

Microplastics in ASEAN Freshwater Sediments: A Review of Methodologies, Occurrence Levels and Effects on Aquatic Organisms

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The presence of microplastics is a growing concern because of their ubiquity, persistence, ability to transmit environmental pollutants, and potential for bioaccumulation through food chains. Although ASEAN countries contribute significantly to the generation of marine litter globally, there has been limited research on microplastics in freshwater environments. This study aims to provide a comprehensive review of the existing state of knowledge regarding methodologies, occurrence levels, and the effects of microplastics on freshwater ecosystems in ASEAN countries. Our review focuses specifically on sediment matrices due to their long-term sink for microplastic pollution, with accumulation in sediment posing a risk to aquatic organisms and human health. Based on publications from 2018 to April 2022, 17 studies were examined. Sediment samples collected from rivers and aquaculture ponds revealed a range of microplastics concentrations between 4 and 66016 particles per kilogram. A comparative analysis was difficult because each study used non-standardised procedures and measurement units. Fibres are consistently found to be the most common shape, with black or blue microplastics being the most common colour across countries. Polyethylene or polypropylene was identified as the most common polymer type in the microplastic samples. The levels of microplastic in freshwater ecosystems have been linked to land use activities such as fishing, tourism, aquaculture, domestic wastewater, and industries. The high accumulation of microplastics in freshwater has been linked to the ingestion by fish species and gastropods. Future research should seek to standardise microplastic collection, extraction, and quality control methodologies to effectively quantify and assess the amounts of microplastic pollution for monitoring purposes, allowing for more comprehensive comparisons and evaluation for risk assessments.

Keywords: Microplastics; freshwater sediments; polymer composition; Southeast Asia

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Plastic production has increased drastically over the past decades. Since its mass production started in the 1950s, global plastic production has increased exponentially with the current production capacity reaching 370 million tonnes in 2019 [1]. Since plastic is widely used in packaging, storing, and transporting goods in an efficient manner, it has become a mainstay in modern society [2]. Currently, most plastics produced are high-density polyethylene (HDPE), low-density polyethylene (LDPE), polystyrene (PS), polypropylene (PP) and polyethylene (PE). Due to its low recycling rate and ineffective waste management, the rapid expansion in plastic production has led to a significant volume of used or unwanted plastic being released into the environment globally. Benson et al. [3] showed that almost 1.6 million tonnes of plastic garbage had been dumped worldwide every day since the COVID-19 pandemic's outbreak. Plastics are synthetic organic polymers with durability, versatility, strength, lightness, ductility, and transparency, making them unique

materials used in industry, construction, medicine, and food production [4]. As a result, 80% of land-based plastic waste ends up in rivers and the ocean [5]. As larger plastic particles (macroplastics) degrade into smaller particles (microplastics), they can be widely dispersed in the water surface, water column, biota and sediment [6].

Microplastics refer to small plastic particles smaller than 5 mm, including primary and secondary microplastics [7]. Primary microplastics are manufactured as small pellets, beads, and fragments for personal care and cosmetic products, which enter aquatic systems via household sewage discharges [8]. In contrast, secondary microplastics break larger plastic into smaller plastic particles. It usually occurs when large plastics are subjected to weathering processes such as ultraviolet light exposure, wave action, and wind abrasion [9]. Secondary microplastics are typically formed as fibres in washing machines and are

primarily composed of polyester (PE), acrylic, and polyamide (PA). Microplastics are typically classified into four categories: microfibrils, microbeads, fragments, and films.

Carpenter and Smith [10] reported the first microplastic pollution on the western Sargasso Sea surface in 1972, and Gregory [11] reported the first record of plastic pellets on New Zealand beaches five years later. Thirty years later, Thompson et al. [12] reported the first microplastic in sediment. Then, more researchers have focused on microplastic pollution in the marine environment with less emphasis on the freshwater environment. In an aquatic environment, microplastics have been discovered in rivers [13], lakes [14], estuaries [15], oceans [16], and ice sheets [17]. Plastic particles in the aquatic environment can float or sink depending on their polymer density, with a density of less than 1.0 g/cm³ floating on the water surface or in the water column, whereas denser polymers sink and settle in the sediment. Furthermore, the formation of biofilms, as well as the adsorption and accumulation of pollutants, have increased the density of plastic polymers, making them the primary source of microplastic in sediments [17]. Thus, sediments are suggested as a long-term sink for microplastics because all accumulated microplastics are eventually deposited in sediment [18]. Microplastic pollution is a growing environmental concern due to its slow degradability, biological ingestion by aquatic living organisms, and acting as carriers to concentrate

and transport synthetic organic substances from the environment to aquatic organisms [17]. Due to their small size and ubiquity, microplastic particles threaten aquatic organisms as they fall in the same size range as their prey and are often mistaken for food [19].

The Association of Southeast Asian Nations (ASEAN) consists of 11 countries: Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand, Vietnam and East Timor. Despite being one of the plastic pollution hotspots [5], microplastic research in the ASEAN region has only recently gained attention, with most microplastic studies focusing on marine [20] rather than the freshwater environments. Until recently, no studies on marine microplastic pollution had been reported in East Timor, Laos, or Cambodia, but only one study on microplastic pollution in open-dumping soils from Laos and Cambodia [21]. The freshwater environment has limited research into microplastic pollution, although most of the world's largest emitting rivers are in ASEAN countries [5], which serve as an important channel from land to sea for plastic wastes [22]. Moreover, few studies have reported on microplastic in freshwater sediment, although long-term microplastic accumulation is more common in sediments than in water and impacts aquatic organisms. Hence, the objectives of this review were to summarise the microplastic studies in freshwater sediment, emphasising (i) the methodology used in each study, (ii) the occurrence levels of microplastics, and (iii) the effect of microplastics towards freshwater organisms.

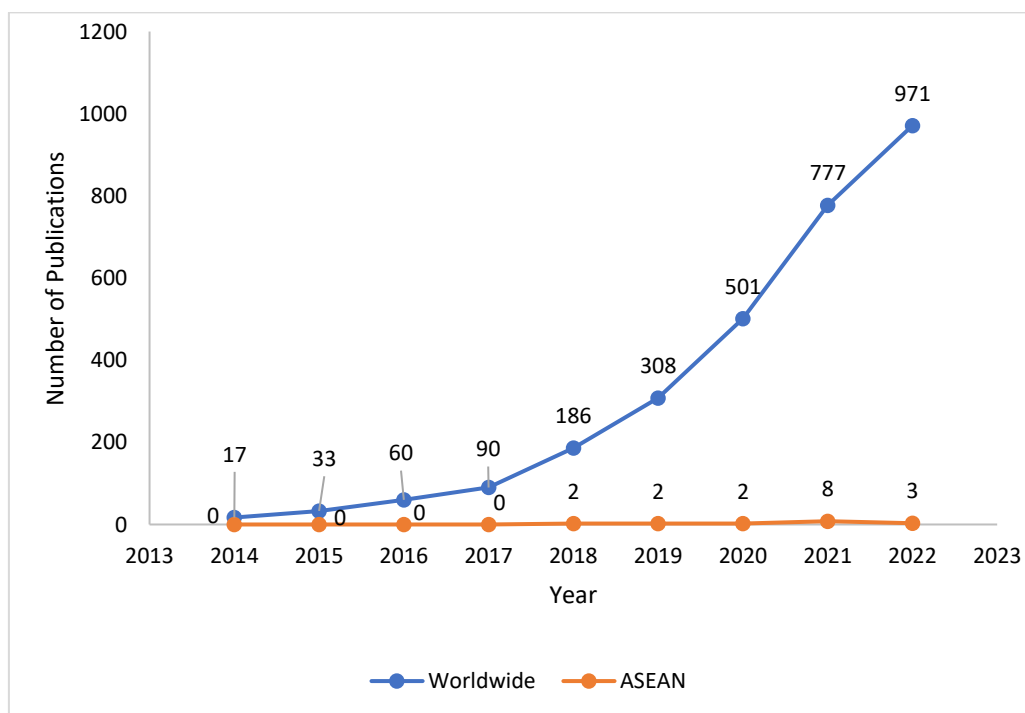


Figure 1. The evolution of publications on microplastics in freshwater sediments from 2014 to April 2022.

Selection of Literature

This review was conducted using Scopus, Science Direct, Google Scholar and Springer Link databases. Various keywords related to microplastics in sediment were applied; "microplastic in sediment, freshwater sediments, microplastic in riverine sediment, microplastic litters in sediment, microplastic pollution in ASEAN and microplastic in aquatic organisms" as search criteria. Based on the search keyword detailed above, 17 studies included journal articles and conference papers in ASEAN were included in this review. From all the publications chosen, the necessary information regarding (i) the sampling technique, (ii) microplastic extraction, (iii) microplastic abundance, (iv) microplastic category and (v) the type of polymer in sediment were extracted. **Figure 1** illustrates the evolution of the global literature on microplastics in freshwater sediments, which began in 2014 and ASEAN from 2018 to April 2022.

Methodologies for Microplastics Analysis

Currently, there are no defined techniques for sampling plastic particles, making data comparability untrustworthy [23,24]. In order to overcome these issues, a unified microplastic analysis in aquatic contexts is required [25]. In this review, we report on the most often used methodologies in the literature (**Figure 2**), addressing the benefits and limitations of the various methods in order to understand the most reliable analytical instruments for assessing the microplastic in freshwater sediment. As the microplastics measurement unit was inconsistent across studies, we converted the reported units to particles/kg sediment for comparison.

1. Sample Collection and Sampling Techniques

The first step in microplastic methodologies is the collection of sediment samples [36]. It must be noted that the distribution of microplastics in sediment is uneven and largely influenced by their properties and environmental factors. Hence, microplastic abundance is typically determined by sampling techniques involving transects, depth, and sampling tools, as some areas may contain higher concentrations of microplastics [36]. According to Hidalgo-Ruz et al. [7], three sampling techniques were commonly used for microplastic studies: volume-reduced, bulk, and selective. In volume-reduced sampling, the amount of the samples is reduced, leaving only the portion of the sample that

needs further processing. Meanwhile, the bulk sampling method refers to samples taken in their entirety without being reduced throughout the sampling procedure, whereas selective sampling is the direct removal of visible objects on the surface of sediments [7]. Microplastic concentrations reported in different studies are often difficult to compare because of the various techniques used. This results in additional calculations based on assumptions or is often impossible [17].

Table 1 summarises the sampling techniques for collecting freshwater sediments used in 17 ASEAN studies. Six studies used volume-reduced sampling techniques, while ten used bulk sampling during sample collection. The sampling depths vary greatly, with most studies collecting sediments from the surface of 0–5 cm or 0–20 cm, whereas three studies collected sediments from the top 2 m or deeper. However, one study did not define the sampling tool and depth of their research. Frias et al. [37] recommended that the location of the sampling sites should be 100 m parallel to the water line, while in freshwater environments, the sampling unit is recommended to be 30 x 30 cm square with a sampling depth of 5 cm and collected with a metal shovel. The Marine Strategy Framework Directive (MSFD) Guidance recommends sediment to be collected from the top 5 cm with a minimum of 5 replicates at least 5 m apart [38]. Most studies used the Ekman grab sampler as their sampling tool, whereas one used the Ponar grab sampler and Box corer sampler, and another four used the Van Veen grab sampler. Surface sediment samples are commonly collected using Ekman grab sampler [39] as it works effectively in soft, sandy, or silty sediments but is less successful on gravelly or rocky substrates. Due to the damage caused by grab samplers during digging and closure, it is recommended that sediments be collected using a van Veen grab sampler or a box corer [40]. Frias et al. [37] have recommended a drill corer for sampling tools as it can avoid disturbing the collected material and causing sediment loss. Microplastic study sediment collection must be conducted using non-plastic sampling tools and containers to avoid cross-contamination. As samples and procedures are being established worldwide, harmonisation and standardisation are urgently needed to improve microplastic research and monitoring in the aquatic environment [17].

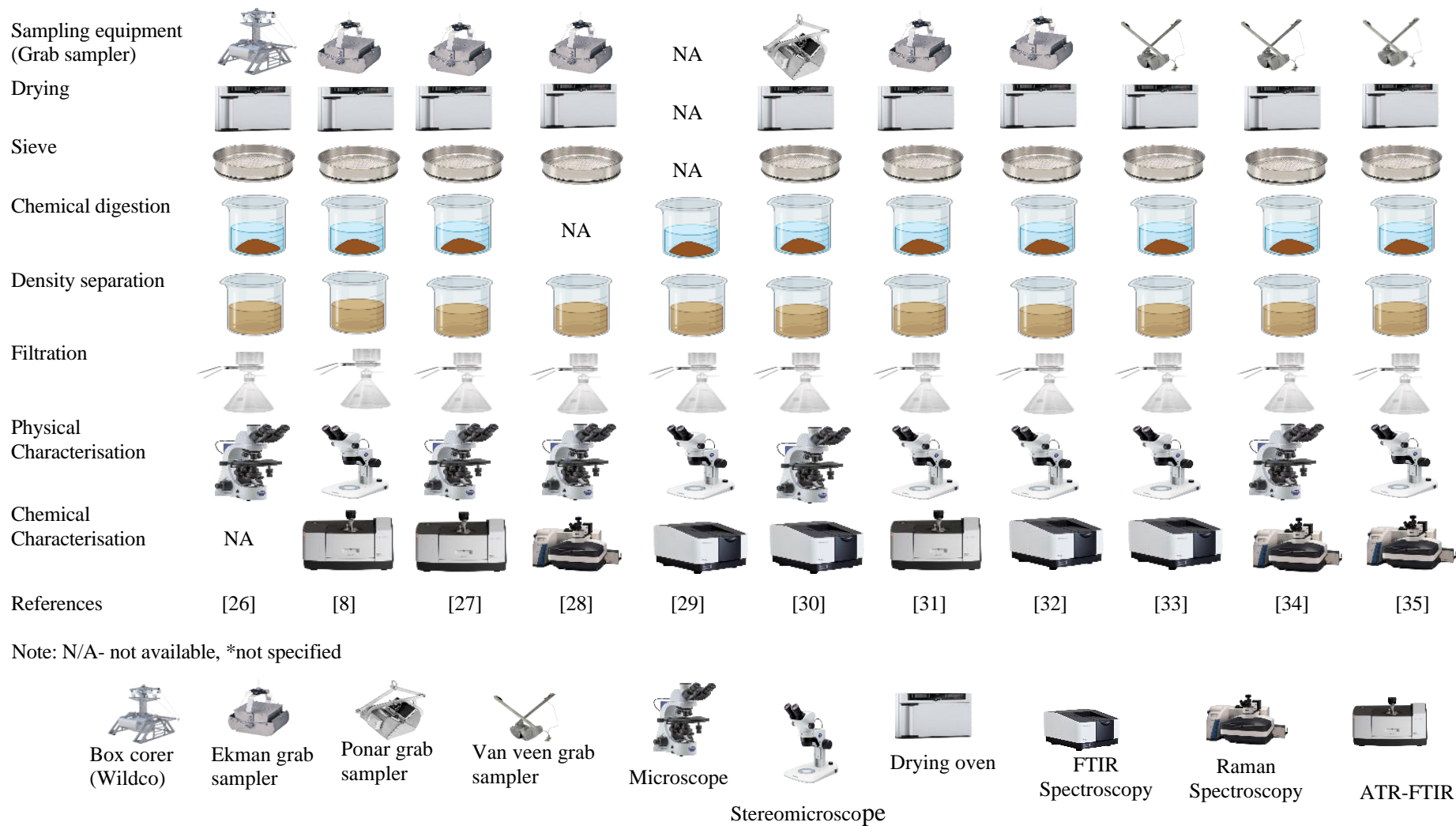


Figure 2. The overview of the methodology for microplastic study in freshwater sediment.

2. Microplastics Extraction

Drying: Due to the water content of sediments collected at various sites along the river, it is advised to express microplastic data as dry weight (microplastic/g sediment [17]). In order to extract microplastics from sediment without any contamination or interfering substances that can affect the quantification and identification of microplastics, the sample should be dried until completely dried or until constant weight. The sediment sample can be dried through two methods: drying at room temperature with a temperature of approximately 25 °C or oven-drying at a high temperature from 40 °C to 100 °C. Oven drying at temperatures less than 60 °C is a good choice because microplastic will deform and may break when heated at temperatures greater than 70 °C [41]. Besides that, the oven-dried method is also the fastest and can retain the physical form of microplastics in the sample. Drying at room temperature should be avoided due to the long drying process, and samples may contaminate via airborne microplastic particles. In addition, it can be incomplete, and the final water content can be varied depending on the humidity of the laboratory [41]. In this review, higher operating temperatures of 90 °C to 100 °C were reported in 12 out of 17 studies (**Table 2**). However, common polymers (such as polyester-based fibres) melt at such temperatures, and chemical deterioration occurs in some cases (such as with polyvinylchloride and PVC). While three studies dried the sediment samples at 40 °C, which is the ideal temperature to use in the drying process, two other studies dried the samples at 60 °C to 65 °C.

Chemical Digestion: As organic matter can be found in sediment samples [36], it can be misidentified as a microplastic, resulting in an overestimation of quantification [42]. Thus, chemical digestion can be employed to improve the separation of microplastics from sediments by removing organic natural matter and biofilms that obstruct the identification of microplastic [43]. Nevertheless, the amount of organic matter in the sample affects the chemical digestion process. About 94% of the 17 studies reviewed have conducted chemical digestion, while the remaining study (1 study) has not done so (**Table 2**). The chemical process can be done using oxidising agents, acid, alkali, and enzymatic degradation. Most studies used 30% hydrogen peroxide (H₂O₂) as an oxidising agent. However, Loder et al. [43] reported that another study combined diluted hydrogen chloride (HCl) and nitric acid (HNO₃) solutions with 30% H₂O₂ treatment to optimise digestion further. Seven studies choose to use Fenton's reagent, which consists of a mixture of 30% H₂O₂ and ferrous iron (Fe²⁺) as the catalyst. Oxidising agents are widely used because of the digestion of organic matter more efficiently than acid and alkali digestion, with little to no polymer degradation [36]. Hurley et al. [44] confirmed that most of the

investigated polymers in their study were unaffected by H₂O₂.

In contrast, Nuelle et al. [45] observed polymer discolouration and size reduction of HDPE, LDPE, PP, polyamide (PA), polyvinyl chloride (PVC), polyethylene terephthalate (PET) and polyurethane (PUR) after treated with 30% H₂O₂ for seven days at room temperature. Furthermore, Karami et al. [46] also reported the degradation of nylon and colour change of PET with the treatment of H₂O₂ at 50 °C for 96 hours. Hence, incubation temperature seems to be a determinant factor for H₂O₂ efficiency [24]. According to Cole et al. [47], incubation at room temperature for 7 days with 35% H₂O₂ only degraded 25% of organic matter. As a result, attention should be paid to the application of higher temperatures during digestion as polymers are unstable at higher temperatures.

On the other hand, Zobkov and Esiukova [48] discovered that the Fenton reaction is an effective method for removing organic matter. As compared to H₂O₂ alone, Fenton's reagent demonstrated superior efficiency in removing all organic matter from complex environmental matrices. It has been demonstrated that microplastic fragments are not affected by peroxide treatment and that infrared spectroscopic identification of microplastics is not compromised by this reagent [41]. However, it has been reported that certain biogenic matter is not digested in sediment samples and thus may need further organic removal [48]. Furthermore, a pH adjustment of 3.0 to 5.0 is required to ensure the dissolution of the Fe²⁺ and to optimise conditions for the degradation of organic matter [49]. These conditions may damage some polymers; therefore, the applicability of Fenton's reagent remains questionable. Acid and alkali digestion was less commonly used, even though it is highly effective in destroying organic matter. Consequently, polymers were degraded and melted due to their effect [47]. Karami et al. [46] found that 37% HCl at 25 °C had a digestion efficiency greater than 95%, but with the melting of PET. Although alkali digestion can damage or discolour plastics, it can also leave oily residues and bone fragments, thereby complicating vibrational spectroscopy analysis of the surfaces [36].

Density separation: Sediment samples usually contain many interfering substances that can affect the quantification and identification of microplastics. Density separation is used to extract microplastics from sediments using a saturated salt solution, usually by carefully mixing the sediment with saturated salt solutions and collecting the microplastic supernatant. Overall, sodium chloride (NaCl) is used as a density separator in the majority of the studies due to the low cost and environmentally friendly compared to others, followed by sodium iodide (NaI), lithium metatungstate (LMT), zinc chloride (ZnCl₂) and calcium chloride

(CaCl₂) (**Table 2**). However, higher-density microplastics cannot be separated by NaCl solution [5] since it has a density of 1.2 g/cm³. Although NaCl solution prevented many higher-density polymers (refer to **Table 3**) from being analysed, MSFD and Frias et al. [37] still advised using NaCl solution for density separation due to the low costs and the low toxicity.

Table 3 shows the specific densities of different polymer types. Each polymer has different density values (g/cm³). One study uses NaI as their density separator with a density of 1.8 g/cm³. NaI has the maximum density, which improves the effectiveness of the density separation process for microplastic particles from the sediment grains while being one of the most expensive solutions [47]. The NaI solution was recycled and reused wherever feasible (loss of 35.9% after 10-times usage) [47]. Based on particle number, NaI solution gives good recovery; however, NaI is very expensive and must be handled cautiously. LMT solution with a density of 1.62 g/cm³ was suggested as an alternative for the density separator because it can separate more polymer than NaCl. However, LMT is very expensive and only one study used LMT for

microplastic study (**Table 2**).

Furthermore, two studies have used ZnCl₂ as a salt solution. ZnCl₂ has been reported as the best salt solution used in studies because it can separate most polymer types and different particle sizes of microplastics [49]. However, Zhang et al. [49] reported that it is more environmentally hazardous than other salt solutions. CaCl₂ with a 1.3 g/cm³ density is also used for density separation. The advantage of CaCl₂ is that it is cheap and environmentally friendly, but a study from Scheurer and Bigalke [50] showed that CaCl₂ is not suitable for sediment with high organic matter since Ca²⁺ can bridge the negative charge of organic molecules. Thus, the filter may be covered with thick brownish material, interfering with the measurement [41,51]. All density separation methods now in use have drawbacks [51]. Li et al. [52] confirmed that the salt solution used impacts microplastic separation efficiency and the form and size of microplastics captured. High-density salt solutions (such as NaI) may help better separate microscopic microplastic fibres with high density, but they have little effect on bulk or fragment microplastics [52].

Table 1. The techniques used in 17 studies for microplastic sampling in sediment.

Country	Study Area	Sampling tools	Depth	Sampling technique	References
Malaysia	Skudai River, Johor	Box corer (Wildco)	2 m	Volume reduced sampling	[26]
	Tebrau River, Johor	A box corer (Wildco)	2 m	Volume reduced sampling	[26]
	Baram River, Sarawak	Ekman Grab sampler	0–20 cm	Volume reduced sampling	[8]
	Miri river, Sarawak	Ekman Grab sampler	0–20 cm	Volume reduced sampling	[27]
Indonesia	Ciwalengke River	Ekman grab sampler	N/A	Bulk sampling	[28]
	Surabaya River	N/A	N/A	N/A	[29]
Philippines	Lawaye River	Ponar Grab sampler	N/A	Bulk sampling	[30]
	Cañas River	Ekman grab sampler	0–5 cm	Bulk sampling	[31]
	Pasig River	Ekman grab sampler	0–5 cm	Bulk sampling	[31]
	Tullahan River	Ekman grab sampler	0–5 cm	Bulk sampling	[31]
	Parañaque River	Ekman grab sampler	0–5 cm	Bulk sampling	[31]
Thailand	Meycauayan River	Ekman grab sampler	0–5 cm	Bulk sampling	[31]
	Tapi-Phumduang River	Ekman grab sampler	0–5 cm	Volume reduced sampling	[32]
	Chao Phraya River, Bangkok	Van Veen grab sampler	2–3 m	Bulk sampling	[33]
Vietnam	Aquaculture ponds in Hanoi city (Ponds 1)	Van Veen grab sampler	0–30 cm	Bulk sampling	[34]
	Aquaculture ponds in Hanoi city (Ponds 1)	Van Veen grab sampler	0–30 cm	Bulk sampling	[34]
	Tô Lịch River	Grab sampler	N/A	Volume reduced	[35]

Note: N/A-not available

Table 2. The techniques used in 17 studies for microplastic pretreatment, extraction and analysis

Study area	Drying	Chemical digestion	Density separation	Physical characterisation	Chemical characterisation	References
<i>Malaysia</i>						
Skudai River, Johor	Oven-dried at 50°C	30% H ₂ O ₂	NaCl	Microscope	N/A	[26]
Tebrau River, Johor	Oven-dried at 50°C	30% H ₂ O ₂	NaCl	Microscope	N/A	[26]
Baram River, Sarawak	Oven-dried at 65°C	Fenton's reagent	LMT, NaCl	Stereomicroscope	ATR-FTIR	[8]
Miri River, Sarawak	Air-dried	Fenton's reagent	NaCl	Microscope	ATR-FTIR	[27]
<i>Indonesia</i>						
Ciwalengke River	Oven-dried at 100°C	N/A	NaCl	Microscope	Raman	[28]
Surabaya River	Oven-dried at 90° C	30% H ₂ O ₂	NaCl	Stereomicroscope	FTIR	[29]
<i>Philippines</i>						
Lawaye River	Oven-dried at 90°C	Fenton's reagent	NaI	Microscope	FTIR	[30]
Cañas River	Oven-dried at 50°C	30% H ₂ O ₂	NaCl	Stereomicroscope	ATR-FTIR	[31]
Pasig River	Oven-dried at 60°C	Fenton's reagent	NaI	Stereomicroscope	ATR-FTIR	[31]
Tullahan River	Oven-dried at 90°C	Fenton's reagent	NaCl	Stereomicroscope	ATR-FTIR	[31]
Parañaque River	Oven-dried at 90° C	Fenton's reagent	NaCl	Stereomicroscope	ATR-FTIR	[31]
Meycauayan River	Oven-dried at 90°C	Fenton's reagent	NaCl	Stereomicroscope	ATR-FTIR	[31]
<i>Thailand</i>						
Tapi-Phumduang River	Oven-dried at 90°C	Fenton's reagent	NaCl	Stereomicroscope	FTIR	[32]
Chao Phraya River	Oven-dried at 90°C	Fenton's reagent	NaCl	Stereomicroscope	FTIR	[33]
<i>Vietnam</i>						
Aquaculture pond, Hanoi city (Ponds 1)	Oven-dried at 40°C	30% H ₂ O ₂	NaCl	Microscope	Raman	[34]
Aquaculture pond in Hanoi city (Ponds 2)	Oven-dried at 40°C	30% H ₂ O ₂	NaCl	Microscope	Raman	[34]
Tô Lịch River	Oven-dried at 40°C	30% H ₂ O ₂	NaCl	Stereomicroscope	Raman	[35]

Note: N/A-not available

According to Scheurer and Bigalke [50], NaCl is the optimal density solution after evaluating the separation efficiency of several common solutions. The authors argue that PET and PVC have a minor influence on the large microplastic fraction of their samples and will not significantly alter the results in Europe [51]. As a result, the separation solutions chosen must be based on the local plastic demand conditions. In this regard, we suggest using ZnCl₂ as a salt solution, as most microplastics can be

separated since it has a density of 1.6 g/cm³ [17, 25]. A higher recovery rate (96–100%) was achieved with larger microplastics (1–5 mm) and 96% for smaller microplastics (<1 mm) using chloride solution [53]. However, compared to other salt solutions, it has been considered to be more environmentally harmful [48,54]. Therefore, careful handling, disposal, and reuse are required to ensure the most efficient and safe use of the product [55].

Table 3. Specific densities of different polymer types.

Polymer	Density (g/cm ³)
Polypropylene (PP)	0.9–0.91
Polyethylene (PE)	0.917–0.965
Polyamide (nylon)	1.02–1.05
Polystyrene (PS)	1.04–1.1
Acrylic (AC)	1.09–1.20
Polyvinylchloride (PVC)	1.16–1.58
Polymethyl acrylate (PMA)	1.17–1.20
Polyurethane (PUR)	1.2
Polyvinyl alcohol (PVA)	1.19–1.31
Polyethylene terephthalate (PET)	1.37–1.45
Polyoxymethylene (POM)	1.41–1.61
Polyester (PES)	1.24–2.3
Alkyd	1.24–2.10

3. Identification of Microplastics

Physical characterisation: Physical characterisation of microplastic particles is mainly based on shape and colour. In most reviewed studies, the physical characteristics of microplastics are used as pre-selection when chemical characterisation is performed. Although physical characterisation may produce wide variation between observers and is highly time-consuming, this method is a good choice for observing microplastics directly on the filter paper without losing microplastics due to transfer [42]. The number of particles and physical characteristics of microplastics are usually observed under a microscope. The microscope includes an optical microscope and a stereomicroscope. Most of the studies used a stereomicroscope followed by a microscope in their physical characteristics phase (Table 2). The stereomicroscope is the most commonly used in manually counting and identifying microplastics. There are several standardised criteria for strict inspection of microplastic which are (i) microplastic particles are roughly divided into five categories (fibre, pellet, foam, film, and fragment), (ii) microplastic particles should have a relatively uniform colour, (iii) size range, (iv) no cellular or organic structures are visible [7, 36, 42].

Chemical characterisation: Fourier-transform infrared (FTIR) and Raman spectroscopy, in particular, are the most often employed spectroscopic methods for the qualitative identification of plastic particles [56,57,58]. A laser light source is typically used in FTIR and Raman spectroscopy, which produces spectra that can be compared to databases that are accessible commercially or in references [25]. The majority of the studies (11 of 17) used FTIR spectroscopy to identify polymers, with 7 studies using Attenuated Total Reflectance FTIR (ATR-FTIR) and the remaining 4 using traditional FTIR (Table 2). However, the two studies did not employ spectroscopy and therefore provided no information about the polymer type.

Compared with traditional FTIR, ATR-FTIR offers minimal sample preparation with greater surface sensitivity for irregular microplastics. In addition, the ATR-FTIR can provide a high signal-to-noise ratio, and the literature contains many spectral data sets. The ATR-FTIR can also analyse microplastic particles greater than 500 µm [51]. The MSFD technical subgroup recommended FTIR and Raman spectroscopy to all the suspected particles ranging from 20 to 100 µm and 10% microplastics of sizes 100–5000 µm [38]. Both spectroscopy methods are non-destructive, efficient, accurate and complementary, producing a spectrum based on the interaction of light with molecules, whereas FTIR produces an infrared spectrum resulting from the change in dipole moment. At the same time, Raman provides a molecular fingerprint spectrum based on the polarizability of chemical bonds [36]. Raman spectroscopy allows the characterisation of wet samples and microplastics with a size of less than 20 µm, but the biggest disadvantage of Raman is the interference of fluorescence from microbiological, organic and inorganic contaminations, which restricts the identification of microplastics [41].

Further, both of these methods can be used in conjunction with optical microscopy for the purpose of imaging the particles in two dimensions in order to determine their morphological characteristics [59,60,61]. After MPs are detected in a sample, FTIR and Raman spectroscopy can be used to compare the sample's spectrum with libraries and standards to identify and quantify the polymers [62]. Overall, FTIR is better for routine analysis, particularly for quick observation of coarser particles (50-500 µm), whereas Raman spectroscopy is more time-consuming but more accurate.

However, none of the studies has examined the polymer concentration as well as the surface

morphology of microplastics. Hence, we propose that both analyses should be included in future studies because the surface morphology of microplastics can be seen very clearly and magnified, allowing one to observe the surface roughness of the particles and distinguish microplastics from organic particles through Scanning Electron Microscope (SEM) analysis. Polymers and inorganic particles can be distinguished using a scanning electron microscope with elemental analysis with energy-dispersive X-ray spectroscopy (EDS) [58,61]. Meanwhile, pyrolysis with gas chromatography coupled to mass spectrometry (GC-MS) can be used to quantify the polymer concentration of microplastics [25,63, 64] which can further be used to evaluate microplastic risk assessment.

Apart from that, all reviewed studies did not specify the use of contamination control protocols throughout their microplastic studies. Given the possibility of airborne microplastic contamination [65], it is reasonable to ensure the implementation of quality control approaches throughout the study process, including during sample collection, extraction and analysis to avoid any error and interference. It is imperative across all matrices that field and laboratory practices are combined with field control and blank procedures in order to minimise airborne plastic particles generated by equipment and personnel. One common background check or control is exposing a filter paper in a petri dish during field sampling and sample processing [66]. The cleaning or disinfecting all the equipment including the sampling devices and workstation using 70% alcohol could prevent cross-contamination among samples [66]. Due to potential contamination from microplastics in the air, it is important to store clean equipment appropriately (covered or sealed with aluminium foil). Microplastic samples are potentially exposed to bioaerosols due to their ability to bind particulate matter (including microplastics) through adsorption [67]. In addition, the large surface area of plastic particles would facilitate their role as carriers [68]. Therefore, we propose to include a quality control procedure in the future study of microplastics.

Microplastics Levels in Freshwater Sediments

There have been global reports of microplastics in freshwater sediment, but research fields in ASEAN countries are only done for rivers and aquaculture ponds. **Table 4** summarises the microplastic abundance, shape, colour, polymer types and potential sources for freshwater sediments across five ASEAN countries; Malaysia, Indonesia, Thailand, Vietnam, and the Philippines.

1. Abundance of Microplastics in Freshwater Sediments

Riverine sediments: In Malaysia, microplastic research

for riverbank sediment has only been conducted in two states; Johor and Sarawak. Sarijan et al. [26] measured the abundance of microplastics in two Johor rivers, discovering 120–280 particles/kg and 140–820 particles/kg for the Skudai river and Tebrau river, respectively. Microplastic levels in both rivers were found to be proportional to population density as well as near-by industries. In Sarawak, the abundance of microplastics in the Baram River ranged from 53 to 870 particles/kg [8], while the abundance in Miri was 284–456 particles/kg [27]. Microplastic fragments dominated each river, with PP being the most prevalent polymer. In Indonesia, the abundance of microplastics in the bottom sediment of the Ciwalengke river was reported to be 15 to 38 particles/kg [28], whereas the Surabaya River had much higher levels of microplastics with 760 to 43110 particles/kg [29]. While in the Philippines, 6 rivers were investigated for microplastic pollution in sediments. The range of microplastics in sediment from lowest to the highest were Lawaye River (4–11 particles/kg), Cañas River (386–557 particles/kg), Pasig River (386–771 particles/kg), Tullahan River (386–848 particles/kg), Parañaque River (386–1033 particles/kg), and Meycauyan River (386–1052 particles/kg) [30,31]. Microplastic abundance has been linked to anthropogenic activities such as domestic discharges, agricultural activities, fish-ponds, industries, and local informal settlers. Despite being the largest contributor to global river plastic [5], the sediment of the Pasig River did not contain the highest levels of microplastic compared to other regions in ASEAN countries (**Table 4**). The presence of plastic manufacturing industries within the watershed of the river system in the Philippines caused the highest concentration of microplastics in the Meycauyan river, which was ranked fifth on the list of the world's largest plastic-emitting rivers [5]. In Thailand, Chinfak et al. [32] found that the abundance of microplastics in the Tapi-Phumduang river ranged from 55 particles/kg to 160 particles/kg. Another study found 2290 particles/kg of microplastics in the Chao Phraya River [33]. A study in the Tô Lịch River in Vietnam found 956 to 66061 particles/kg microplastic in sediment samples due to untreated domestic and industrial wastes from Hanoi, the capital city of Vietnam [35]. Overall, the Tô Lịch River in Vietnam has the highest abundance of microplastic in freshwater sediment across the ASEAN countries.

Aquaculture ponds sediments: Aquaculture is significant to the economy especially in ASEAN countries. The production and quality of aquaculture products may be impacted by the water and sediment quality of an aquaculture pond [34]. Species could consume microplastic accumulation in the hydro system at low to high trophic levels [35]. Therefore, research on microplastic pollution in aquaculture ponds is crucial for environmental management and the advancement of sustainable aquaculture. At present, only Le et al. [34] have investigated the presence of

microplastics in aquaculture pond sediment. In their study, microplastic was found in the surface sediment of two fishponds in Hanoi, with concentrations ranging from 2527 to 3007 particles/kg and 2657-3009 particles/kg. Like Tô Lịch River, human anthropogenic activities in Hanoi are suspected of being the source of microplastic for this study [35].

2. Physical Characterisation of Microplastics

Shape: Microplastics have been discovered in various shapes, including fibre, fragment, film, pellet, and foam [5,50]. According to de Souza Machado et al. [69] and Zhang et al. [70], the shapes are determined by the original form of primary microplastics, the erosion and degradation processes of the plastic particle's surface, and the time of the microplastics have existed in the environment. Fragment, foam, fibre, and film were identified in varying percentages across all studies (**Table 4**). The most common shape of microplastic found in freshwater sediments is fibre, while the pellet is the least common shape discovered in all studies. The sediment of the Tapi-Phumduang River, Thailand contained the highest percentage of fibre, accounting for 93.9% whereas just 6% of it was made of fragments [32].

Meanwhile, the original material of microplastics can be inferred to a large extent from their shape, as certain shapes can be derived from specific items [71, 72]. For example, fibre, typically derived from textile manufacturing is released into the environment during washing activities [73]. Meanwhile, fragmented microplastics may be produced due to the breakdown of larger plastic pieces into small pieces caused by exposure fatigue or UV light [74]. The surface roughness of fragments and pellets (such as grooves, cracks, attached particles, and flakes) strongly indicates mechanical wear and chemical weathering [75, 76]. Then, the film is primarily derived from plastic bags and packaging materials, whereas foam-shaped microplastics are derived from styrofoam damage [77, 78]. Microplastic pellets were generated from personal care products such as toothpaste, scrub, and others [72].

Colour: Colour is a concern in microplastic research because aquatic species mistake it for food [79,80]. Furthermore, colour is expected to provide potential sources of microplastics during sample preparation. In this review, seven different colours of microplastics were identified; black, blue, transparent, white, red, yellow, and red (**Table 4**). Microplastics of transparent and white colour are typically derived from disposable plastics such as single-use plastic bags, plastic cups, polystyrene food containers, and bottles, all of which are disposable and have a limited lifespan [36,54,81]. Meanwhile, the colours blue and black are expected to be derived from bottle caps, fishing nets, rope, and a black shirt [78]. The original

coloured microplastics most likely come from plastic consumer products with long service lives may be altered during sample preparation and extraction as well as weathering processes [71,80, 82]. Blue was found to be the most common colour in 12 studies (**Table 4**). Furthermore, 4 studies did not report the colour discovered in their research. However, it is important to use caution when using colour to identify the potential source of microplastics. There has been some disagreement in other research on microplastics' colour. As a result, it is not easy to compare different findings. However, it has been suggested to categorise microplastics into four obvious colours (transparent, black, white, and others) rather than analysing additional, more contentious colours (such as yellow, green, blue) [76].

3. Chemical Composition of Microplastics

Polymer types: Plastics were manufactured using a wide range of polymers. The polymer kinds used in plastic materials greatly influence their properties and performances. Polymer types can thus significantly impact the lifetime and buoyancy of microplastics, influencing their fate in the environment [19]. Overall, 15 out of 17 studies have reported on polymer types of microplastic in freshwater sediment. However, only 8 studies have reported the detected polymer types in percentage. Based on **Table 4**, PP dominates the type of particles detected in the Parañaque River of the Philippines, accounting for 63% of the total microplastic found in their study [31]. The synthetic polymers reported in other rivers in the Philippines are PE, HDPE, LDPE, and PS, ranging from 9 to 63% of total microplastics (**Table 4**). In another study, the dominant microplastics found in Thailand have been classified as PS [33], whereas LDPE predominated in freshwater sediment of Indonesia [29]. Aside from that, aquaculture ponds in Hanoi, Vietnam, only found PS and PP in their study, which was 40% and 50%, respectively [34]. The polymers discovered from Miri River, Sarawak, included PE, PU, PP, ethylene propylene diene monomer (EPDM), butyl Branham, and ethylene vinyl acetate (EVA) [27] whilst PE, PET, and PS were found in Baram River [8]. Most PE wastes came from foils, bottles, and tire cords [81]. PE can also be found in disposable plastic and food packaging bags, which billions of people use daily. So far, there is no clear link or explanation for why the types of polymers in freshwater sediment are not always the same. Continuing research is needed to identify the main microplastic polymer group that pollutes freshwater sediments and how the group varies depending on location and travel distance.

Effects of Microplastics Pollution on the Freshwater Ecosystem

Microplastic can directly and indirectly influence aquatic ecosystems because it can absorb toxic compounds

and be absorbed by living organisms, making it a dangerous contaminant to freshwater ecosystems. Microplastic persistence and ubiquity highlight possible concerns. Thus, plastic pollution in freshwater can harm aquatic ecosystems and human health due to consuming contaminated food and drinking water [32,76].

Microplastics of various types have been found in fish species [83,84]. The small microplastics influences the microplastics ingested by a wide variety of aquatic species, disrupting their physiological processes, which then travel up the food chain and cause adverse health effects in humans [19]. There were few studies in ASEAN countries that have found microplastics in biota. Microplastics were discovered in fish digestive tracts and tissues in Citarum River, Muara Gembong, Indonesia, with concentrations ranging from 1.3 to 2.2 items/fish (digestive tract) and 1.1 items/fish (tissues) [84]. Another study in Surabaya River reported microplastics ingested by fish with a microplastic abundance of *O. niloticus* was found at 155.50 ± 61.96 particles/individual and the highest microplastic abundance of *B. gonionotus* was found at 155.00 ± 81.71 particles/individual [28]. The presence of transparent microplastics along the Surabaya River in Indonesia increases the likelihood that aquatic biota will consume it because the colour of the microplastics is similar to that of their natural prey. Cabansag et al. [85] demonstrated that *K. rupestris*, a typically freshwater fish species from the Lawaan River in the Philippines, was susceptible to microplastic ingestion in the same way as marine species (*S. canaliculatus*) but with a lower percentage of microplastic ingestion which ranges from 0.62 microplastics/fish to 1.24 microplastics/fish. Next, Sarijan et al. [86] reported microplastics found in fish digestive tracts, with 40% of the fish community ingesting microplastics in the Skudai River, Malaysia. In another study in Malaysia, Zaki et al. [87] discovered microplastic in the gastropod *Nerita articulate* with a mean abundance of 0.92 particles/g. The investigation of microplastics and trace metals in fish and shrimp from Songkhla Lake, Thailand, during the covid-19 pandemic discovered microplastics with a variety of colours, and polymers in all investigated fish and shrimps, with the mean concentration of *M. brevicornis shrimp* being 0.76 ± 0.48 pieces/g (wet weight), followed by *P. hardwickii* shrimp possessing 0.55 ± 1.19 pieces/g (wet weight), and for fish being 0.018 ± 0.27 pieces/g (wet weight) [88]. Another study also reported microplastic ingestion by freshwater fish in Thailand's Chi River, with the percentage occurrence of microplastics in each species ranging from 50.0 to 86.7%, with *Puntioplites proctozysron* having the highest microplastics abundance [89]. Hu et al. [90] have reported that microplastic consumption by fish can alter gut function, resulting in obstruction, tissue damage, decreased swimming velocity or energy reserves, and a false sense of satiation, which limits nutrient intake, causes hunger,

limits growth, and reduces the ability to evade predators. Since the majority of microplastics accumulate in fish digestive organs [91], it is recommended that digestive organs should be removed for human consumption to limit microplastic ingestion.

Furthermore, the chemical impacts of microplastics may be induced by the polymers, additives, and persistent organic pollutants (pollutants of particular concern to human health) absorbed by the microplastic surface. Since benthic invertebrates provide up to 90% of fish food biomass [93], and sediment becomes a sink for various organic and inorganic pollutants [94,95], the effects of microplastic intake in freshwater benthos is an important concern. Because of this, the build-up of microplastics in sediment might increase the bio-magnification of contaminants. In addition, the consumption of microplastics by benthic freshwater invertebrates could affect the bioturbation of sediment [96]. Microplastics affect freshwater organisms by releasing additives that aggravate water pollution and absorb toxic pollutants [97]. Additives like plasticisers, heat stabilisers, colourants, foaming agents, and even heavy metals are commonly found in plastics [98]. Thus, it can potentially cause cancer and harm the endocrine system of the organism. Other than that, the leaching of microplastic additives may cause water pollution, harming aquatic organisms. Due to their large surface area and hydrophobicity [33,99,100], microplastics would absorb persistent organic pollutants (e.g., PCBs, PAHs, DDTs, and PBDEs) and heavy metals (e.g., Cr, Cu, Ni, Pb, Cd, and Zn) at the same time increasing their toxicity. Continual exposure to organic pollutants and heavy metals can cause abnormal secretions, mutations, and cancer. These pollutants may eventually be absorbed into high-nutrient organisms through the food chain [100].

Other than that, a study from Ireland has also been conducted to investigate the effects of microplastic in freshwater on producers such as plants and algae. Both Mateos-Cárdenas et al. [101] and Kalčíková et al. [102] discovered that the presence of microplastic on the leaf surfaces of *Lemna minor* had no influence on the plant's ability to produce photosynthesis, and they also discovered that there were no effects on the rates at which leaves grew. Despite this, Kalková et al. [102] discovered that there were effects on the growth of roots and the vitality of root cells. The impacts of microplastic have also been examined in *Myriophyllum spicatum*, a sediment-rooted macrophyte. The researchers found that the shoot length of this species was significantly reduced [103]. It has been demonstrated that exposure to microplastics disrupts photosynthesis and causes damage to the cell walls of the algal *Scenedesmus obliquus* [104]. Additionally, photosynthesis in *Chlorella pyrenoidosa* was found to be reduced by microplastics when present at large concentrations, as discovered by Wu et al. [104]. On the other hand, Canniff and Hoang [105] discovered

that the microalga *Raphidocelis subcapitata* was unaffected by the deleterious impacts of microplastic. Nevertheless, none of the ASEAN studies has addressed the impact of microplastics on organisms, as all studies only report the microplastic level in fish species and gastropods. As a result, the risk to ecological health and humans remains difficult to assess in the ASEAN region, where basic information on contamination levels in freshwater is extremely limited [106].

CONCLUSION

This review examined the literature from 2018 to April 2022 in order to improve our understanding of microplastics in freshwater sediments, including their occurrence levels, physical and chemical properties, and impacts on aquatic life. Various abundances have been reported in the studies, reflecting the heterogeneity of river systems and a lack of standardisation across sampling techniques. Therefore, comparing sediment studies comprehensively and addressing the environmental effects of microplastics is problematic. Land-based activities coupled with population density lead to the discharges of significant amounts of microplastic with complex chemical composition contributing to

elevated microplastic pollution levels and diversity of shapes, sizes, and colours. From a contamination control perspective, it is necessary to develop comprehensive quality control to be included in routine sampling protocols and analysis to prevent cross-contamination while ensuring quality in microplastic analytics. Especially in ASEAN countries, where drinking water and food depend on the freshwater ecosystem, micro-plastics and their impact on aquatic organisms are growing concerns due to their ubiquitous presence in the environment. Therefore, more extensive research is needed to fully characterise the sources, fate, and effects of microplastic contamination in freshwater environments in ASEAN nations, which are among the top plastic polluters in the world.

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Table 4. The abundance of microplastic in freshwater sediment across ASEAN countries.

Study Area	Abundance (particles/kg)	Size range	Shape	Colour	Polymer Types	Sources	References
<i>Malaysia</i>							
Skudai River, Johor	120–280	1000–5000 µm	Film, fragment, fibre	Yellow and white	N/A	Discharge of local plastic manufacturer and sewage treatment plant	[26]
Tebrau River, Johor	140–820	1000–5000 µm	Film, fragment, fibre	Blue	N/A		
Baram River, Sarawak	53–870	N/A	Fragment (67.8%) fibre (18.7%), film (8.4%) pellets (3.0%) foam (2.0%)	Blue, black, red, green, transparent, yellow	PE, PET, PS	Heavy industrial, shipyards, and other wood-based industries, fishing activities and domestic wastewater	[8]
Miri River Estuary	284–456	<1 mm–4 mm	Fragment (57%), fibre (36%), foam (4%), pellet (3%)	Black (22.7–35.9%), blue (23.6–24.1%), and transparent (11.9–14.8%)	PE, PU, PP, EPDM	Discharge of domestic sewage, industries manufactured, shipyard industry, and agriculture	[27]
<i>Indonesia</i>							
Ciwalengke River,	15–38	50–2000 µm.	fibre (91%), fragments (9%)	N/A	polyester (PE), Polyamide (PA)	Laundry, industries, and domestic activities	[28]
Surabaya River	760–43110	1–5 mm	film (63.4–88.7%), fragments (4.7–35.6%), foam (0.8–20.7%), pellets (0.5–2.6%), fibres (0.8–12.4%)	Transparent (33.1–79.9%), white (7–56.5%), blue (4.7–27.2%), red (1.7–10.6%), black (6.9–13%) yellow (0.3–8.2%)	LDPE (39–73%)	Domestic activities, industrial activities	[29]

*Note: N/A-not applicable

Table 4. continued

Study Area	Abundance (particles/kg)	Size Range	Shape	Colour	Polymer Type	Sources	References
<i>Philippines</i>							
Lawaye River	4–11	N/A	Filaments and fragments	N/A	PE (HDPE or LDPE) with silica	Discharge of domestic's activities, agricultural activities, fishponds, and local informal settlers	[30]
Cañas River	386–557	0.075–5 mm	Fragment, films	Transparent and blue	PP (45%), LDPE (24%), HDPE (23%), PS (9%)	Residential homes mostly informal settler	[31]
Pasig River	386–771	0.075–5 mm	Fragments, Films	Transparent and blue	PP (59%), HDPE (22%), LDPE (19%)	Manila port and Baseco Compound, a relocation site for informal settler families	[31]
Tullahan River	386–848	0.075–5 mm	Fragments	Transparent and blue	PP (44%), PS (29%), HDPE (18%), LDPE (9%)	Residential houses, pumping stations and bancas and canoes; Presence of plastic manufacturing industries within the watershed	[31]
Parañaque River	386–1033	0.075–5 mm	Fragments, fibres	Transparent and blue	PP (63%), HDPE (20%), LDPE (17%)	Near to Las Piñas-Parañaque Critical Habitat and Ecotourism Area (LPPCHEA) and a seafood market.	[31]
Meycauayan River	386–1052	0.075–5 mm	Fragments	Transparent and blue	PP (47%), HDPE (33%), LDPE (10%), PS (10%)	Presence of plastic manufacturing industries within the watershed of the river system	[31]
<i>Thailand</i>							
Tapi-Phumduang River	55–160	0.424–1.356 mm	Fibres (93.9%), fragments (6%)	blue (44%), white (25%), black (20%), green (9%) and red (2%)	PP, PE, PET, PA, Rayon	Areas of intensive palm and rubber farming, agriculture activities, and intensive tourism activities	[32]

Table 4. continued

Study Area	Abundance (particles/kg)	Size Range	Shape	Colour	Polymer types	Sources	References
<i>Thailand</i> Chao Phraya River, Bangkok	2290	0.053–0.5 mm	Fragments	N/A	PS (42.86%), PP (28.57%), PE (14.29%), PES (7.14%)	Tourism and urban activities	[33]
<i>Vietnam</i> aquaculture ponds in Hanoi city (Ponds 1)	2657–3009	N/A	Fibre (62%) and fragment (38%)	green, white, black, red, white, yellow, blue	PE (40%) and PP (50%)	Untreated wastewater or sewage from Hanoi city, domestic	[34]
aquaculture ponds in Hanoi city (Ponds 2)	2657–3009	N/A	Fibre (81%) and fragment (19%)	green, white, black, red, white, yellow, blue	PE (40%) and PP (50%)	Wastewater, agriculture activities, and other polluted sources	
Tô Lịch river	956–66061	300–500 µm	Fragments, flms, foams, fbres and pellets	N/A	PS, PVC, PET, PP and HDPE	Untreated domestic and industrial waste from Hanoi city.	[35]

*Note: N/A-not available

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