bibliographic review conducted in 2019 (Lyons, 2019) highlighted a shortage of scientific knowledge regarding plastics pollution in ASEAN countries, the situation has been gradually improving year by year (see Fig. 1). However, paucity of sampling locations across various environments in each country remains a problem, with the accessibility of these locations substantially influencing the extent of the research conducted. Areas with greater accessibility tend to attract more research, leading to a discrepancy in the comprehensiveness of studies and suggesting that research activity is likely to be short-term. For instance, there has been notably less survey activity in offshore regions, including the South China Sea, than in coastal and riverine areas. Although monitoring efforts in the open ocean have increased (e.g., in the Indonesian Throughflow region; Cordova et al., 2024a; Cordova and Hernawan, 2018; Yuan et al., 2023), the amount of monitoring is still low. This discrepancy is likely due to the limited opportunities for offshore surveys, which in turn stem primarily from the scarcity of large research vessels. To address this issue, collaborative surveys organized by institutes equipped with large vessels, coupled with the involvement of local researchers, could greatly enhance data acquisition.

Table 2
Particle shape categories used in the reviewed papers.

Category	Number
Fiber	121
Fragment	120
Film	68
Pellet	48
Foam	40
Granule	23
Filament	15
Sphere	12
Line	5
Other Categories	32

4.2. *Use updated harmonized methods and definitions*

Although increasing microplastics monitoring is essential, it is equally important to collect data by using harmonized techniques to ensure comparability among research worldwide. For instance, through a questionnaire in ASEAN regions, a recent study demonstrated the need for such harmonization (Omeyer et al., 2022). That study found that the extent of the plastic waste entering the oceans remains unclear, and it emphasized the need for data-driven research. Furthermore, it underscored the importance of harmonized protocols across all stages of research, including sampling, sample preparation, and identification. Achieving this update would require access to recent scientific papers and the fostering of international collaboration. Additionally, the formulation of domestic guidelines or manuals based on international guidelines that are modified to suit localities in terms of the available items (e.g., Michida et al., 2019) could facilitate the harmonization of plastics pollution data. Establishing domestic networks would further enhance knowledge sharing at an international level, allowing local researchers to exchange resources and expertise.

The results of our analysis of digestion methodologies revealed that the need to update methodologies is one of the key issues in microplastics research in the ASEAN region. Therefore, researchers should refer to papers focusing on protocol development (e.g., Munno et al., 2018; Table 1 in Tanoiri et al., 2023; Uddin et al., 2020) and guidelines (Center for Ocean Plastic Studies, 2024; Michida et al., 2019).

A variety of terminologies were used in the shape categories in ASEAN countries. GESAMP (2019) recommended shape categories such as fragment, film, foam, fiber/line, and pellet. If we consider the origin of fibers, the category "line" should be used only for fishing gear. Likewise, spherical microplastics were categorized into multiple terminologies such as "foam, pellet, granule, and sphere" (Table 2). Of these terminologies, "foam" should be separately reported because foams have a different transport pathway, as pointed out by Kuroda et al. (2024). Likewise, "pellet" indicates only resin pellets or microbeads (i.e., primary microplastics; Andrady, 2011), which are manufactured for specific end products such as cosmetics. In fact, it is hard to consider that spherical microplastics are generated in nature, so descriptions of pellets should be individualized. Overall, the shape categories of fragment, film, foam, fiber, and pellet (e.g., GESAMP, 2019) are reasonable. Note that microscopic examination can be subjective in the identification of whether particles are microplastics. Although the shape is basic information, advanced technologies are required (see Section 4.3).

4.3. *Consider the limitations of each protocol and apply advanced technologies*

In the field of spectroscopy and microplastics, analysis requires precise and efficient methodologies to ensure the accuracy and reliability of data. A common approach is the combination of ATR-FTIR spectroscopy with various microplastics collection techniques. However, the appropriateness of this combination substantially influences the quality of the resulting data, warranting careful consideration and optimization of the methods employed.

If researchers' combination of ATR-FTIR and microplastics collection is inappropriate, the quality of the data may be debatable. For example, ATR-FTIR spectroscopy, which can easily measure large particles (> 5 mm), has been used in many studies to identify plastic materials, but so has grab sampling, which is an effective method for collecting fibers and small particles from surface water and sediment samples. In the case of ATR-FTIR, most research papers focus on tiny particles (colored and colorless) that can be seen under a microscope to enable chemical analysis; during the analysis, the particles are handled by being placed on the measurement stage with tweezers. However, handling very small particles requires highly skilled experts, a good laboratory apparatus, and the ability to analyze the characteristics of microplastics. Furthermore, counting large numbers of small particles that are invisible to the naked eye can be subject to human error and pressures to reduce the amount of manual labor in long-runtime experiments. Under these circumstances, valuable research results can be lost from samples taken by using methods that can efficiently collect smaller particles (e.g., grab sampling for the surface water). To address this issue, the model of the FTIR spectrometer and the type of technique (function or mode) used in the particle measurements should be reported.

In addition to there being a need to disclose this information, conventional ATR-FTIR spectroscopy in accordance with established guidelines (e.g., Michida et al., 2019) should be performed when microplastics larger than 300 μm are targeted. Additionally, to accurately assess smaller microplastics (< 100 μm) pollution, the adoption of advanced techniques such as imaging analysis (e.g., a focal plane array) with a transmission mode should be encouraged (e.g., Primpke et al., 2017a, 2017b). Note that point measurements on filter paper by using μFTIR would be useful, but they have the potential to underestimate abundance because of human error (e.g., failure to select particles).

Furthermore, reporting the technique used to determine the detectable size in research papers is essential, because each function's detectable particle size and resolution are different. For example, the transmission mode of μFTIR can detect particles of around 20 μm (depending on the aperture size) and cannot detect particles more than ~100 μm thick, because the spectra are saturated (Fig. S4b). Also, the reflection mode can be used to detect smooth surfaces but, in the case of irregularly shaped particles, diffuse reflection under rough surface conditions leads to weak reflection toward the detector, producing a low signal-to-noise ratio (Fig. S4c). Therefore, low correlation coefficients or a low hit quality index could be calculated when the spectra are compared with library data. We advise aiming for a hit quality index of at least 0.7 or 70 % similarity in microplastics identification. However, some publications accept a minimum of 60 % similarity to classify particles as microplastics confidently. This degree of spectral similarity ensures a high level of certainty that the analyzed particles are indeed microplastics and no other visually similar organic or inorganic materials (Circelli et al., 2024). When ATR mode is used, if the prism is larger than the targeted particle, the filter paper can be simultaneously measured. As mentioned above, operator-associated losses during measurement can happen more easily with ATR than with other methods such as the imaging method. During measurement, these points should be considered, and the method limitations should be clarified in the methods sections of future scientific papers.

In summary, recognizing the limitations of each method and disclosing them in scientific papers is crucial for enhancing the reliability of microplastics pollution assessments. By adopting appropriate sampling techniques and transparently reporting on the spectroscopy methodologies used, microplastics researchers can improve their data quality and contribute to more accurate environmental monitoring and analysis.

4.4. Optimize QA/QC and LOD/LOQ

According to our review results in Section 3.6, QA/QC has not been well documented in studies performed in the ASEAN region. Blank and recovery tests (negative and positive controls) are important to ensure the quality of abundance data; however, in many papers, the numbers produced by these tests have received only a brief mention.

To improve this situation in terms of blank tests (negative control), Horton et al. (2020) proposed the use of the LOD and the LOQ to consider abundance detection limits during laboratory experiments. They found polyethylene, polypropylene, and polyethylene terephthalate particles in their blanks, with fewer particles of polymethyl methacrylate, polyurethane, polyvinyl chloride, polyamide, and polystyrene, suggesting that contamination occurs, with a discrepancy among polymer types; such contamination affects clean samples more substantially than higher-microplastics-content samples. They also introduced LOD/LOQ calculations based on the background contamination (the number of particles in blank samples) (see Table 3). For example, using the condition that the number of particles in a sample is statistically significantly larger than the contamination (3.3 times the standard deviation of blank samples) works to check whether the experimental data is acceptable. Therefore, at least three blanks should be tested.

In recovery testing (positive control), because microplastics have different particle densities and characteristics, a wide range of microplastic sizes and polymers is needed to achieve a more accurate recovery rate (Horton et al., 2020). In fact, the results of recovery tests performed by Wang et al. (2021) showed that the recovery rate during the positive control decreased with decreasing particle size. Also, recovery tests using synthetic polymers of varying sizes mixed with clay powder to simulate natural environmental conditions can be used to assess the efficiency of extraction and identification processes (Cordova et al., 2022, 2024a, 2024b). Such recovery studies help validate the methodology, ensure that the reported results are reliable, and represent the actual microplastics contamination in the study area.

To determine the limitations on particle size selectivity during sample collection, it is important to consider which sampling tools are to be used. For example, Tokai et al. (2021) proposed mesh selectivity; in other words, they associated the retention rate of particles with the mesh size of the net. According to their experiments, almost all particles can be trapped in the net when the shortest diameter is the same as the size of the mesh opening. Particles with a shortest length that is shorter than the mesh opening of the net can pass through the net. Therefore, the dominant shape collected by Neuston nets was "fragment" (e.g., Nakano et al., 2021a); in contrast, the dominant shape collected by grab sampling tools such as water samplers was "fiber" (e.g., Prarat and Hongsawat, 2021). Mesh selectivity should be considered in size-detectability limitations (or shape limitations). Nakano et al. (2021b) indicated that identification by spectroscopy significantly improves data quality over digestion methods, and their results show that the digestion step boosts the working efficiency of the research procedure. The use of microscopic observation alone should be avoided because of the potential for overestimation due to false positives or underestimation due to the overlooking of plastic particles. Munno et al. (2018) also checked the effectiveness of different chemicals (H_2O_2 and KOH) and found that high-temperature wet peroxide oxidation damages polymers. Therefore, moderate conditions are recommended for sample processing. Furthermore, the chemical substances for the digestion should be selected to suit the sample (e.g., H_2O_2 for surface water and KOH for biota samples). Given the working efficiency of complex samples (e.g., proteins, cellulose), the potential applications of the two-step digestion are considerable (e.g., Alfonso et al., 2021; Tanoiri et al., 2023).

On the basis of the previous studies mentioned above, along with the QA/QC results of our review and the discussion in Section 4.3, Table 3 summarizes the recommended QA/QC and LODs/LOQs.

4.5. Current conditions in the ASEAN region

To truly tackle the urgency of microplastics pollution in the marine ecosystems of Southeast Asia, a comprehensive and holistic research study is essential in this area. Such a study should encompass thorough investigations into the sources, pathways, and fate of microplastics in the coastal and marine environments of the region (Omeyer et al., 2022, 2023). It should utilize advanced analytical techniques to precisely

Table 3
Recommendations (or minimum requirements) for QA/QC and LOD/LOQ with microscopic observation.

Purpose	Recommendation	Category	How to conduct (example)
To prevent contamination	Clean up equipment	QC	Use mild detergent to wash glassware. Ultrasonicators work to remove particles attached to the glassware surface. After that, rinse the glassware with particle-free water (filtered distilled water or milli-Q).
	Clean up laboratory	QC	Regular clean-up should be conducted. Use ethanol to clean the working space.
	Personal protective equipment	QC	Wear a cotton lab coat or a lab coat that prevents particle production, and use gloves that minimize contamination.
	Filter chemical solutions	QC	Use a filtration unit with a vacuum pump; remove contaminants before use
	Check for potential contaminants	QC	Collect samples that can be the source of contaminants and check the material by spectroscopy. Remove these materials from the experimental results or mention the contamination possibilities
To guarantee data quality	Material identification	QA	Use spectroscopy or a similar method. Mention the percentage particle abundance adopted in the identification method. (e.g., Nakano et al., 2021b)
To explain data limitations	Detectable size	Resolution	Consider the size of particles that can be collected by the monitoring method (e.g., mesh selectivity, Tokai et al., 2021) Consider the size of particles that can be detected by the identification method
To check for background contamination	Negative control (blank test)	QA	Set up a beaker of particle-free water (filtered distilled water or milli-Q) during field sampling and in a laboratory, and monitor particle contamination throughout field sampling and lab procedures. (at least three times to calculate LOD/LOQ below)
	Minimum detectable abundance	LOD/LOQ	LOD: 3.3 × standard deviation of blank samples, or 1.1ˆ particles, whichever is higher LOQ: 10 × standard deviation of blank samples, or 3.3ˆ particles, whichever is higher (Horton et al., 2020)
To assurance abundance	Positive control (recovery/spike test)	QA	Set up a beaker with particle-free water (filtered distilled water or milli-Q) and add several polymer types and sizes of plastic particles. Conduct laboratory procedures with this sample and check the particle recovery rate

characterize the chemical composition, size, and distribution of microplastics, facilitating a detailed understanding of their environmental impact (Omeyer et al., 2022, 2023). Furthermore, the study should examine potential mitigation strategies, which may involve developing effective waste management systems and promoting environmentally friendly consumer behaviors (Lebreton and Andrady, 2019; Pilapitiya and Ratnayake, 2024). Adopting a multidisciplinary approach that involves collaboration among scientists, policymakers, and local communities will be pivotal in delivering robust, evidence-based solutions to address the microplastics crisis in Southeast Asian marine ecosystems (Wagner et al., 2014).

To effectively address the region's urgent issue of microplastics pollution, there is a crucial need for increased research and development funding and enhanced research collaboration among ASEAN nations (Fiala, 2022). Investing in these areas will facilitate the development of more targeted, effective, and efficient strategies for managing the escalating problem of microscopic plastic waste in the region's marine ecosystems. By strengthening their research capabilities and fostering cross-border cooperation, Southeast Asian countries can make substantial progress in mitigating the environmental and ecological impacts of plastics pollution.

Also, research in Southeast Asian countries—particularly in Thailand, Indonesia, Malaysia, the Philippines, and Vietnam—has experienced notable growth in recent years. This growth can be attributed to the advancement of their respective economies and the increased emphasis of national governments on promoting scientific research and innovation (Sukoco et al., 2023). This higher research production will help researchers to better understand plastics pollution and will provide scientific evidence to help create mitigation plans.

5. Conclusions

We have provided a comprehensive review of current microplastics research protocols in the ASEAN region and have identified several challenges that must be addressed to fill existing data gaps and facilitate evidence-based actions. There are several streams in the microplastics research protocol (Fig. 3). The research results highlight the importance of updating the methodologies used in microplastics research. We identified several challenges, including the need to 1) have strategic research plans based on demand in the targeted research area; 2) update methods and definitions to harmonize the data at an international level; 3) recognize the limitations in each protocol and apply advanced technologies—especially spectroscopy; and 4) update knowledge of QA/QC and LOD/LOQ. Such efforts should be continuously conducted, and the recommended protocols (minimum requirements) presented in Table 3 should be used in all laboratories.

To the best of our knowledge, most ASEAN nations have goals for evidence-based mitigation in their road maps or policies (Table S1). To achieve these goals, the challenges elucidated in this review should be overcome. For this, it is imperative for governments to provide technical and budgetary support to encourage local researchers in the ASEAN region. Furthermore, similar challenges may be prevalent in other regions, such as the Middle East and African nations. Therefore, addressing the gaps in microplastics research in the Global South is an urgent and critical challenge worldwide. The microplastics data and research produced by such efforts and updated knowledge will facilitate global comparisons.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.117342.

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported mainly by the Science and Technology Research Partnership for Sustainable Development (SATREPS), Japan, in collaboration with the Japan Science and Technology Agency (JST, JPMJSA1901) and the Japan International Cooperation Agency (JICA), for S. Chavanich, V. Viyakarn, and A. Isobe. This work was also partly supported by a microplastics data center project (The Atlas of Ocean Microplastic (AOMI) of the Ministry of Environment), for A. Isobe, H. Nakano, M.B. Alfonso, and S. Jandang. The research was partly supported by JSPS KAKENHI grant numbers JP23K17030 for H. Nakano and JP23K17048 for M.B. Alfonso. Additional support was from a Malaysian research grant for Geran Putra-Inisiatif Putra Muda (GP-IPM/2023/9741500 and GP-Fast Track/2024/9791800) for M.R. Abdull Manap. This research is also partially supported by the NERC UKRI funding scheme (Grant No. NE/V009516/1), the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDA19060204, No. XDB42000000), and the Key Collaborative Research Program of the Alliance of International Science Organization (Grant No. ANSO-CR-KP-2022-08), for M.R. Cordova. The publication fees of this international collaborative research paper were supported by the Tokuo Fujii Research Fund of Kyushu University for H. Nakano.

Data availability

Data will be made available on request.

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