MICROPLASTICS IN THE LANGAT RIVER BASIN, MALAYSIA: SOURCES, LOADS AND FATE

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Abstract

Microplastics (commonly defined as plastic particles smaller than 5mm in size) are widely recognised as a global concern. However, studies of the occurrence of microplastics in freshwater systems, particularly in Malaysia, are limited.

This study aims to assess the sources and levels of microplastic contamination within the Langat River Basin, Malaysia. This study presents the first comprehensive empirical assessment of the contribution of different sources of microplastic as well as the first systemic data on spatio-temporal variability in microplastic concentrations in a Malaysian river. Such data is crucial for the identification of contamination hotspots and, in turn, the development of management strategies. This study is also the first to evaluate the degree of correspondence between microplastics in river water and bed sediments, which is critical for monitoring and assessment of potential risks posed by microplastics to acquatic organisms and human health.

A total of 656 water and sediment samples were collected from 33 sites spread across the Langat River Basin. The samples were treated with Fenton reagent to remove organic material and filtered onto glass microfiber filter papers before enumeration under a stereoscopic microscope.

Road runoff, residential and industrial areas, atmospheric deposition and wastewater treatment plants were significant sources of microplastics in the Langat River, with road runoff being the main contributor. The Langat River had a mean concentration of 4.39±5.11 particles/L, which varied spatially (associated with differences in land-use) and temporally (associated with flow changes). Microplastic was deposited on the bed of the Langat with a mean of 6027.39±16585.87 particles/m^2, which did not correspond to the concentrations in the water either at site-scale or patch-scale, suggesting that sampling designed to assess risks posed by microplastic should assess both concentrations in the water and on the bed. Efforts to reduce microplastic contamination should be focused on upstream intervention, including law

enforcement, endorsing proper management systems and creating public awareness. Such efforts are sorely needed in Malaysia, due to the rapid pace of development set against limited awareness and poor waste management.

List of Published Papers

Published thesis chapters

Chapter 2

Chen, H.L., Selvam, S.B., Ting, K.N. and Gibbins, C.N., 2021. Microplastic pollution in freshwater systems in South East Asia: contamination levels, sources and ecological impacts. *Environmental Science and Pollution Research*, 28, pp.54222-54237.
DOI: 10.1007/s11356-021-15826-x

Chapter 3

Chen, H.L., Selvam, S.B., Ting, K.N., Tshai, K.Y. and Gibbins, C.N., 2022. Relative contributions of different local sources, to riverborne microplastic in a mixed landuse area within a tropical catchment. *Environmental Research, 210,* 112972.
DOI: 10.1016/j.envres.2022.112972

Chapter 4

Chen, H.L., Gibbins, C.N., Selvam, S.B., and Ting, K.N., 2021. Spatio-temporal variation of microplastic along a rural to urban transition in a tropical river. *Environmental Pollution, 289,* 117895.
DOI: 10.1016/j.envpol.2021.117895

Chapter 5

Chen, H.L., Selvam, S.B., Ting, K.N. and Gibbins, C.N., 2023. Microplastic concentrations in river water and bed sediments in a tropical river: implications for ecological status and water quality monitoring. *Environmental Monitoring and Assessment, 195,* 307.
DOI: 10.1007/s10661-022-10856-5

Other published papers

Lee, K.S., Chen, H.L., Ng, Y.S., Maul, T., Gibbins, C.N., Ting, K.N., Amer, M. and Camara, M., 2021. U-Net Skip-Connection Architectures for the Automated Counting of Microplastics. *Neural Computing and Applications, 34*(9), pp.7283-7297.
DOI: 10.1007/s00521-021-06876-w

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List of Acronyms

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
DID	Department of Irrigation and Drainage Malaysia
EVA	Ethylene vinyl acetate
FTIR	Fourier-transform infrared spectroscopy
GKL	Greater Kuala Lumpur
GLMM	Generalised linear mixed model
HDPE	High density polyethylene
LDPE	Low density polyethylene
LOESS	Locally weighted least squares regression
OLS	Ordinary least squares regression
PCCP	Personal care and cosmetic product
PE	Population equivalent
PET	Polyethylene terephthalate
PP	Polypropylene
PS	Polystyrene
PU	Polyurethane
RE	Removal efficiency
SBR	Styrene butadiene rubber
SEA	South East Asia
SEM	Scanning electron microscope
SSB	Sum of squares between groups
SSC	Suspended sediment concentration
SSW	Sum of squares within groups
TRWP	Tyre and road wear particle
WQI	Water quality index
WWTP	Wastewater treatment plant

1

Introduction

1.1 Background

1.1.1 Plastics

Plastics are synthetic polymers that are commonly derived through the polymerisation of monomers of petrochemicals (Ivleva et al., 2017). Plastics are typically manufactured using a wide range of additives, including plasticisers, flame retardants and pigments, to make them fit their intended purpose (Lambert and Wagner, 2018). These additives provide plastics with outstanding durability and versatility, as well as their lightweight and thermal insulation properties (Ivleva et al., 2017). Due to these properties, as well as the relatively low cost of production, plastic has replaced many traditional materials in the automotive, manufacturing, textile, packaging and construction industries (Ivleva et al., 2017). While plastic improves our daily lives in an almost uncountable number of ways, it also creates one of the most pressing global environmental problems we face (Chen et al., 2021a).

Plastics were first produced in the 1800s, but it was not until 1909 that the mass production of plastics started (Lambert and Wagner, 2018). Since then, plastic production has increased exponentially (Ostle et al., 2019). Currently, the annual production of plastics is more than 320 million tonnes, and the ubiquity, durability and persistence of this material pose a major threat to the environment (Barboza et al., 2019). Together with the increasing plastic production, the generation of plastic waste has also increased significantly. Owing to the worldwide transition to single-use plastics, plastic production is currently dominated by the packaging industry, where plastics have a short useable life and are often discarded within the same year of production (Chen et al., 2021b). In 2016, plastic constituted over 12% of global waste generation, third only to food and paper wastes (Kaza et al., 2018); the result is that up to 12.7 million metric tonnes of plastic may be entering oceans annually (Jambeck et al., 2015).

From the polar regions to the deep-sea sediments, every ocean in the world is now contaminated by plastic (Gall and Thompson, 2015; Hurley et al., 2018; Obbard, 2018; Van

Cauwenberghe et al., 2013). Plastics have been recognised as a threat to the marine environment since the 1970s, with early research focusing on larger plastic fragments, otherwise known as macroplastics; such plastics carry risks of entanglement and ingestion by marine organisms (Barboza et al., 2019; Xanthos and Walker, 2017). It is estimated that more than one million marine animals are killed by plastic pollution annually (World Wildlife Fund Malaysia, 2019). Plastic can affect individual organisms, populations and trophic interactions, threatening marine biodiversity (Eerkes-Medrano et al., 2015; Gall and Thompson, 2015); hence, plastic waste has become a highly emotive issue, capturing media and public attention. However, it is only recently that small fragments of plastic, almost invisible to the naked eye, have been found in oceans and marine organisms (Browne et al., 2017) and this discovery has raised questions about their ecological and human health impacts.

Plastics break down into smaller fragments, known as microplastics. The degradation process, typically relating to plastics that are exposed to the environment and which subsequently lose their original properties (Horie et al., as cited in Vohlídal, 2020), depends on several biological, physical or chemical factors (Klein et al., 2018). The degradation of plastics can be categorised into (1) biodegradation, (2) photodegradation and (3) mechanical degradation. Biodegradation involves the degradation of plastics by microorganisms and is governed by three criteria: (1) the presence of microorganisms, (2) the environmental conditions, and (3) the morphology of plastic particles (Klein et al., 2018). The slow pace of degradation can mean that plastics remain in the natural environment for several decades (Vohlídal, 2020).

In the aquatic environment, plastics typically break down through mechanical degradation. Mechanical degradation involves the breakdown of plastics into smaller plastic particles through friction forces. This process increases the total surface area of plastics, which, in turn, encourages the rate of mechanical degradation and further decreases the sizes of plastics.

1.1.2 Microplastics

Microplastics have been defined as any plastics that is smaller than 5 mm in size. The lower size limit, however, has yet to be formalised and in practice often depends on the size of the mesh used during sample collection (Ivleva et al., 2017; World Health Organisation, 2019). In recent years, smaller particles have been referred to as 'nanoplastic'. Although a size range of 1 nm to 1 μm for nanoplastic has been proposed (Gigault et al., 2018), the dividing line between microplastic and nanoplastic remains unclear and varies between studies. As authors have yet to adopt consistent methods for size ranges, reported contamination loads are often not directly comparable (Jiang et al., 2020; Ramírez-Álvarez et al., 2020; Zhang et al., 2020). This is discussed further in Chapter 2.

Microplastic can be categorised into four types: (1) beads or pellets, (2) fibres or lines (3) films or sheets and (4) fragments. Some examples of these microplastic types are shown in Fig 1.1. Beads and pellets are categorised by their spherical shapes, which include polystyrene beads used for beanbags and microbeads used for nail art. Fibres or lines are categorised by having an equal diameter throughout their long axis (Hidalgo-Ruz et al., 2012) and are primarily sourced from synthetic textiles and fishing nets. Films are categorised by their 2-dimensional structure, often originating from plastic bags or films, while fragments are categorised by their 3-dimensional, irregular structure, and come from a wide range of plastic items.

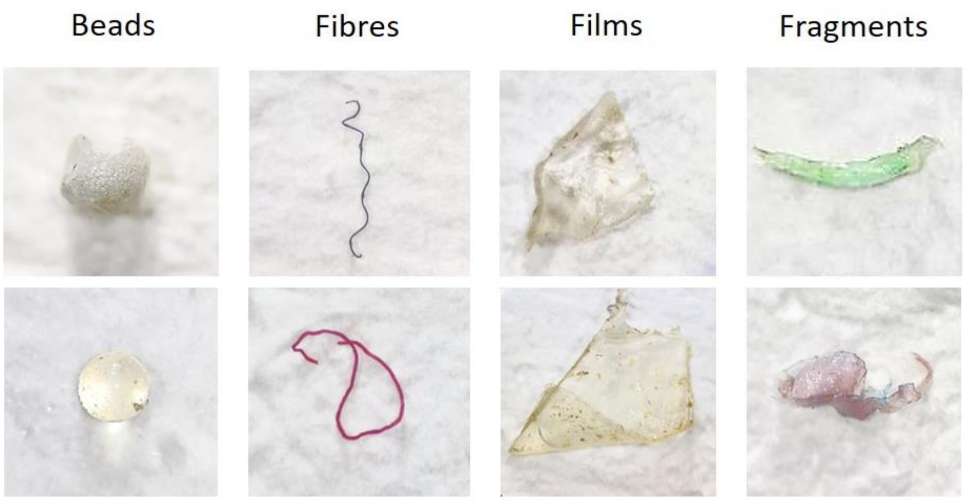

Fig 1.1 Examples of types of microplastics (from samples collected in this study).

Microplastic can also be classified by its origins, and so is often termed either primary or secondary. Primary microplastics, which account for 15-31% of microplastic in the oceans, are materials produced originally at a small size (European Commission, 2017). Plastic resin pellets are an example of primary microplastic. Used primarily as raw materials for the production of plastics, the introduction of these pellets into the environment is often linked with spills during production and transportation (Karlsson et al., 2018). Another common example of primary microplastics is material used in personal care and cosmetic products (PCCPs) such as toothpaste, body scrubs, hand sanitisers and other cosmetics. The functions of microplastics in PCCPs are variable, including (1) exfoliants (i.e. in the form of microbeads) to replace natural ingredients such as dried almonds, (2) glitter (i.e. in the form of hexagonal films) and (3) capsules for the controlled release of active ingredients in PCCPs (Leslie, 2014). Microplastics from PCCPs are emitted directly into the rivers via discharges from domestic drains.

Secondary microplastics, resulting from the fragmentation of larger plastics, are what make up most microplastics worldwide; they comprise up to 81% of microplastics found in oceans (European Commission, 2017). One of the major sources of secondary microplastics is the fragmentation of microfibres from synthetic textiles through domestic textile washing; e.g. a recent study has estimated that up to 1.5 million fibres may be released from a single load of laundry (De Falco et al., 2019). Tyre and road wear particles (TRWPs) from general wear and tear are another major source of secondary microplastics, with studies reporting concentrations of up to 14472 particles/L in road runoff (Knight et al., 2020). Although these secondary microplastics may be intercepted in wastewater treatment plants (WWTPs), WWTPs with inadequate facilities will, in turn, act as a source of microplastics in the environment. Though studies have reported removal efficiencies as high as 97% to 99.9% in WWTPs (Tang et al., 2020), these data were obtained from developed countries and may vary greatly, as a function of the nature of the treatment processes and technologies employed.

1.1.3 Microplastics in the environment

Microplastics are omnipresent. They have been found in all the world's oceans, from the seawaters of the Pacific and the Atlantic Oceans to the sediments of the Arctic deep-sea and Australian seafloor (Bergmann et al., 2017; Courtene-Jones et al., 2017; Desforges et al., 2015; Eerkes-Medrano et al., 2015; Ling et al., 2017). The first study on microplastics was carried out by Thompson et al. (2004), where the authors pointed out the likely increase of microplastic contamination in marine ecosystems. Thompson et al. (2004) also highlighted the possibility of microplastic ingestion by marine organisms after exposing barnacles, amphipods and lugworms to microplastics in laboratory trials. Throughout the years, studies on microplastic ingestion by marine organisms have confirmed this concern (examples are Browne et al., 2008; Cole et al., 2013; Graham and Thompson, 2009 and von Moos et al., 2012), and the impacts of microplastics on organisms have been increasingly documented (Wright et al., 2013).

Aside from marine ecosystems, microplastics have also been observed in terrestrial, freshwater and mangrove ecosystems (Deng et al., 2021; Wong et al., 2020), where all three ecosystems have been regarded as both sources and sinks of microplastics. Over recent years, however, an increase in studies addressing the occurrences of microplastics in freshwater ecosystems is evident (Horton et al., 2017; Rochman, 2018), as rivers often act as a major source of drinking water and fisheries and food. Studying microplastics in rivers is also crucial as rivers act as a major conduit, transporting microplastics from terrestrial ecosystems into the oceans.

1.2 Problem statement

Although research on freshwater microplastics has increased throughout the years, marine ecosystems have remained the focus of microplastic research. Based on the literature review carried out (Chapter 2), there remains a lack of spatio-temporal studies on microplastic contamination in river channels, and how microplastics relate to discharge and fine sediments. Moreover, the relative contributions of sources of microplastics to rivers are extremely poorly known; an extensive review of existing literature shows that only two studies have been carried out to date (Dris et al., 2015; Dris et al., 2018), both of which were conducted in a defined geographic area. Such studies are crucial for the identification of contamination hotspots to assess associated ecological and human health risks, yet a general paucity of information remains.

Research on microplastics in freshwater ecosystems also remains geographically fragmented (Chen et al., 2021a). Blettler et al. (2018) have pointed out the research gap on microplastics in Asia, in particular, in countries with low to middle income. Research needs to be focused on developing countries, primarily in countries where waste management and treatment systems are inadequate (Blettler et al., 2018) and where drainage systems may be poorly developed (hence wastewater goes directly to water courses). In Malaysia for instance, despite the recent increase in microplastic research, only 3 published works are related to microplastic contamination in rivers. The sources of microplastics in Malaysian rivers have yet to be studied, and basic knowledge and monitoring of contamination levels of microplastics in Malaysian freshwaters remain scarce.

1.3 Objectives of study

Based on the problem statement discussed in Section 1.2, a series of aim and objectives of this thesis were developed. The overall aim of this thesis is to assess the sources and levels of microplastic contamination in rivers in the Langat River Basin, Malaysia. The objectives are:

1. To assess the contribution of different sources of microplastic contamination in river water (Chapter 3).
2. To assess the spatial and temporal variability as well as the influence of hydrological conditions on microplastic in surface water (Chapter 4).
3. To evaluate the degree of correspondence between microplastics suspended in surface water and deposited on bed sediments, as well as their implications for microplastic monitoring and assessment (Chapter 5).

1.4 Significance of study

This thesis presents the first comprehensive empirical assessment of the relative contribution of different sources of microplastics in Malaysian rivers. Understanding the local-scale contribution of microplastics from different point- and non-point sources is crucial to help prioritise measures to reduce river contamination levels and develop mitigation strategies. This is especially important in rapidly developing countries, where drainage systems and water treatment facilities are often poor and have limited capacity to remove microplastics, therefore representing a major source of river contamination.

As even basic levels of contamination of microplastics in Malaysian rivers are almost unknown, the findings reported in this thesis will shed light on the severity of microplastic pollution in Malaysia. This thesis presents the first systematic data on spatio-temporal variation in microplastic concentrations in a Malaysian river. The catchment-wide study (i.e. assessment of spatial variation) is critical for the identification of contamination hotspots to help gauge associated ecological and human health risks, while the repeat survey (i.e. assessment of temporal variation) is crucial for highlighting peak concentrations to improve both estimates of microplastic concentrations and risk assessments.

This thesis is also the first to elucidate the relationship between microplastic concentrations in surface water and sediments in Malaysian rivers. This will provide insights into whether microplastic concentrations on the bed reflect what is suspended in the water column and direct the monitoring of microplastic contamination in rivers. This is important as existing studies on microplastic contamination often focus on microplastics in the water column, but the assessment of ecological risks requires information on the amount of material deposited on the bed. Therefore, understanding this relationship helps to elucidate the most appropriate way to monitor microplastic contamination.

1.5 Thesis scope and structure

This thesis comprises a total of six chapters, i.e. an introduction chapter, a literature review chapter, three research chapters and a synthesis chapter. Chapters 2 to 5 of this thesis have been prepared as scientific papers and have been published in scientific journals. The contents of the published articles have been retained in this thesis, hence several sections (e.g. method sections) may include some repetition. Minor edits to Chapter 2 were also made to ensure that the literature cited is up-to-date (i.e. to include newly published literature after the review was published). These chapters can be read and understood independently. However, together they advance knowledge of microplastic loads, sources, pathways and potential associated health risks in Malaysia.

The structure and questions addressed by the thesis are summarised in Fig 1.2.

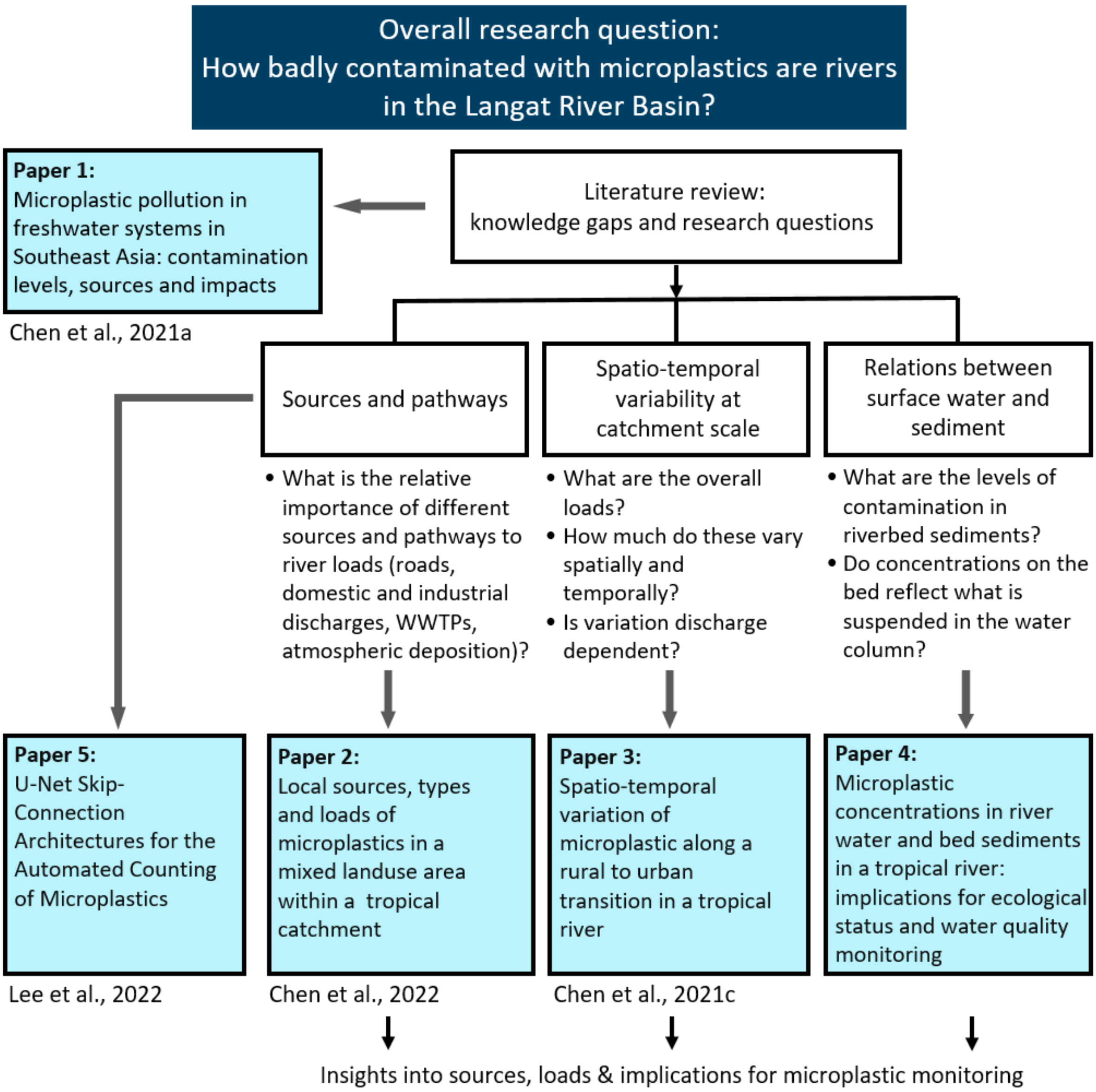

Fig 1.2 Research topics, key research questions and research outputs of this thesis.

The thesis starts by reviewing the literature on microplastic contamination in freshwater systems (Chapter 2). This chapter synthesises important background information on the sources and occurrences, fate and transport of microplastics in freshwater ecosystems, as well as the impacts of freshwater microplastics on organisms and potentially human health. In particular, the lack of freshwater microplastic studies in the South-east Asian (SEA) region and Malaysia has been highlighted. The knowledge gaps highlighted in this chapter have led to the development of the subsequent chapters. This chapter has been published in the Environmental Science and Pollution Research journal.

Chapters 3 to 5 are structured around the objectives of this thesis, with each objective addressed in a separate chapter. Fig 1.3 shows the overall map of the sampling sites in Chapters 3, 4 and 5.

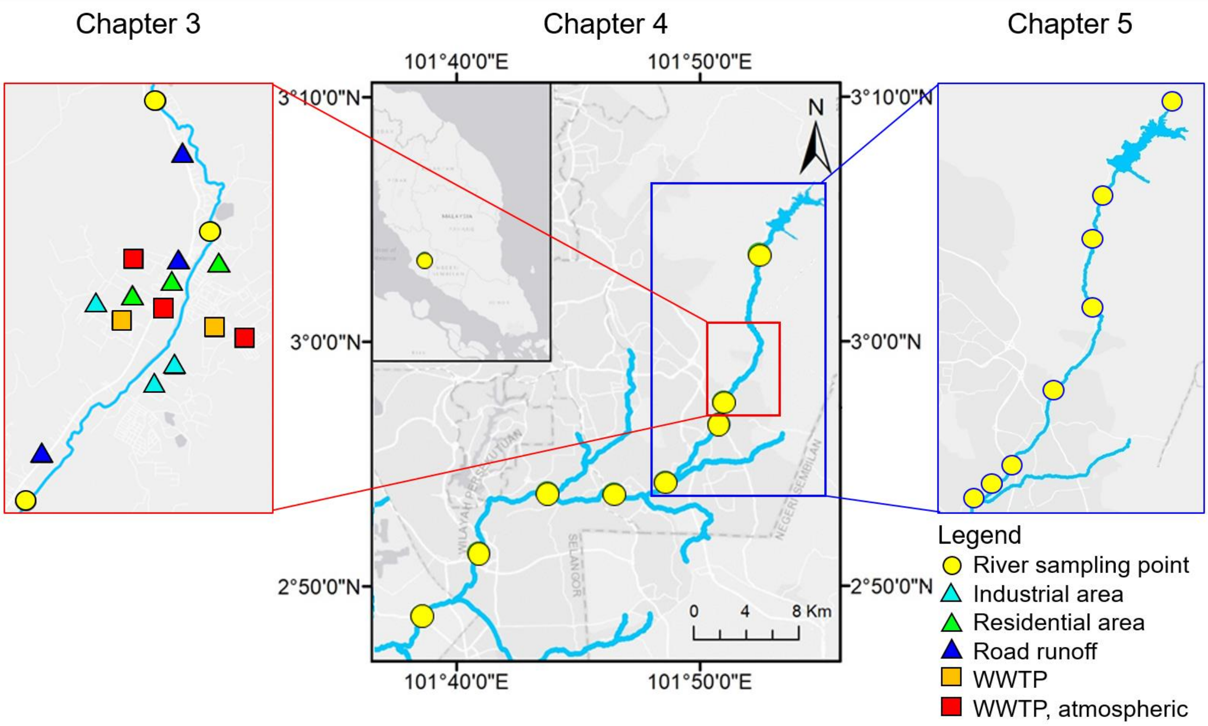

Fig 1.3 Overall map of the study area, showing the sites of Chapters 3, 4 and 5.

Chapter 3 assesses the sources of microplastics in a small part of the Langat basin. It investigates and compares discharges from road runoff, industrial and residential areas, atmospheric deposition and WWTPs as sources of microplastics into the mainstream river. It establishes the proportions of load originating from respective areas and examines the efficiency of microplastic removal from WWTPs, giving a basis for targeted remediation. This chapter has been published in the Environmental Research journal. Images of several sites for Chapter 3 are shown in Fig 1.4.

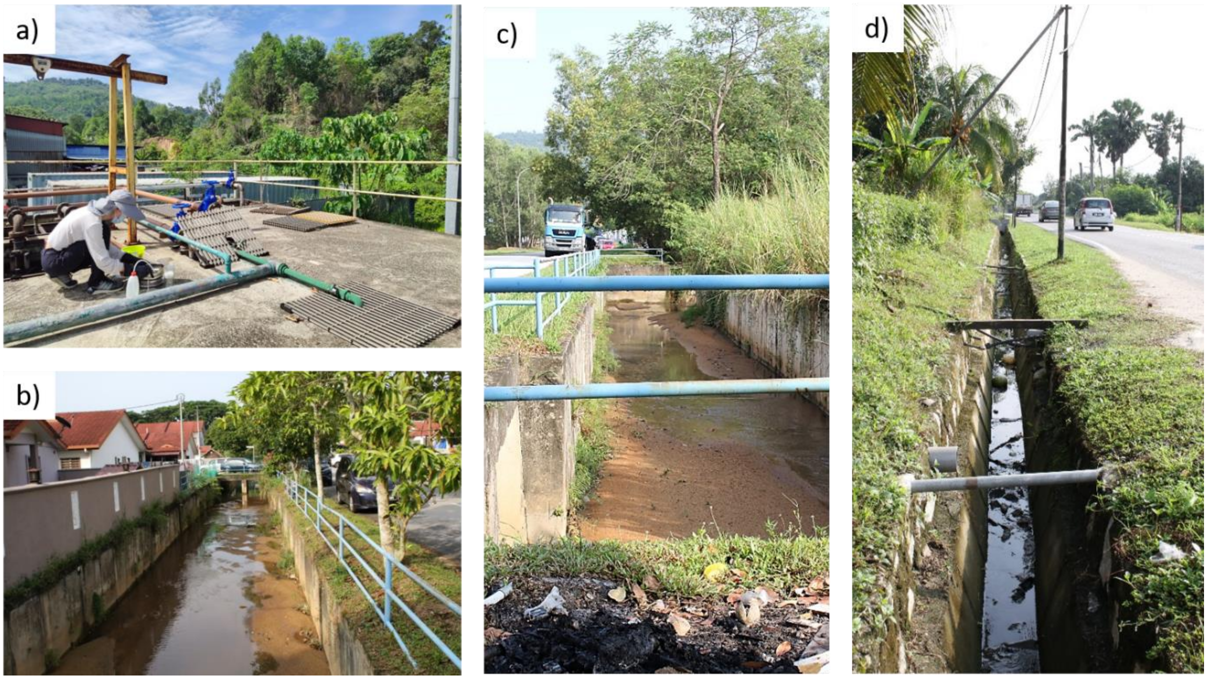

Fig 1.4 Sites of Chapter 3, showing examples of a) a wastewater treatment plant as well as drains adjacent to b) a residential area, c) an industrial area and d) a main road.

In Chapter 4, the spatio-temporal variation of microplastics across a tropical river, i.e. the Langat River, is studied. It comprises a 12-month study of microplastics spanning a rural to urban transition along the river. This chapter examines the relationship between microplastic concentrations, discharge and suspended sediment concentrations, to which a daily estimate of the total amount of microplastics delivered by the Langat to the ocean was provided. The need for this is discussed in the literature review. This catchment-wide study was also designed to assess how catchment-wide flooding affects microplastic concentrations as well as whether suspended sediments may be used as a potential surrogate to provide daily estimates of microplastic loads in rivers. This chapter has been published in the Environmental Pollution journal. Images of several sites for Chapter 4 are shown in Fig 1.5.

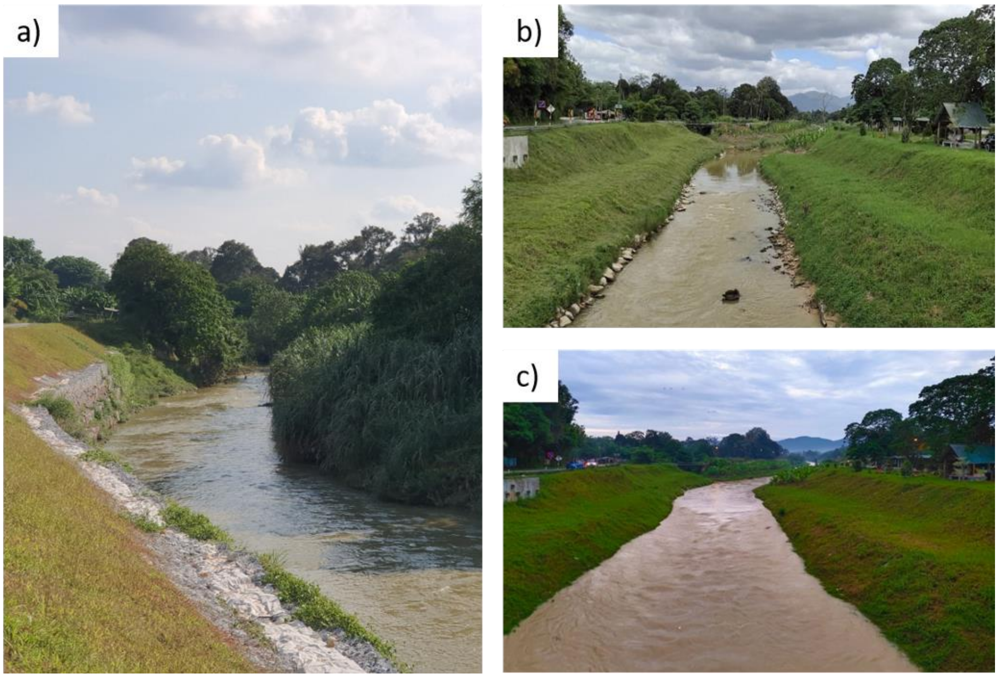

Fig 1.5 Sites of Chapter 4, showing a) the Langat River and the river b) before and c) during a flood event.

In Chapter 5, the levels of microplastic contamination in riverine surface water and bed sediments were compared. This was to assess the extent to which microplastic concentrations on the bed reflect what is suspended in the water column and vice versa. It provides recommendations for the most appropriate way to monitor microplastic contamination in rivers, particularly to assess ecological risks. The overall goal is to determine whether the most appropriate way to monitor microplastic contamination is to focus on assessing concentrations in the water or material on the bed. Images of several sites for Chapter 5 are shown in Fig 1.6.

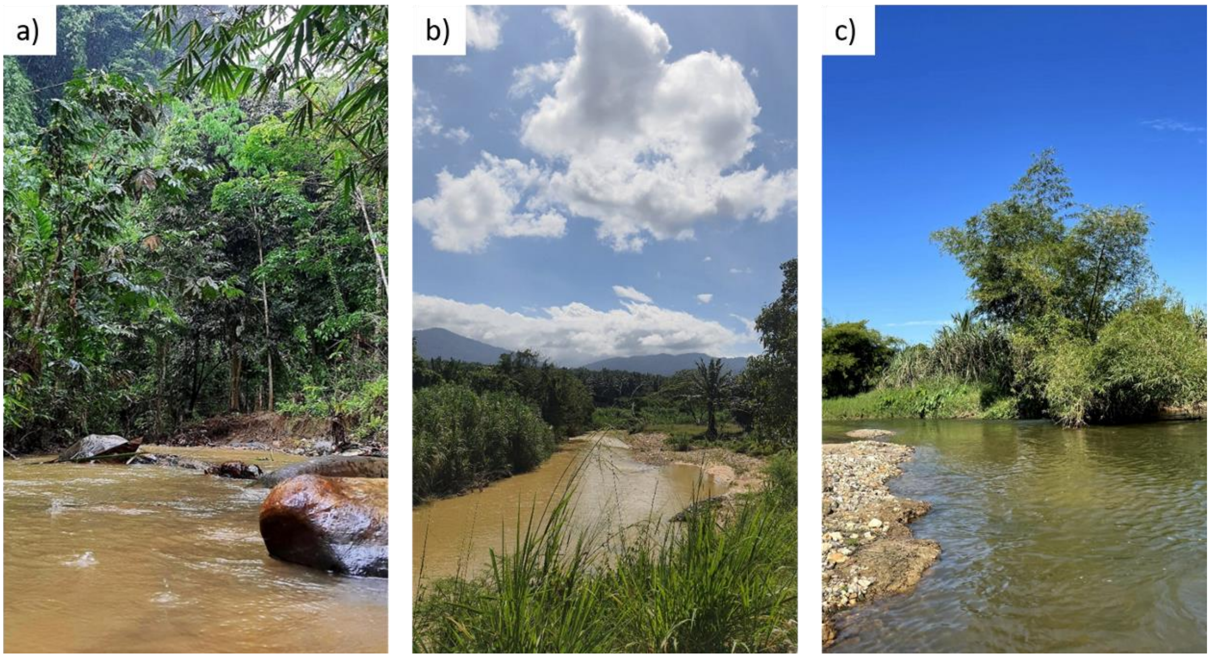

Fig 1.6 Sites of Chapter 5, showing the a) upstream, b) midstream and c) downstream sites of the Semenyih River.

Finally, in Chapter 6, the general conclusions of this thesis are discussed. The objectives of this thesis are first revisited. The main findings of each chapter are then summarised, highlighting the implications of the research findings on policy and practice. Lastly, the chapter discusses possible limitations and provides recommendations for future research needs.

Note that the findings of this thesis mainly covers research in the Langat River Basin, Malaysia, and may not be generally applicable to rivers in different ecosystems. However, the Langat is typical of many rapidly developing parts of Malaysia and other tropical rivers in SEA, hence may be used to understand the sources and fate of microplastics in other suburban/urban rivers in SEA.

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2

Microplastic pollution in freshwater systems in South East Asia: contamination levels, sources and ecological impacts

2.1 Abstract

Plastics are synthetic polymers known for their outstanding durability and versatility and have replaced traditional materials in many applications. Unfortunately, their unique traits ensure that they pose a major threat to the environment. While the literature on freshwater microplastic contamination has grown rapidly, research undertaken in rapidly developing countries, where plastic production and use are increasing dramatically, has lagged behind that in other parts of the world. In the South-east Asian (SEA) region, basic information on levels of contamination is very limited and, as a consequence, the risk to human and ecological health remains hard to assess. This review synthesises what is currently known about microplastic contamination of freshwater ecosystems in SEA, with a particular focus on Malaysia. The review: (1) summarises published studies that have assessed levels of contamination in freshwater systems in SEA, (2) discusses key sources and transport pathways of microplastic in freshwaters, (3) outlines what is known of the impacts of microplastic on freshwater organisms, and (4) identifies key knowledge gaps related to our understanding of the transport, fate and effects of microplastics.

2.2 Introduction

Plastic waste is a major global problem. Plastics found in the world's oceans have been causes of concern for around 50 years, but it is only quite recently that attention has turned to plastic waste and its impacts on freshwater systems. This applies to plastic waste in general, but in particular to tiny fragments of plastic, hardly visible to the naked eye, which have been grossly understudied in freshwater ecosystems (Wong et al., 2020).

The first studies of these tiny fragments, now commonly referred to as 'microplastic', focused on marine contamination. This focus was largely due to concerns that microplastics could enter the human food chain (Thompson et al., 2004). Over the last decade or so, marine microplastics have been studied extensively; they have now been found in all the world's oceans (Bergmann et al., 2017; Courtene-Jones et al., 2017; Desforges et al., 2015). More recent attention has focused on other environments, with microplastics now recorded in terrestrial and freshwater ecosystems (Wong et al., 2020). Studies of microplastics in freshwater ecosystems are quite recent, with the first papers published less than a decade ago (Faure et al., 2012). Microplastic research in marine ecosystems has remained the main focus (Horton et al., 2017), with publication rates five times higher than work related to freshwater systems (Blettler et al. 2018).

Understanding microplastic contamination of freshwater ecosystems is important for three principal reasons. First, these systems support great biodiversity but are already threatened by a wide range of human stressors (Dudgeon, 2019); microplastics have been highlighted as a 'newly emerging' threat to freshwater organisms (Reid et al, 2019). The second reason is that human populations depend on freshwaters for a range of ecosystem services, including food and drinking water. Contamination by microplastics therefore potentially poses a significant human health risk. Finally, rivers convey water and sediments to the world's oceans and, in so doing, also deliver plastic waste (Jambeck et al., 2015; Wong et al., 2020).

Rivers have been considered the main source of marine plastics (Roebroek et al., 2021; Simon-Sanchez et al., 2019), delivering an estimated 1.2-2.4 million tons annually to the oceans (Lebreton et al., 2017). Assessment of microplastic loads in rivers is, therefore, necessary to better understand and mitigate contamination of coastal and marine environments.

Assessment of microplastic contamination is needed as a matter of great urgency in the world's rapidly developing regions. South East Asia (SEA) is currently witnessing urban growth and industrial development typically associated with rapid economic transformation, and these changes are threatening ecosystems and ecosystem services in the region (Lechner et al., 2020). By 2050, rivers in SEA are expected to have the world's highest microplastic loads (Xu et al., 2020). Malaysia typifies the situation in the region; it is developing rapidly, but the speed of this change risks outpacing its ability to put in place mechanisms to manage plastic waste. Malaysia is a major plastic user and a large importer of plastic waste (Chen et al., 2021) and is partly responsible for the 0.37 million metric tons of plastic being delivered to the ocean annually (Jambeck et al., 2015). Hence, countries such as Malaysia are useful case studies to help understand what is known about freshwater microplastic contamination in rapidly developing countries in general, and in particular across SEA.

This review synthesises what is currently known about microplastic contamination in freshwater ecosystems, with a specific focus on SEA and Malaysia. It summarises published studies of (1) levels of contamination in freshwater systems here, (2) the sources of microplastics found in freshwaters, and (3) what is known of the impacts of microplastics on freshwater organisms. The review aims to identify important research gaps that need to be addressed to improve our understanding of how microplastics move through freshwater systems and the risks they pose to ecological and human health. It stresses how much work has been done in SEA, and in turn, what this means for our understanding of the magnitude of the problem in the region. The review ends by setting a research agenda, to identify the work needed to improve our understanding of human and ecological health risks and to support mitigation strategies in rapidly developing countries.

2.3 Literature search

A search was conducted in March 2021 using the Google Scholar database. Google Scholar was used because it is the most comprehensive academic search engine (Gusenbauer, 2019). To compare the number of papers focusing on microplastic in marine and freshwater ecosystems globally, the words “microplastic”, “marine”, “sea”, “coast”, “beach”, “freshwater”, “lake”, “river” and “stream” were used in the search. The search included the additional keywords and terms “South East Asia”, “Malaysia”, “Philippines”, “Vietnam”, “Thailand”, “Myanmar”, “Singapore”, “Indonesia”, “Laos”, “Cambodia”, “Brunei” and “Timor-Leste”. This search generated quantitative information on numbers, dates and geographic foci of published work, with the content then scrutinised to summarise and synthesise findings.

2.4 Microplastic contamination in SEA

2.4.1 Definitions and related issues

Microplastic is normally defined as any polymer that is smaller than 5 millimetres (mm) in size (Horton et al., 2017; Ivleva et al., 2017). Over the last decade, the very finest of particles have come to be referred to as nanoplastic (e.g Gigault et al. 2018), but the assessment of environmental contamination by this material is very challenging (Cai et al., 2021) and so far very few studies have been undertaken (Zhang and Xu, 2020). There is no consensus yet on where the dividing line between microplastic and nanoplastic should be (Ivleva et al., 2017), with studies of ‘microplastic’ using a variety of different lower size limits. As a result, reported contamination levels are often not directly comparable (Jiang et al., 2020; Ramírez-Álvarez et al., 2020; Zhang et al., 2020). This issue is discussed further in Section 2.5, concerning contamination loads in Malaysia.

2.4.2 Timeline of published literature on microplastic

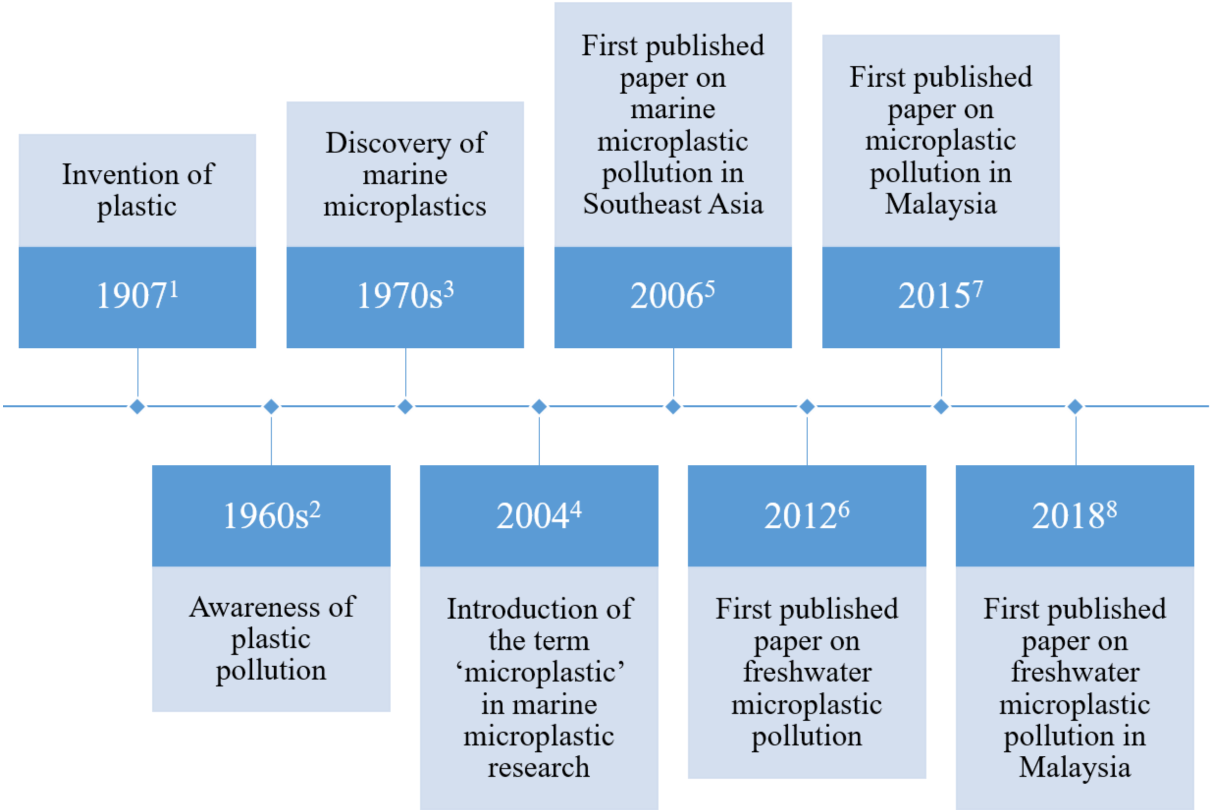

Fig 2.1 Key milestones in the history of plastic waste and knowledge of its environmental effects. Indicative papers for key points in the timeline are [1]Knight, 2014; [2]Ostle et al., 2019; [3]Carpenter and Smith, 1972; [4]Thompson et al., 2004; [5]Ng and Obbard, 2006; [6]Faure et al., 2012; [7]Barasarathi et al., 2014; [8]Sarijan et al., 2018.

Plastic was first developed at the beginning of the 20th century but was not used widely until the middle part of the century (Fig 2.1). Since the 1950s, there has been an exponential increase in plastic production (Ostle et al., 2019). The earliest report of marine plastic waste was in 1957, while the earliest reported ecological impact (on turtles and seabirds) was in the late 1960s (Ostle et al., 2019). Ostle et al., (2019) charted a significant increase in marine plastics between 1957 and 2016, paralleling the increase in global production and use of plastics.

The first discovery of microplastic pollution was reported in the early 1970s (e.g. Carpenter and Smith, 1972; Colton et al., 1974). However, the term 'microplastic' has yet to be introduced then. The term 'microplastic' is first introduced in 2004 by Thompson et al. (2004). This paper reported on the spatial distribution of microplastics in beach and estuarine sediments and highlighted that microplastics were ingested by amphipods, barnacles and lugworms. The first paper on marine microplastics in SEA was published two years later (Ng and Obbard, 2006); it reported similar findings to those of Thompson et al. (2004) but also suggested that microplastics may act as a vector for persistent anthropogenic chemicals.

Studies of microplastics in freshwater ecosystems are more recent, with the first papers published only nine years ago (Faure et al., 2012). Since then, publication rates have grown, not least because rivers and streams are important sources of marine microplastic (McCormick et al., 2014; Simon-Sanchez et al., 2019). The first paper on microplastic pollution in Malaysia was published in 2014 (Barasarathi et al., 2014), with the first one focused on freshwater ecosystems coming four years later (Sarijan et al., 2018).

2.5 Levels of contamination

To date, research on microplastic in marine ecosystems has remained the main focus (Fig 2.2) and although the literature on freshwater contamination has grown significantly, studies remain geographically fragmented (Blettler et al., 2018). Freshwater microplastic has been reported as widely as the Crocodile River in South Africa, the Amazon River in Brazil, and the Ottawa River in Canada (Gerolin et al., 2020; Umlauf, 2019; Vermaire et al., 2017) but most studies have been undertaken in Europe, including the Seine in France, the Rhine in Germany and the Danube in Austria (Horton et al., 2017; Mani et al., 2019; Treilles et al., 2018). The average concentration of microplastics in European rivers has been found to range from 0.00035 particles/L in the Seine, to 0.32 particles/L in the Danube (Horton et al., 2017). Very few freshwater studies have focused on lakes (only 15% of papers returned in the Google Scholar search).

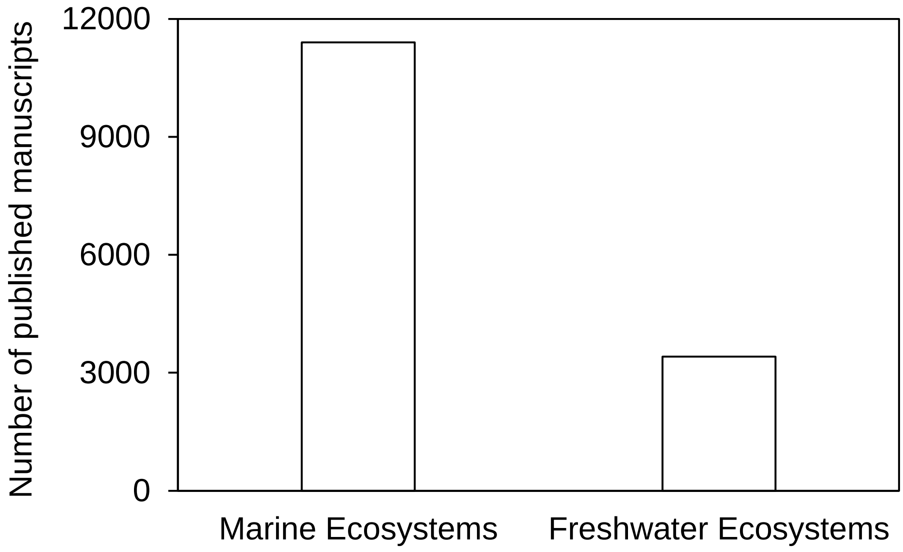

Fig 2.2 Comparison of the number of papers (Google Scholar, March 2021) focusing on microplastics in marine and freshwater ecosystems globally. Search criteria: “microplastic”, “marine”, “sea”, “coast”, “beach”, “freshwater”, “lake”, “river”, and “stream”.

In Asia, research comes mostly from China, including the Hanjiang, Yangtze, Buqu, Naqu, Lahasa, Nyang and Pearl rivers (Jiang et al., 2019; Lin et al., 2018; Wang et al., 2017a). Additional studies concern rivers in Japan and South Korea (Eo et al., 2019; Kataoka et al., 2019), as well as lakes in Mongolia, Pakistan and India (Free et al., 2014; Irfan et al., 2020; Sruthy et al., 2017). The average concentration in freshwaters in Asia ranges from 0.00012 particles/L in the relatively pristine Lake Hovsgol, Mongolia, to 0.71 particles/L in the urban Pearl River, China (Fan et al., 2019; Free et al., 2014).

Little is known about microplastic contamination in rivers in SEA; concentrations have only been studied in nine rivers in the region (Table 1). Notably, all these papers concern urban rivers. The highest freshwater concentrations in SEA have been reported in the Saigon River, Vietnam, where 0.01 to 519 particles/L have been recorded (Lahens et al., 2018). Authors attributed these high values to the presence of textile and plastic industries in the catchment (Alam et al., 2019; Lahens et al., 2018).

Table 2.1 Studies of the occurrences of microplastics in freshwater ecosystems in SEA (Google Scholar; March 2021). To allow comparison, the units of microplastic concentrations have been converted.

Country	Location	Sample	Microplastic size range observed	Microplastic types observed	Microplastic concentration (units converted)	Reference
Indonesia	Citarum River	Surface water	125-5000 µm	Films and fragments	0.00004–0.00009 particles/L	Sembiring et al., 2020
		Sediment	125-5000 µm	Films and fragments	12452–20316 particles/kg	Sembiring et al., 2020
	Ciwalengke River	Surface water	50-2000 µm	Fibres, fragments and others	2.57-9.13 particles/L	Alam et al., 2019
		Sediment	50-2000 µm	Fibres, fragments and others	14.446.2 particles/kg	Alam et al., 2019

	Surabaya River	Surface water	333-5000 µm	Fibres, fragments, films, foams and pellets	0.0008 – 0.04311 particles/L	Lestari et al., 2020
Malaysia	Cherating River	Surface water	100-5000 µm	Fibres. Fragments, films, foam and beads	0.000004–0.00001 particles/L	Pariatamby et al., 2020
	Dungun River	Surface water	60-5000 µm	Fibres, fragments, and films.	0.04-0.30 particles/L	Hwi et al., 2020
	Skudai River	Sediment	1-5000 µm	Fibres, fragments, beads, foams and films	120-280 particles/kg	Sarijan et al., 2018
	Tebrau River	Sediment	1-5000 µm	Fibres, fragments, beads, foams and films	540-820 particles/kg	Sarijan et al., 2018
Thailand	Chao Phraya River	Surface water	50-5000 µm	Hard plastics, soft plastics, foams and beads	0-0.052 particles/L	Johansson and Ericsson, 2018
		Surface water	53-5000 µm	Fibres, films and fragments	41.77 particles/L	Ta and Babel, 2019
Vietnam	Saigon River	Surface water	50-300 µm	Fibres and fragments	0.01-519 particles/L	Lahens et al., 2018

Very little work on microplastics has been conducted in Malaysia. Only a few studies have reported environmental concentrations of microplastics: two in marine ecosystems (Khalik et al., 2018; Taha et al., 2021), five in river estuaries (Choong et al., 2021; Liong et al., 2021; Zahari et al., 2022; Zainuddin et al., 2022; Zaki et al., 2021a), one in mangrove ecosystems (Barasarathi et al., 2014) and three in freshwater ecosystems (Hwi et al., 2020; Pariatamby et al., 2020; Sarijan et al., 2018). Most of the studies in Malaysia focused on the characteristics and abundance of microplastics in vertebrates (Foo et al., 2022; Ibrahim et al. 2017, Jaafar et al., 2021; 2017; Karbalaei et al., 2019) and invertebrates (Amin et al., 2020; Hamzah et al., 2021; Husin et al., 2021; Ibrahim et al. 2016; Taha et al., 2021; Zaki et al., 2021b), with another focusing on loads in commercial fish meals (Karbalaei et al., 2020). Auta et al. (2017) explored the development of a microplastic mitigation plan using bacterial isolates. Other papers in Malaysia focused on the concentration of heavy metals found in marine microplastic samples (Noik et al., 2015) and estimated the influx of microplastics from personal care and cosmetic products (PCCPs) into the marine environment (Praveena et al., 2018). The latter authors estimated that 199 billion particles of microplastic are released into the environment annually in Malaysia; 95% from direct sources and 5% from wastewater treatment plant (WWTP) discharges (Praveena et al., 2018). However, these estimates were based on extrapolations from questionnaire surveys focused on product use, rather than environmental field data.

Despite Malaysia being a major contributor to marine plastics, delivered by its rivers, almost nothing is known about microplastic loads in the rivers themselves. To date, only three Malaysian studies can be considered as assessments of environmental contamination levels in freshwater systems; a one-off spot sampling program in Skudai and Tebrau Rivers, Johor (Sarijan et al., 2018) and spatial studies of microplastic variation in the Dungun River, Terengganu (Hwi et al., 2020) and Cherating River, Pahang (Pariatamby et al., 2020). Although these authors related their data to that of other rivers, comparisons are compromised by the fact that size ranges differed. The lower limits of microplastic adopted in the Malaysian studies were 1µm (Sarijan et al., 2018), 60µm (Hwi et al., 2020) and 100µm (Pariatamby et al., 2020), whereas the works they cite and compare data to used lower limits as high as 1mm. This is indicative of a wider problem in the literature, with the use of different size ranges making it hard to make comparisons of contamination loads in different areas or between different parts of environmental systems (i.e. in water, sediments and organisms).

2.6 Sources, pathways and transport of microplastics in freshwater systems

2.6.1 Sources

The main sources, transport pathways and sinks of microplastic in freshwater systems are shown in Fig 2.3. Microplastic contamination stems from wastewater discharges from residential areas and industrial activities such as plastic and textile manufacturing, in which microplastics are either released into the atmosphere or discharged into wastewater (Kataoka et al., 2019). The fragmentation of macroplastics in landfills, as well as discharges from domestic textile washing and WWTPs, are major sources of microplastics (Kataoka et al., 2019).

Like many other freshwater contaminants, microplastics can originate from point sources and non-point (diffuse) sources. The former are more easily identified and include WWTPs, domestic drainage systems and industrial discharges (Kataoka et al., 2019). Conversely, diffuse sources such as agricultural activities and atmospheric fallout are harder to quantify and more difficult to control (Kataoka et al., 2019). Depending on the source and transport pathways taken, microplastic may be deposited or intercepted by terrestrial parts of catchments through wind, rainfall or surface water runoff before eventually making its way to freshwater systems (Fig 2.3). In river channels, the transport of microplastics is governed by the hydrological and hydraulic conditions that influence its entrainment and deposition. Although most will eventually be transported to the ocean sinks, freshwater ecosystems may act as short-term reservoirs for microplastics (Peng et al., 2018).

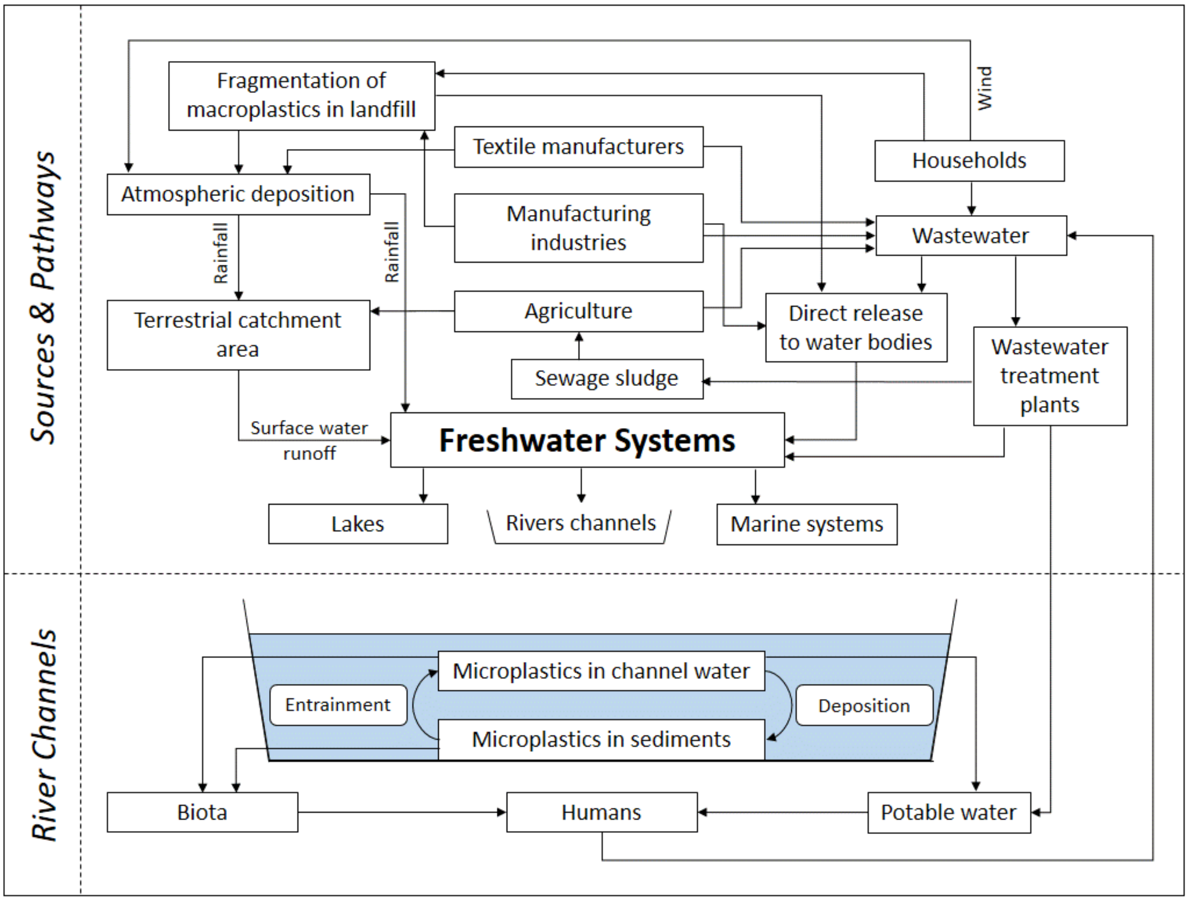

Fig 2.3 Pathway of microplastics into freshwater systems and conveyance to marine systems. The lower part of the figure shows more details of dynamics and pathways within river channels.

The importance of the dispersal and deposition of airborne microplastics has been stressed by several authors (Cai et al., 2017; Kay et al., 2018). Microplastics can be released into the atmosphere from textile manufacturing processes, domestic textile washing, incomplete plastic waste incineration and resuspension of road dust particles (Chen et al., 2019;

Dris et al., 2016; Liu et al., 2019a). Several authors have suggested that the concentration of microplastics in the atmosphere correlates with population density (Dris et al, 2016; Liu et al., 2019a); microplastics in atmospheric fallout are dominated by synthetic textiles (microfibres), which are largely associated with the use of domestic washing machines (Cai et al., 2017; Dris et al., 2016; Liu et al., 2019a). Airborne microplastics are largely transported by wind and can settle on terrestrial areas or lake surfaces (Chen et al., 2019; Liu et al., 2019a). The significance of airborne sources is influenced by rainfall, with lower rainfall periods reported to result in less deposition and, consequently, reduced loads in streams and rivers (Dris et al., 2016).

Terrestrial ecosystems can be contaminated as a result of agricultural practices, and plastic waste disposal (Rodríguez-Seijo et al., 2018). In many countries across SEA, processed sewage sludge is used as a fertiliser in farmlands. This sludge may contain microplastic that can contaminate terrestrial systems (Nizzetto et al., 2016a). A small number of studies have reported the accumulation of microplastics in agricultural soils as a consequence of the application of sewage sludge (Corradini et al., 2019; Kay et al., 2018); no such studies have been carried out in SEA. Plastics contained in mulches and silage covers contribute to contamination in agricultural areas (Kyrikou and Briassoulis, 2007). Although the use of plastic in agriculture has increased crop yields and played a role in suppressing weeds, plastic wastes may accumulate in soils, contributing to microplastic contamination (Duis and Coors, 2016; Kyrikou and Briassoulis, 2007) and eventually find their way to freshwater and marine systems.

Due to urban expansion, paved surfaces have become a major non-point source of microplastic in freshwaters. Mechanical abrasion of vehicle tyres is one of the key sources of microplastic, estimated to constitute 35% of contamination (Kole et al., 2017; Magnusson et al., 2016). Plastic in building materials, as well as the abrasion of road paint and shoe soles further contribute to the release of secondary microplastic (Lassen et al., 2015). Additionally, plastic debris in the general litter can break down into fragments through photo- and hydrodegradation (Kataoka et al., 2019). All of the microplastics are conveyed to streams, rivers and standing water bodies through rainwater runoff (Kataoka et al., 2019), with drains and storm-sewage overflow acting as conduits. This problem is exacerbated in SEA due to the rapid development and expansion of many cities, combined with the limited capacity of drainage systems to deal with the amount and intensity of precipitation associated with the tropical climate.

One of the main point sources of microplastic is domestic wastewater (Baldwin et al., 2016; Dikareva and Simon, 2019; Dris et al., 2018). Water contaminated with textile fibres from domestic washing (Murphy et al., 2016) or microplastic beads from toothpaste and skin cleansers may drain into rivers directly as a result of uncontrolled discharges in drains and culverts (Duis and Coors, 2016), especially when sewer and storm-water systems are not well developed or water does not go through WWTPs (Kataoka et al., 2019). WWTPs can, themselves, act as point sources of microplastics (Estahbanati and Farenfeld, 2016; McCormick et al., 2014; Murphy et al., 2016). The presence of microplastics in discharges from WWTPs depends on processes used for wastewater treatment, and the types of plastics present (Kataoka et al., 2019). Conley et al. (2019) found that synthetic fibres from textiles are less efficiently removed by WWTPs than microplastic fragments and beads. Although the majority of the microplastic present in domestic wastewater is removed, an estimated 5-17% of synthetic fibres and microbeads are still being released to watercourses from WWTPs (Dris et al., 2018; McCormick et al., 2014; Murphy et al., 2016). Integrated over time, the large volume of domestic wastewater discharged from WWTPs represents a major source of riverborne microplastic (Conley et al., 2019). To date, no empirical field assessments of microplastic contamination by discharges from domestic wastewater and WWTP have been undertaken in Malaysia.

2.6.2 Transport

Studying the transport and fate of contaminants is important to help understand their impacts on both ecosystems and human health (Hemond and Fechner, 2014). Fate refers to the final reservoirs where contaminants are deposited and stored, while transport refers to the processes that convey them through the environment (Hemond and Fechner, 2014). Rivers have been widely regarded as a key conduit, transferring microplastic from terrestrial ecosystems to the ocean (Besseling et al., 2017; Lebreton et al., 2017; Jambeck et al., 2015). Rather than being a continuous process, the transport of microplastic is interrupted by short-term periods of storage in soils and riverbed sediments (Horton and Dixon, 2018; Liu et al., 2018; Nizzetto et al., 2016b). The following text reviews the literature on the transport of microplastic in rivers, but as no such work has been conducted within the region, it is based entirely on studies conducted outside of SEA.

Modelling studies have indicated that rivers can store microplastic at least temporarily, with material accumulating in riverbeds for various lengths of time (Besseling et al., 2017; Nizzetto et al., 2016b). Numerous other authors have reported river sediments as sinks that accumulate microplastic (Horton et al., 2017; Wang et al., 2018), with Hoellein et al. (2019) suggesting that concentrations in bed sediments can be twice as high as those in river water. Due to differences in the characteristics of riverbed sediments and topography, the characteristics of the microplastic particles themselves, and differences in river flow regimes and hydraulics, the transport, settlement and entrainment dynamics of microplastics will differ between rivers (Nizzetto et al., 2016b). Nizzetto et al. (2016b) predicted that deposition, storage and retention of microplastic are dominant in streams with low stream power. Large particles tend to be heavier than water, so settle out of suspension more rapidly than small ones (Besseling et al., 2017; Nizzetto et al., 2016b); however, despite being denser than water, microfibres with a complex 3-dimensional structure tend to stay afloat (Hoellein et al., 2019).

Time in suspension can be extended for small particles due to surface adsorption (Besseling et al., 2017; Li et al., 2019). Such differences affect transport distances.

Turbulent flow conditions encourage the aggregation of microplastic with sediments or other colloidal particles, which may then settle out more rapidly from the water column (Li et al., 2019). Decreasing sizes and densities of microplastics, along with higher concentrations, may increase aggregation rates and, in turn, rates of settlement and accumulation on the bed (Besseling et al., 2017; Li et al., 2019). The flocculation of finer, more cohesive microplastic particles with one another may also cause them to settle and accumulate on the bed (Besseling et al., 2017). Net accumulation occurs when deposition rates are greater than entrainment (Nizzetto et al., 2016b). Burial by coarser sediments may cause long-term storage of microplastic in riverbeds. In the absence of flows that can disturb the bed, this burial traps microplastic (Horton et al., 2017). Burial is significant as material in the subsurface zone may experience minimal weathering; this can increase the time taken to decay and enhance bioavailability (Kooi et al., 2018; Li et al., 2019), creating a greater risk to benthic and hyporheic organisms (Li et al., 2019).

The resuspension of microplastics sitting on or buried within riverbeds and its subsequent transport downstream are governed by the fundamental hydraulic geometry relationships of river channels; that is, the relations between flow magnitude and channel dimensions (Besseling et al., 2017). In order for settled microplastics to be resuspended during high-flow periods, the critical entrainment threshold of the microplastic particles (or, if buried, the bed sediments) must be exceeded (Kooi et al., 2018; Nizzetto et al., 2016b). The physical properties of microplastics, including their size, density and shape, also play a role in determining entrainment and transport (Waldschläger and Schüttrumpf, 2019). Globally, only three studies of the behaviour of microplastics under different flow hydraulic conditions have been conducted. All three of these were laboratory flume studies – two looked at the settlement of microplastics from the water column (Khatmullina and Isachenko, 2017; Waldschläger and Schüttrumpf, 2019a) and the other at entrainment from the bed (Waldschläger and Schüttrumpf, 2019b). Due to differences in the density and shape of microplastic fragments compared to fine sediment, they settle and are entrained under different hydraulic conditions.

Nevertheless, just like fine sediment, the behaviour of microplastics (e.g. whether they are entrained or not) can be explained using hydraulic parameters such as shear stress and represented using Shield's diagrams (as in Fig 6 of Waldschläger and Schüttrumpf, 2019b). Although equations to describe the rising and settling velocities of microplastics have been established (Waldschläger and Schüttrumpf, 2019), these were developed for standing water and so do not help to understand the accumulation in river channels where water is moving. A particularly important field-based study demonstrated that microplastic concentrations in riverbed sediments may be significantly reduced after floods (Hurley et al., 2018). Great temporal variation in microplastic concentrations has been reported in river water (Stanton et al., 2019), with this variation partly explained by inputs and partly by changes due to flushing. This variation raises concern that periodic spot sampling is insufficient to characterise ecological and human health risks posed by microplastics, as it may misrepresent longer-term patterns of exposure.

The limited literature indicates that the general dynamics of microplastic transport within river channels are linked to patterns of precipitation and runoff as well as high flows. In tropical climates, rainfall timing and intensity differ from those in other climate regions, and correspondingly river flows differ in terms of magnitude, timing and duration of periods of low and high flow. For instance, in Mediterranean rivers, flows are highly seasonal, with relatively low flows during the summer and high flows during winter (Simon-Sanchez et al., 2019). This seasonal rhythm differs markedly from that in many tropical rivers. While such differences suggest that the dynamics of microplastic transport may vary between climate regions, just as fine sediment loads do (Chong et al., 2021), no studies have yet assessed patterns of microplastic transport in tropical rivers in SEA, or the changes that occur during and after individual flood events.

2.7 Ecological impacts

2.7.1 Uptake and accumulation of microplastics

Microplastics have been reported in human lung tissues, blood and placenta (Jenner et al., 2022; Leslie et al., 2022; Ragusa et al., 2021). Pang et al. (2021) recently reviewed the evidence of the human health risks of microplastics and gave examples of work showing chronic inflammation, oxidative stress and cancers arising from ingestion, inhalation and dermal contact with microplastic. Microplastic enters the food chain because of uptake by organisms (Waring et al., 2018). In humans, exposure to freshwater microplastics comes from food items such as fish and freshwater mussels as well as potable water (Revel et al., 2018). Senathirajah et al. (2021) suggested that humans may be ingesting as much as 5g of microplastic per week. In SEA, a key issue is that a large number of less-affluent people, including those living in heavily urbanised areas, are reliant on self-caught food derived from rivers; these rivers may be badly contaminated with microplastic, but to a degree that at present is simply unknown. The following sections focus on the evidence and its impacts on several organisms.

The ingestion of microplastics has been studied in diverse organisms, from fishes, amphibians and riverine macroinvertebrates to benthic amphipod species and planktonic crustaceans (Boyero et al., 2020; Iannilli et al., 2019; Jemec et al., 2016; Windsor et al., 2018; Zhang et al., 2019). Microplastics may be ingested either by incidental ingestion (i.e. when incorporated with food) or as a consequence of organisms mistaking microplastic for food (Peters and Bratton, 2016; Raza and Khan, 2018). Microplastics can also enter the food chain via passive ingestion (Batel et al., 2018; Raza and Khan, 2018; Welden and Cowie, 2016), e.g. by filter feeders such as mussels (Li et al., 2020). Factors that influence microplastic ingestion include river flow dynamics, the characteristics of microplastics (i.e. shape, size and density), as well as the position of an organism in the food chain (Cverenkárová et al., 2021; Windsor et al., 2018).

Depending on gut passage times, ingested microplastics may be remobilised and translocated from the digestive system to other organ systems (Rehse et al., 2018). For freshwater organisms such as *Gammarus fossarum* and *Daphnia magna*, microplastic is egested completely and so is not retained in the digestive tract (Blarer and Burkhardt-Holm, 2016; Rehse et al., 2018). In juvenile palm ruffs, *Seriolella violacea*, most ingested microplastics were rejected by a gustatory trap and egested (Ory et al., 2018). However, the inability of several organisms to egest microplastic may lead to bioaccumulation (Li et al., 2020). For instance, Zhang et al. (2019) observed accumulations of microplastics in the brain and liver of red tilapias, *Oreochromis niloticus.* In organisms such as *Daphnia magna*, irregular-shaped microplastics tend to have a longer residence time in the gut (Frydkjær et al., 2017) and so have greater potential to cause damage. The bioaccumulation of microplastics varies as a function of exposure time, particle size and concentration, and food intake (Ding et al., 2018). The potential for bioaccumulation is greater for small particles (Wagner et al., 2014).

2.7.2 Impacts of microplastics

Microplastic may release toxic substances that can be harmful, may act as a vector for contamination through pollutant adsorption or may have direct physical effects. The following texts deal with each of these.

Properties such as rigidity, elasticity and stability that make plastics useful are made possible by the incorporation of additives such as plasticisers (Horton et al., 2017). Plasticisers may be endocrine disruptors or toxic and have been confirmed to leach out of plastics (Horton et al., 2017; Schrank et al. 2019). These additives, which include heavy metals and flame retardants, are often toxic and may be desorbed upon ingestion (Horton et al., 2017; Li et al., 2018).

Microplastic can form complexes with metal ions, silt and clay. This can increase the transport of heavy metals (Wang et al., 2017b). Such metals accumulate in riverbed sediments (Wang et al., 2017b). Khan et al. (2015) observed that metals adhered to microplastics can be desorbed upon ingestion. When combined with other contaminants, microplastic has been shown to enhance toxicity. For instance, microplastics and sorbed contaminants such as polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) can affect organ homeostasis (Rainieri et al., 2018).

The ingestion of microplastics also causes physical effects such as inflammatory responses and lacerations (Horton et al., 2017; Lei et al., 2018a). Additionally, false satiation, caused by the accumulation of microplastics in the guts of organisms, will cause reduced feeding, ultimately causing mortality (Horton et al., 2017).

Few studies of the impacts of microplastics on organisms have been carried out in freshwater ecosystems, and the majority of these have focused on consumer species. Table 2.2 summarises the key findings of these studies. Microplastics have been reported to affect reproduction, feeding and growth rates in invertebrates such as *Caenorhabditis elegans, Daphnia magna, Gammarus pulex, Hyalella Azteca* and *Hydra attenuata*, (Au et al., 2015; al Lei et., 2018a; Martins and Guilhermino, 2018; Murphy and Quinn, 2018; Rist et al., 2017; Weber et al., 2018). Microplastics are also reported to cause alteration of gene expression in both *Caenorhabditis elegans* and *Daphnia magna* (Imhof et al, 2017; Shang et al., 2020). Microplastics have been shown to decrease enzyme activity in *Pomatoschistus microps and Oreochromis niloticus* (Ding et al., 2018; Fonte et al., 2016). Only 2 studies, which were carried out on *Gammarus duebeni* and *Gammarus pulex,* showed no significant impacts of microplastics on the focal organisms (Mateos-Cárdenas et al., 2019; Weber et al., 2018).

Microplastics have been reported to disturb energy and lipid metabolism, cause lipid accumulation in the liver, and cause intestinal enterocyte damage in *Danio rerio* (Lei et al., 2018b; Lu et al., 2016). In addition, microplastics can alter the larval gene expression and transfer plasticisers to embryos in this species (Batel et al., 2018; LeMoine et al., 2018). In *Oryzias latipes*, decreased growth rates have been reported in the presence of microplastics (Chisada et al., 2019). Zhu et al. (2020) found that microplastics caused morphological alteration of the buccal cavity, kidney and spleen as well as swollen intestinal enterocytes and increased lamellae mucous production in *Oryzias latipes.*

Few studies have also looked into the effects of chronic exposure on the mortality of freshwater organisms. In *Pomatoschistus microps*, microplastic exposure has led to decreased predatory performance and increased mortality (Fonte et al., 2016). Depending on exposure times and concentrations, microplastics can cause a decrease in feeding rates in *Daphnia magna*, leading to mortality caused by false satiation (Martins and Guilhermino, 2018; Rist et al., 2017). Pacheco et al. (2018) reported the impairment of reproduction and growth of *Daphnia magna* due to exposure to microplastics and suggested mortality as a long-term consequence. Individuals of the same species subjected to different microplastic sizes, exposure times and concentrations are affected differently.

Table 2.2 Impact of microplastic on freshwater invertebrates and vertebrates.

Species	Impact	Range of microplastic sizes	Range of exposure concentration	Maximum exposure time	Reference
Invertebrates					
Caenorhabditis elegans (Annelid)	Intestinal damage, inhibition of survival rates, reproduction	0.1-5 μm	0.5-10 mg/m2	2 days	Lei et al., 2018a
	Alteration of gene expression and shortened defecation interval	1-5 μm	10^7-10^{10} particles/m2	6 days	Shang et al., 2020
Daphnia magna (Amphipod)	Alteration of gene expression	23.5-55.5 μm	11-55 particles /individual	2 days	Imhof et al., 2017
	Decreased feeding rates	0.1-2 μm	1 mg/L	1 day	Rist et al., 2017
	Immobilisation	10-106 μm	0.1-10,000 mg/L	1 day	Frydkjaer et al., 2017
		1-100 μm	12.5-400 mg/L	4 days	Rehse et al., 2016
	Impaired reproduction and development	1-5 μm	0.09-6 mg/L	21 days	Pacheco et al., 2018
	Mortality and decreased growth	1-5 μm	0.1 mg/L	21 days	Martins and Guilhermino, 2018

Gammarus duebeni (Amphipod)	No impact on mobility or mortality	1-1000 µm	$5x10^{7}$ particles/L	7 days	Mateos-Cárdenas et al., 2019
Gammarus fossarum	Decreased assimilation efficiency	1.6 µm	$5x10^{5}$-$6x10^{7}$ particles/L	32 hours	Blarer and Burkhardt-Holm, 2016
(Amphipod)		32-250 µm	10-10,000 particles/ individual	1 day	Straub et al., 2017
Gammarus pulex (Amphipod)	No impact on feeding activity, metabolism, development and mortality	10-150 µm	$8x10^{3}$-$4x10^{6}$ particles/L	1 day	Weber et al., 2018
Hyalella Azteca (Amphipod)	Decreased growth rate, reproduction rate and ability to process food	10-27 µm	4.64-71.43 particles/L	42 days	Au et al., 2015
Hydra attenuate (Cnidarian)	Decreased feeding rates and alteration of morphology	<400 µm	10,000-80,000 mg/L	1 hour	Murphy and Quinn, 2018
Vertebrates					
Alytes obstetricans (Amphibian)	Mortality and impaired growth	10 µm	0-$1.8x10^{6}$ part/L	14 days	Boyero et al., 2020
Danio rerio (Fish)	Alteration of larval gene expression	5 µm	5-20 mg/L	14 days	LeMoine et al., 2018
		4.6-17.6µm	0.005-0.5 mg/L	20 days	Karami et al., 2017

	Disturbed lipid and energy metabolism, lipid accumulation in liver and inflammation	0.07-20 µm	4.5×10^6-2.9×10^8 particles/L	7 days	Lu et al., 2016
	Intestine enterocyte damages	0.1-5 µm	0.001-10 mg/L	10 days	Lei et al., 2018b
	Transfer of plasticiser to fish embryo	1-20 µm	6×10^8-10^8 particles/L	1 day	Batel et al., 2018
Oreochromis niloticus (Fish)	Decreased blood cell count	>100m	1-100 mg/L	15 days	Hamed et al., 2019
	Disturbed metabolism and inhibition of brain enzyme (AChE) secretion	0.1 µm	0.001-0.1 mg/L	14 days	Ding et al., 2018
Oryzias latipes (Fish)	Decreased egg number and growth rate	10-63 µm	0.065-0.65 mg/L	12 weeks	Chisada et al., 2019
	Swollen intestine enterocytes, increased mucous production in lamellae and alteration of buccal cavity, kidney and spleen	10 µm	500-2000 µg/g	10 weeks	Zhu et al., 2020
Physalaemus cuvieri (Amphibian)	Morphological changes and mutagenic effects	35 µm	60 mg/L	7 days	Da Costa Araujo et al., 2020
Pomatoschistus microps (Fish)	Mortality, decreased predatory performance and enzyme activity	5 µm	0.184 mg/L	4 days	Fonte et al., 2016

The effects of microplastics on producers such as plants and algae have also been studied. Mateos-Cárdenas et al. (2019) found that microplastics on leaf surfaces of Lemna minor did not affect photosynthetic efficiency, while Kalčíková et al. (2017) found no effects on leaf growth rates. However, impacts on root growth and viability of root cells were found (Kalčíková et al., 2017). The effects of microplastics have also been tested in *Myriophyllum spicatum*, where a decreased shoot length of this sediment-rooted macrophyte has been reported (van Weert et al., 2019). Microplastics have been reported to affect photosynthesis and destroy cell walls in the algae *Scenedesmus obliquus* (Liu et al, 2019b). Wu et al. (2019) have also found that photosynthesis in *Chlorella pyrenoidosa* is inhibited by microplastics at high concentrations. Conversely, Canniff and Hoang (2018) found no harmful effects of microplastic on the microalga *Raphidocelis subcapitata*.

2.7.3 Ecological impact studies in SEA

Very few studies of the effects of microplastics in freshwater organisms have been conducted in SEA; note for instance that none of the studies listed in Table 2.2 was carried out in the region. In marine systems in SEA, microplastics have been reported in rabbitfish, *Siganus fuscescens* (Bucol et al., 2020) and various commercial marine fishes, including *Alepes* sp., *Leiognathus* sp., *Scomberiodes* sp., *Johnius* sp. and *Sarginella* sp. (Azad et al., 2018). These studies focus only on the concentration of microplastics in the organisms, rather than the biological effect per se. Only one study in SEA had a specific focus on organisms in freshwater ecosystems (Kasamesiri and Thaimuangphol, 2020); this work examined the presence of microplastics in the fishes *Mystus bocourti, Puntioplites proctozysron, Hemibagrus spilopterus* and *Cyclocheilichthys repasson* in the Chi River, Thailand, but as with the marine studies, did not assess the biological effects of the observed contamination.

In Malaysia, 10 studies have examined the presence of microplastics in organisms, but none of them concerned freshwater environments. A maximum concentration of 558 particles/g of dry weight tissues, which were dominated by polyethylene and polyamide microfibers, have been reported in the bivalve *Scapharca cornea* (Ibrahim et al., 2016). Similarly, polyamide microfibres have also been reported to be the most abundant microplastic particle in the Asian sea bass, *Lates calcarifer* (Ibrahim et al., 2017). The concentration of microplastics in the gastrointestinal tract of wild *L. calcarifer* was found to be significantly higher than cage-cultured ones (Ibrahim et al., 2017). In zooplanktons, average microplastic ingestion of 0.03 to 2.04 particles/m^3 across six different zooplankton groups was reported by Amin et al. (2020), while Taha et al. (2021) reported an average microplastic ingestion of 0.01 to 0.2 particles/individual across seven different zooplankton groups. These microplastics were dominated by microfibres, which were considered to originate from fishing and other offshore recreational activities (Amin et al., 2020; Taha et al., 2021). This was in accordance with the findings of Zaki et al. (2021a), where authors reported an abundance of microplastics that originated from fishing gear in gastropods sampled from the Klang River estuary. Microplastics in commercial marine fishes have also been studied in Malaysia. For instance, Karbalaei et al. (2019) found microplastic particles in the viscera and gills of 9 out of the 11 fish species examined, while Foo et al. (2022) found microplastics in all 72 individuals of the four examined species of commercial fish guts. In the study by Jaafar et al. (2021), microplastics were found in up to 92% of the gills and gastrointestinal tract of the 16 examined commercial fish species.

Overall, there remains a paucity of data on freshwater microplastic contamination levels across SEA. Given that such data are crucial for understanding the threat posed to ecological and human health (Wright and Kelly, 2017), assessing the magnitude of this threat is difficult at present. Part of the problem is that most of the toxicological studies of the effects of microplastics have used concentrations appreciably higher than found in the environment (i.e.

up to seven orders of magnitude higher) (Xu et al., 2020). Windsor et al. (2019) highlighted that although studies have looked at the biological effects of microplastic ingestion, both directly and indirectly, there is a paucity of empirical field studies assessing these biological effects. Thus, not only do we have limited data across SEA to show what contamination levels are, but existing toxicological studies are of limited use for understanding the risks posed by these levels.

2.8 Key knowledge gaps and research needs

Most work on microplastic contamination has focused on marine systems, with relatively few studies dealing with freshwater species and ecosystems. Below we identify four key knowledge gaps and discuss the research needs related to each of these.

1. Processes governing the dispersal of microplastic across catchments and its transport within river channels, primarily in SEA, are poorly known. Policies to tackle the problem of microplastic contamination in rivers and potable water require knowledge of the relative contribution from different sources and the factors that influence the storage and transport dynamics of microplastic in river channels. However, less than 10 studies in the global literature have tried to elucidate the hydrologic and hydraulic factors that influence the transport and storage dynamics of microplastic in rivers, and none have been conducted in tropical countries. Studies examining inputs from different sources are needed at the catchment scale to help develop and direct mitigation measures.

   This is particularly important in the developing countries of SEA, where rapid urban development has resulted in the deterioration of chemical water quality and the ecological status of rivers. Specific issues in the region relate to the poor water infrastructure, from the control and management of urban runoff to the treatment of domestic and industrial wastewater. Integrated studies are needed of catchments, capable of isolating key sources, transport routes and the nature and causes of spatio-temporal variability in microplastic concentrations. In turn, such studies can identify hotspots of contamination and key transport pathways, both of which can be targeted as part of management. As well as badly contaminated urban rivers, it would also be useful to have data on the degree of contamination of non-urban rivers, to help understand the human and ecological risks in rural and more remote areas across the region.

2. Published assessments of temporal variation of microplastic carry great uncertainty. For example, two published studies have dealt explicitly with temporal variation in microplastics in river systems; one of these assessed variations in concentrations in water (Stanton et al., 2019) and the other in sediment (Hurley et al., 2018). Both studies found significant temporal variation. Stanton et al. (2019) used this variation to caution over the use of spot sample data when estimating total fluxes carried by rivers. A better understanding of temporal variation is needed both to improve risk assessment by highlighting peak concentrations and to improve estimates of microplastic fluxes (e.g. annual loads) delivered to oceans.
3. The lack of consistent sampling methods and size ranges (especially lower limits of microplastic) means that few studies are directly comparable (Section 2.5). Standardisation of methods is needed to help identify the most badly contaminated rivers and, in turn, help direct mitigation. We call on scientists involved in microplastic research to work toward the development of standardised protocols that can be used globally.
4. The majority of laboratory toxicological studies have been carried out using unrealistic concentrations (Xu et al., 2020). Although such studies can isolate cause

and effect, to date negative effects are only evident at the highest doses, where concentrations of microplastic are several orders of magnitude higher than observed in natural ecosystems (Zhang et al., 2019). Further toxicity studies are needed to assess the chronic effects of sub-lethal doses that better match observed levels of environmental contamination. This means that robust data on contamination levels are needed not just to better understand health risks but to help design more realistic toxicity studies.

5. Despite the rapidly developing literature, most published work on microplastic has dealt with coastal and marine systems. While studies of freshwater contamination have raised significant concerns, these remain relatively few and are geographically biased. In Malaysia for example, despite being a major contributor to marine plastics, only a handful of published studies of microplastic loads carried by rivers, uptake by river organisms or risks to human health from water or food derived from rivers have been conducted in Malaysia. Rivers in Kuala Lumpur, Malaysia's rapidly expanding capital city, may be badly contaminated by microplastic, but at present, there is simply no data that can be used to properly assess this. This situation is mirrored in many major cities across SEA. We suggest that agencies responsible for routine water quality monitoring should incorporate an assessment of microplastic in their work. This is important globally, but especially in low- and middle-income countries in SEA. As well as experiencing major population growth, many such countries have undergone marked urbanisation. These changes impact the quality of life and pose new and complex environmental challenges in urban areas. So-called 'blue-green spaces' in cities are recognised as being important for human health and well-being (Lechner et al., 2020) but the services they provide are threatened by water contamination. It is therefore important that

studies are conducted in rapidly developing cities in SEA to help understand the extent to which microplastic contamination is impacting the ecosystem services provided by urban rivers and wetlands. Including microplastic as part of routine water quality assessment would help provide a clearer picture of contamination loads and risks in urban areas.

Overall, there is a clear need for more systematic research, using standardised collection and processing techniques. Research is needed on a country-by-country basis in SEA to assess loads and the human and ecological health risks posed by microplastic. While there is a need for microplastic to be included in the list of contaminant monitors as part of routine government sampling programs, there also is a need for more detailed catchment-scale studies to better inform management. These studies should focus on assessing the relative contribution of point and diffuse sources, as well as temporal variation in loads in river water and sediments. Data from such studies will allow the identification of contamination hotspots across catchments, as well as allow better estimates of fluxes of microplastics being conveyed annually to the oceans. Catchment studies capable of identifying key sources and pathways of microplastics form the starting point for improved waste management; by highlighting ‘hotspots’ of contamination where the risk to human and ecological health is greatest, such work can flag priority areas for remediation and, where necessary, limit human use of water until the contamination can be reduced.

2.9 References

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3

Relative contributions of different local sources to riverborne microplastics in a mixed landuse area

3.1 Abstract

Information on the relative contributions of microplastics coming from different sources is important to help prioritise measures to reduce river contamination levels and limit human and ecological health risks. This chapter reports on work which aimed to quantitatively assess the relative concentrations and types of microplastic delivered to the Semenyih River from different sources. The study was undertaken in a mixed landuse area within a rapidly urbanising part of the Semenyih catchment. Over six weeks, water samples were collected from road culverts and drains in residential and industrial areas to assess microplastic concentrations, while inputs from atmospheric deposition and wastewater treatment plants (WWTPs) were also quantified. Microplastic fibres and fragments were the dominant material in all sources, with the majority consisting of styrene-butadiene rubber and nylon. Culverts draining main roads were the main contributor to riverborne microplastics, delivering 42.20±35.29 particles/L directly to the river channel. Road inputs were up to seven times greater than those from residential (8.53±9.91 particles/L) and industrial (5.67±4.88 particles/L) areas. The five WWTPs had removal efficiencies of between 30.95±5.51% and 69.94±22.17%, with their outflows delivering microplastics to the river in concentrations similar to those in uncontrolled residential and industrial drains. Atmospheric deposition across the study area was estimated to be 76.07±32.85 particles/m^2/day (8.35±5.11 particles/L). Mitigation strategies in the study area should focus on improving the management of water draining roads, and re-routing discharges from domestic and industrial areas to WWTPs rather than allowing them to flow directly to the river. The low efficiencies of some of the WWTPs are not unusual and indicate the need for additional water treatment to deal with the microplastic present in wastewater.

3.2 Introduction

Annual global plastic production is estimated to be 360 million tonnes (PlasticsEurope, 2019), with the environmental impacts of plastic waste now recognised as a major problem (Chen et al., 2021a). Small fragments of plastics, termed ‘microplastics’ (i.e. plastics that are smaller than 5mm in size), have now been reported on every continent, and contaminate terrestrial, marine and freshwater ecosystems (Horton and Dixon, 2017) as well as being present in the atmosphere and cryosphere (Zhang et al., 2020a).

Microplastics originate from anthropogenic activities, and so contamination levels tend to correlate with population density (Liu et al., 2019a). Like many other contaminants, microplastics can be released into the environment in many ways. Microplastics from road runoff, mainly consisting of tyre and road wear particles (TRWP; e.g. tyre fragments, polymer-modified bitumen and detached road markings), are regarded as one of the major sources of contamination (Järlskog et al., 2020). Effluents from wastewater treatment plants (WWTPs) and domestic wastewater discharges are also important contributors (Alvim et al., 2020), with concentrations largely dependent on the removal efficiency of WWTPs (Booth and Sorensen, 2020). Atmospheric dispersal and deposition have also been considered a major source of microplastic contamination; particles, especially those smaller than 2.5 microns (PM 2.5), can remain in the atmosphere for several weeks and may travel distances exceeding 1000 km (Kole et al., 2017). Atmospheric dispersal is a function of wind direction, with settlement occurring in still conditions or as a result of rainfall (Hale et al., 2020; Truong et al., 2020). Once in watercourses, microplastics are transported in suspension in the water column and conveyed towards the oceans (Chen et al., 2021b) in ways that correspond with river flow conditions (Chen et al., 2021c).

Understanding the local-scale contribution of microplastics from different point- and non-point sources is crucial for the formulation of policies to reduce river contamination. Most published studies infer sources and pathways based on the types of microplastics found in river water, rather than assessing inputs from different sources directly through targeted sampling (examples of such studies are Campanale et al., 2020 and Ramírez-Álvarez et al., 2020). Some studies of the relative contributions from different sources have relied on modelling and extrapolations rather than empirical data (examples are Siegfried et al., 2017; Van Wijnen et al., 2019 and Whitehead et al., 2021). Of the studies that have assessed sources directly, most have focused on understanding the contribution from only one or two sources; for example, Tang et al. (2020) assessed inputs from WWTPs, Piñon-Colin et al. (2020) those from domestic activities, and Klöckner et al. (2010) inputs from roads.

To date, only two empirical studies (Dris et al., 2015 and Dris et al., 2018) have assessed the relative contribution of multiple sources within a defined geographic area. These papers both stem from the same work in Paris. The former focused on quantifying inputs from WWTPs and atmospheric fallout across the city, with authors reporting concentrations of up to 50 particles/L and 280 particles/m^2/day from WWTPs and atmospheric fallout, respectively. The latter study was more comprehensive, and also included an assessment of contributions from urban storm-water and road runoff. The lack of other similar studies means that it is not currently possible to assess whether the relative contributions observed in Paris are typical of urban areas, or whether inputs vary markedly from place to place as a function of the nature and mixture of landuse types present, and the characteristics of local drainage networks. Thus, there remains a need for empirical assessments of the relative contributions of multiple sources to mainstream rivers, especially in rapidly developing countries where (1) drainage systems are often poor, with many drains and culverts discharging directly to rivers without water treatment, and (2) due to their age or design, water treatment facilities may have limited capacity to remove microplastics and so may represent major sources of river contamination.

Such studies are important to help develop mitigation strategies that focus on the most important sources.

This chapter aims to assess the relative contribution of local sources of microplastic contamination in a mixed landuse area in a tropical catchment. The objectives are: (1) to analyse the quantities and characteristics of microplastic draining roads, residential and industrial areas, (2) to assess the contribution of atmospheric fallout to the land surface across the study area, and (3) to evaluate the removal efficiency of microplastics by WWTPs as well as the quantity and composition of material these plants are discharging to the river. The overarching goal of the work was to produce a microplastic 'budget' for this rapidly urbanising tropical study area, indicating the proportions of the load that originate from these different sources.

3.3 Methods

3.3.1 Study area

The Semenyih catchment is located on the South-eastern edge of Greater Kuala Lumpur (GKL), Malaysia (Fig 3.1). The study area was selected as being typical of many rapidly developing parts of Malaysia and cities in other countries in South East Asia (SEA); natural land cover is being lost as a result of rapid and poorly controlled urban expansion (the town of Semenyih is expanding as part of the growth of the GKL conurbation), and ageing water and drainage infrastructure designed to cope with much lower population densities and less industrial activity than it now experiences. Thus, the area provides an opportunity to assess microplastic contamination in a rapidly urbanising area where infrastructure was not designed to cope with the contamination it may now be experiencing.

The Semenyih is one of two main upper sub-catchments of the Langat basin. Flows in the Semenyih are regulated by the Semenyih Dam, which has a capacity of 60.6 MCM (Saadon and Ali, 2014; Selangor Water Management Authority [SWMA], 2019). The Semenyih flows from undisturbed forested headwater areas to progressively more degraded land (extensive agriculture and aquaculture), eventually to industrial and housing areas in the town of Semenyih. It joins the Langat River near Putrajaya before flowing to the sea (Atiqah et al., 2017; SWMA, 2019). The Semenyih River has a mean discharge of 3.89 m^3/s and a mean annual rainfall of 2309 mm (Department of Irrigation and Drainage Malaysia [DID], 2018; 2021). The area chosen was on the outskirts of Semenyih town, with residential and industrial areas in close proximity, and multiple small WWTPs that treat water from some of these areas. The main road in the catchment runs through the centre of the study area. Like many parts of GKL, the town of Semenyih is expanding rapidly, and the study area is typical in having a dated drainage system that reflects historic (more rural) landuses; thus, many open drains and culverts route untreated water from new industrial and housing developments directly to the river. The Semenyih River has a channel width of approximately 10.5 m as it flows through the study area.

The approach was to collect samples from examples of each of the major types of contributing sources (Fig 3.1) and repeat this on several different dates (Appendix 3A). This approach follows that of Dris et al. (2015). We collected 17 samples on each date, split between five different source types and the main river, and collected samples on four occasions over a six-week period between March and May 2021. Thus, our sampling was more intensive than that of Dris et al. (2015) who sampled 10 points on three dates over a nine-month period.

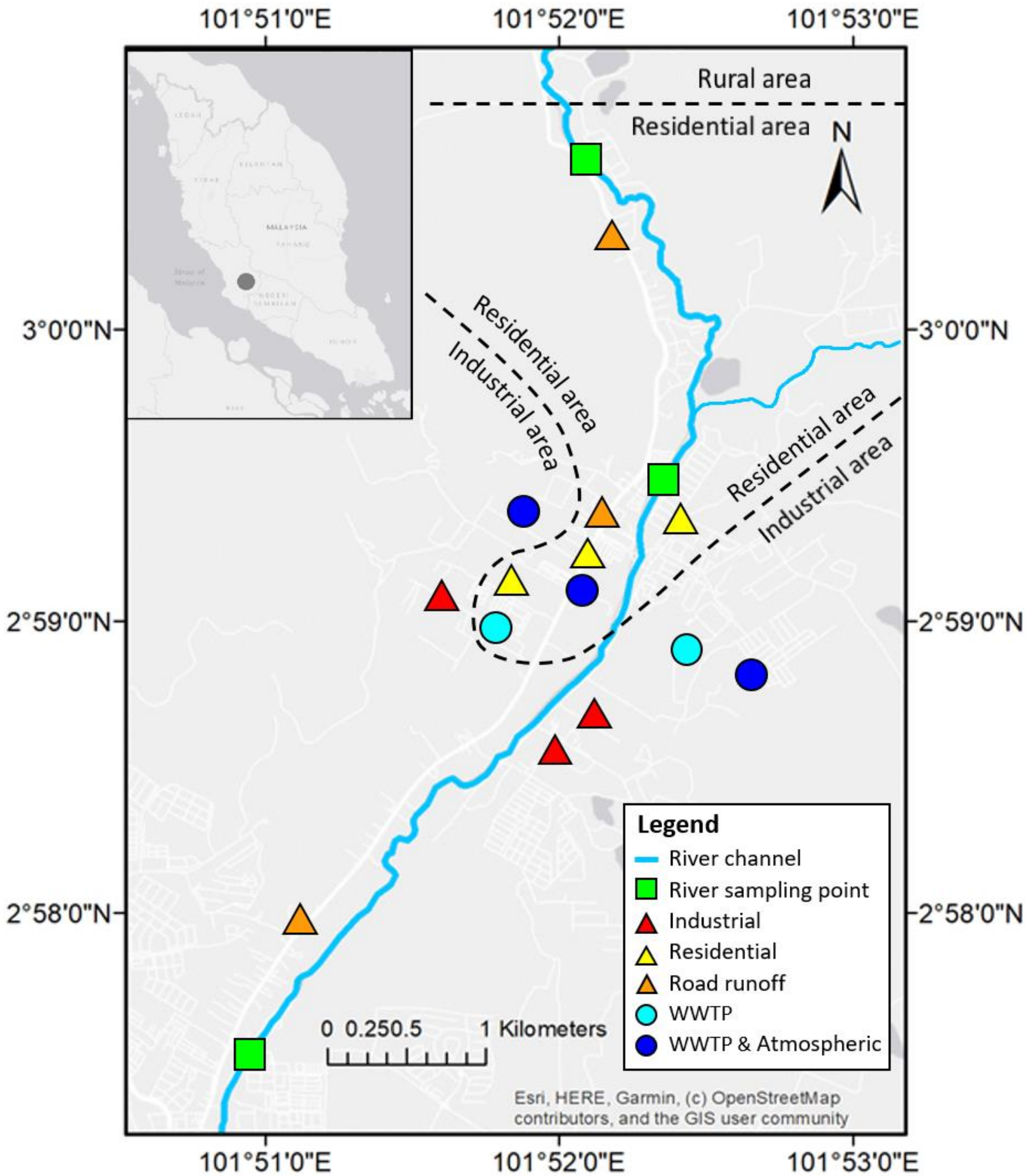

Fig 3.1 Map of the study area and sampling points (adapted using ArcMap; ArcGIS Desktop 10.8.1).

The 17 sampling points represented different source areas (Fig 3.1). The river sites were spread over a distance of approximately 5 km and were located 13.61 km, 16.8 km and 18.42 km from Semenyih Dam. Wastewater samples were collected from five WWTPs; water entering (inflows) and leaving (outflows) each plant was sampled. The atmospheric fallout samples were collected within the grounds of the WWTPs. These plants had open areas ideal for collecting rainwater samples (i.e. free from obstructions) and also were closed to the public, which meant sampling equipment left on site was safe from theft. These small plants were located within the housing and industrial areas, and so samples placed here can be considered representative of the fallout in these areas. The remainder of the sampling points were culverts feeding directly into the Semenyih River, taking runoff directly from the main road, as well as domestic discharges and runoff from small industrial estates that included plastic manufacturers, paint and food products. All five WWTPs receive wastewater from both residential and industrial areas; water is released to the Semenyih River after secondary treatment, i.e. settlement and extended aeration processes. Together, the sampling points allowed the representation of the main input types of microplastic over the study area. Most sampling days were dry (Appendix 3A), with no rain on the day of sampling or over the three preceding days. The exception was the final occasion, which saw 19 mm of rainfall.

3.3.2 Sample collection

Samples from the three main river sites were collected from bridges. At each of these sites, a 10 L sample of water was collected on each sampling occasion by lowering a 2 L sample bottle attached with weights from the bridge, collecting water from the full depth of the water column as it was lowered to the bed and raised. This was to ensure that the samples collected are ‘depth-integrated’, as is standard for sampling and estimating suspended sediment concentrations (Wang and Ribberink, 1986). Samples from drains, culverts and WWTPs were collected by hand using a small bottle; in total, 5 L was collected from each of these points. This smaller volume (compared to the river) reflected the higher microplastic concentrations in these sources and their small discharges. Water in drains and culverts, and the WWTP inflow and outflow channels typically ranged from a few cm deep to a maximum of 0.5 m. These samples were also collected by lowering the 2 L bottle to the full depth. The difference in concentration of microplastics between inflows and outflows of each WWTP was used to determine the microplastic removal efficiency of these plants. Atmospheric deposition samples were collected using 10 L glass jars with openings of 11 cm in diameter (area of 0.0095 m^2) (Appendix 3B). The bottles were left to collect rainwater over the periods between sampling occasions (passive collection). On each sampling occasion, the contents of each jar were collected by rinsing out the jar with distilled water. Due to some jars being lost/blown over, the assessment of atmospheric input is based on data for two time periods rather than all four.

Immediately after collection in the field, all water samples were filtered through a 53 μm mesh size stainless steel sieve to remove suspended clay and silt particles (Stanton et al., 2019). Materials remaining on the sieve were then washed into glass vials for later processing in the laboratory.

3.3.3 Sample processing

To digest organic materials, water samples were treated with 20 ml of 30% hydrogen peroxide and 20 ml of 0.05 M Iron (II) Sulphate solution (catalyst) and heated to 60°C for 30 minutes. Water samples were then filtered through 0.7 μm glass microfibre filter papers (Whatman GF/F) and oven-dried at 60°C for 24 hours. Microplastic particles on the filter papers were enumerated under a stereoscopic microscope (Leica EZ4) with 8x to 35x magnification. The removal efficiencies (RE) for WWTPs were calculated using the formula:

$$\mathrm{RE} = \frac{\text{Influent concentration (particles/L)} - \text{Effluent concentration (particles/L)}}{\text{Influent concentration (particles/L)}} \mathrm{X}100\%$$

In total, 91 pieces of suspected microplastics were picked out randomly from the filter papers and analysed for polymer composition using Fourier transform infrared spectrometer (FTIR; PerkinElmer Frontier). Four scans per sample with a spectra range between 4000 and

400 cm^{-1} under transmission mode were performed. The spectra were then matched with known polymer references. This analysis provided a reliability assessment of the visual sample processing, as well as information on the composition of a sample of microplastics from the study area. Additionally, a field emission scanning electron microscope (FESEM; FEI Quanta 400F) was used to observe the surface morphology of six representative microplastic particles. The particles were mounted on a double-sided adhesive tape before scanning at magnifications ranging from 200x to 20,000x at an accelerating voltage of 20 kV under a low vacuum.

Field blanks were collected to analyse possible contamination from atmospheric fallout from the field and the laboratory processing. In the field, the cleaned stainless steel sieves were rinsed with deionised water and possible materials on the sieve were washed into glass vials. The field blanks were processed alongside the rest of the samples; no contamination was found. To minimise potential contamination, cotton clothing was worn while handling all samples in the field and the laboratory. All apparatus in contact with the samples were rinsed thoroughly with deionised water before the introduction of the samples and all samples were kept covered in the laboratory.

3.3.4 Data analysis

Microplastic concentrations from drains and WWTPs are reported in particles/L. Those from atmospheric deposition are reported in particles/m^2/day to allow comparison with other published work but also converted to particles/L using rainfall to allow comparison with other sources sampled in this study. As the assumptions of analysis of variance (ANOVA) were met (i.e. data were normal and homoscedastic), one-way ANOVA and Tukey's honestly significant difference (HSD) tests were used to assess whether microplastic concentrations and types varied significantly between sources and between WWTPs. The ANOVAs provide: (1) F-values, where a high F-value shows that between-group variation is larger than within-group variation, (2) df-values (degrees of freedom), the number of independent pieces of information used to calculate the statistic, and (3) p-values (probability values), where $p>0.05$ is the probability that the null hypothesis is true. As the data is normally distributed, Pearson's correlation was used to assess the relationship between WWTP removal efficiencies and capacities. The correlation provides r-values (Pearson correlation coefficient; values closer to -1 denotes strong negative correlation, while values closer to +1 denotes strong positive correlation) as well as a p-value. All statistical analyses were carried out using RStudio (Desktop Version 1.4.1717).

3.4 Results

3.4.1 Contribution of microplastics from different sources

Microplastics were present in every sample collected from the study area. Culverts draining the main roads had significantly higher concentrations (42.20±35.29 particles/L) than those draining residential (8.53±9.91 particles/L) and industrial areas (5.67±4.88 particles/L) and were higher than in the water discharged from WWTPs (7.47±3.52 particles/L) (ANOVA; $F=10.624$, $df=4$, $p=0.001$; Tukey HSD; $p=0.001$; Appendix 3C). Atmospheric deposition contributed an estimated 76.07±32.85 particles/m^2/day to the ground surface across the study area, equivalent to 8.35±5.11 particles/L from rainwater.

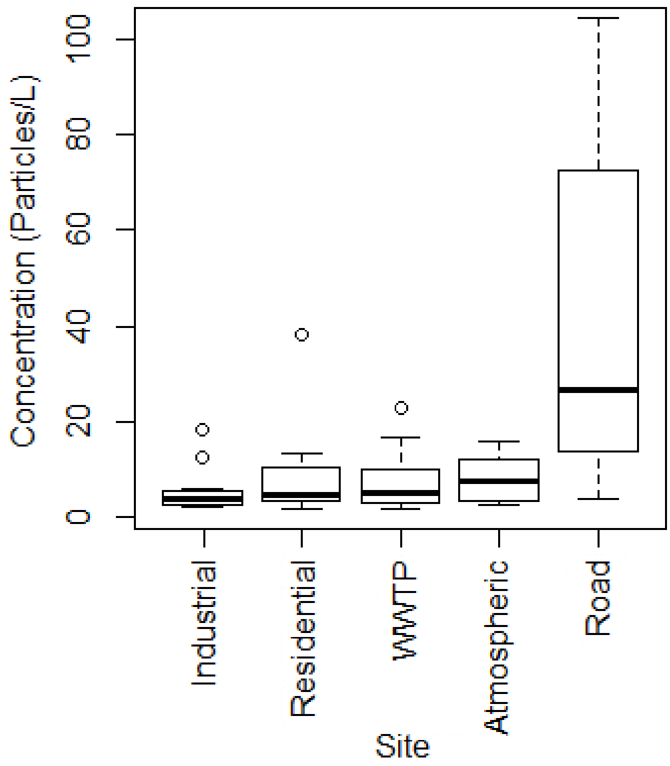

Fig 3.2 Microplastic concentration (particles/L) in different sources across the study area.

3.4.2 Wastewater treatment plants

The average concentration of microplastics in WWTP inflows and outflows were 17.63±6.2 particles/L and 7.47±3.52 particles/L respectively. Removal efficiencies for individual WWTPs over the sampling period ranged from 30.95±5.51% to 69.94±22.17% (Fig 3.3), with WWTP 2 the lowest and WWTP 4 the highest on average. However, these differences proved not to be significant (ANOVA; $F=2.459$, $df=1$, $p=0.128$; Tukey HSD; $p<0.050$), most likely due to low test power stemming from the small number of samples from each plant and the variability in efficiency between dates. Pearson's correlation showed weak negative correlation between removal efficiency and WWTP capacity, but the correlation was not significant (Pearson's correlation; $r=-0.430$, $p=0.058$), so there was no suggestion that their performance was related to size.

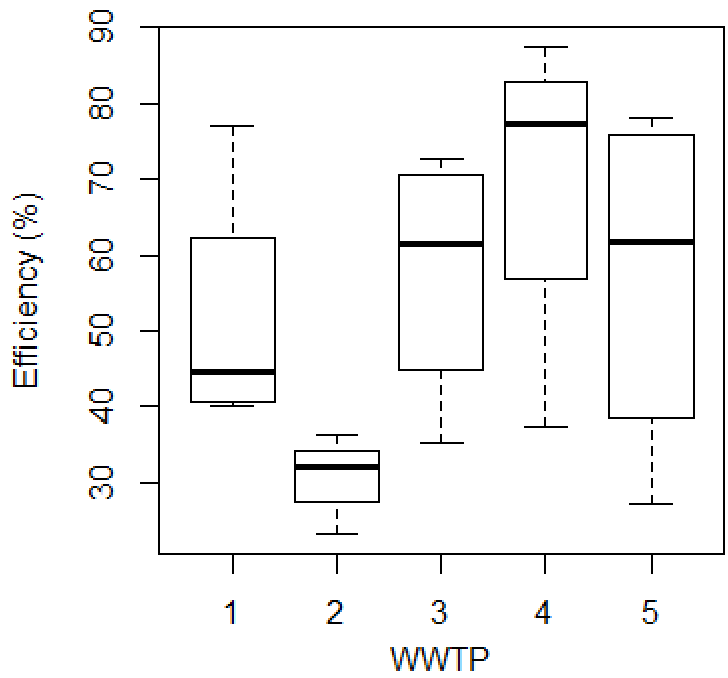

Fig 3.3 Removal efficiency (%) of wastewater treatment plants.

There was no significant difference in the removal of the different types of microplastics (ANOVA; F=4.091, df=1, p=0.058). The mean removal efficiencies for fibres and fragments were 57.01±17.93% and 53.46±30.47%. Note that removal efficiencies of films and beads were not recorded as these types were not detected in either the inflow or outflow of any of the WWTPs.

3.4.3 Microplastics in the Semenyih River

The average microplastic concentration in the Semenyih River was 1.93±0.84 particles/L. The mean value of the most downstream site was higher than the other two (Fig 3.4a) but the difference was not significant (ANOVA; F=0.058, df=2, p=0.944). There was temporal variability in the relative concentrations in samples collected from these sites, with concentration at the upper site higher than the downstream ones on some dates (Fig 3.4b).

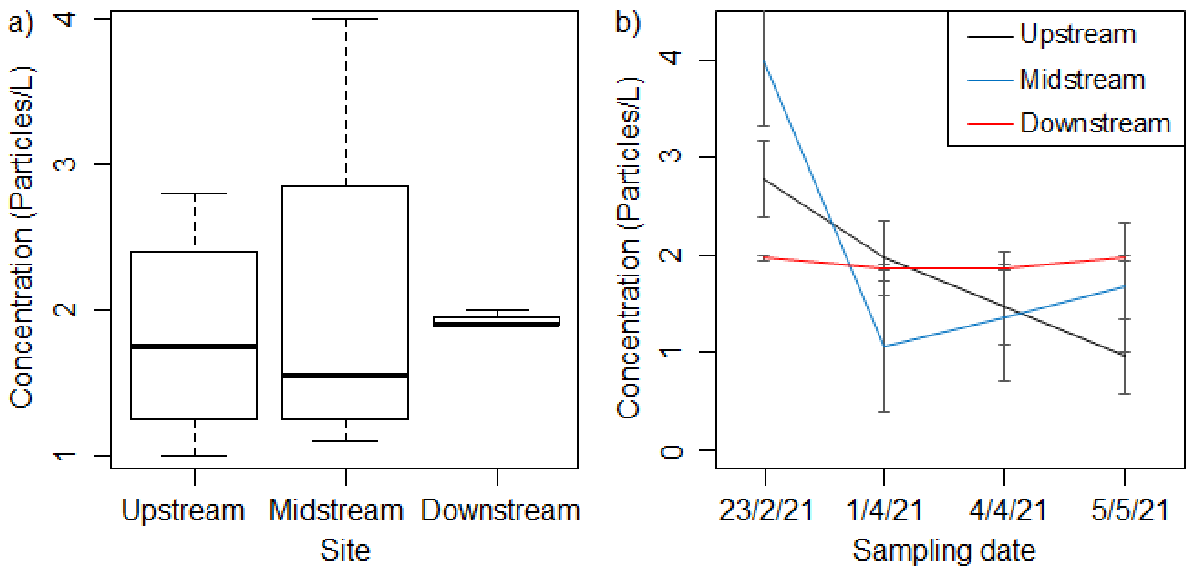

Fig 3.4 a) Spatial and b) temporal variation in microplastic concentration (particles/L) in the Semenyih River.

3.4.4 Characteristics of microplastics

Microplastic in the water samples was dominated by fibres, although the composition varied greatly between the different sources (Fig 3.5a). Fibres were dominant in river inputs from WWTPs, atmospheric deposition and industrial areas; they were rare in road runoff, where the material was dominated by fragments. Inputs from residential areas were most diverse, with an even mix of fibres and fragments but with a notable presence of beads and films (which were absent from other sources). All of the beads were polystyrene. Microplastics in the river consisted mainly of fibres and fragments, with beads and films rare and absent from many samples. No significant differences were found in the types of microplastics between the three river sites (ANOVA; $F=1.820$, $df=2$, $p=0.191$).

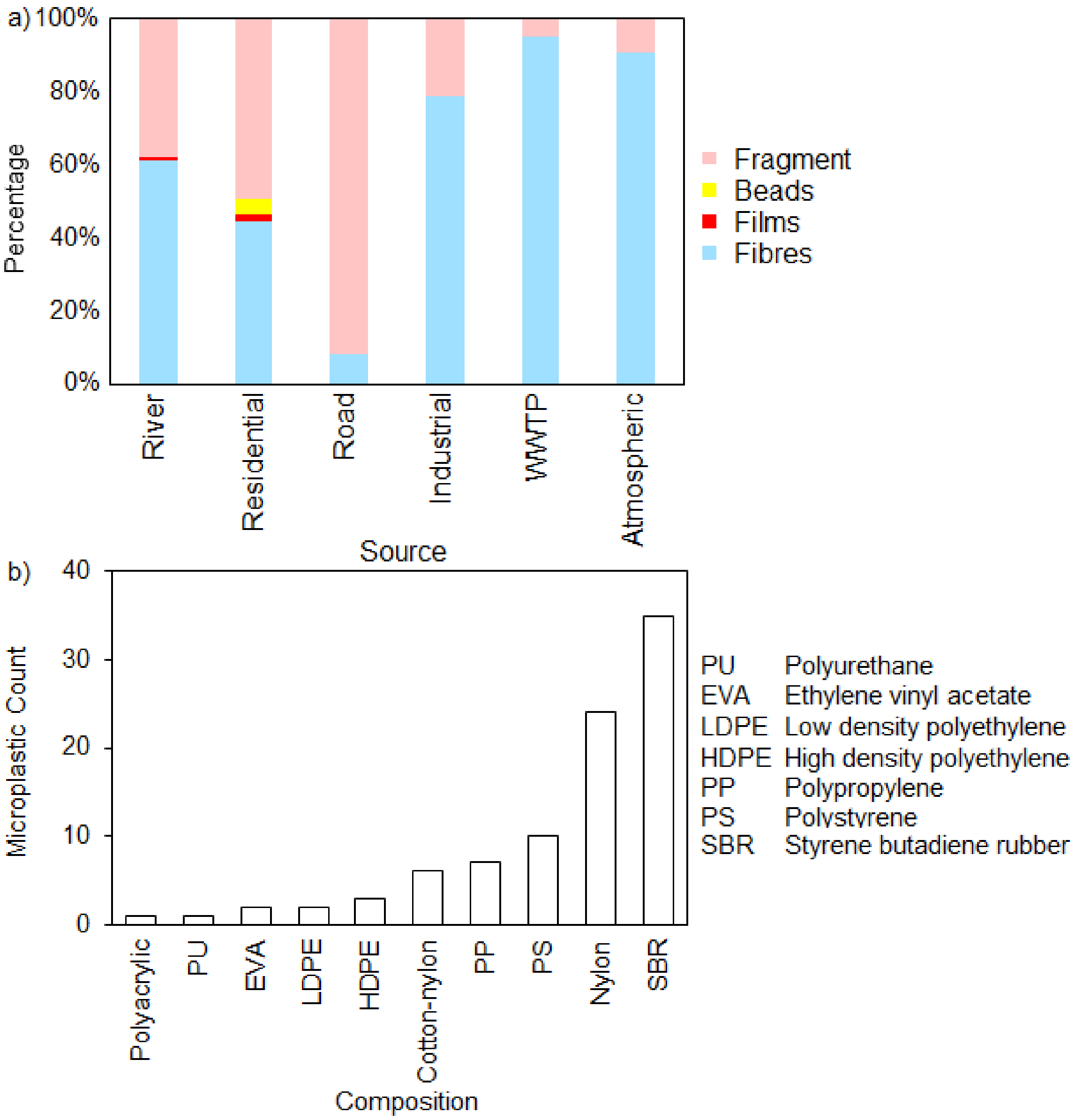

Fig 3.5 a) Type of all (N=5172) detected microplastics and b) composition of representative samples (N=91) of microplastics in the Semenyih River and its contributing sources.

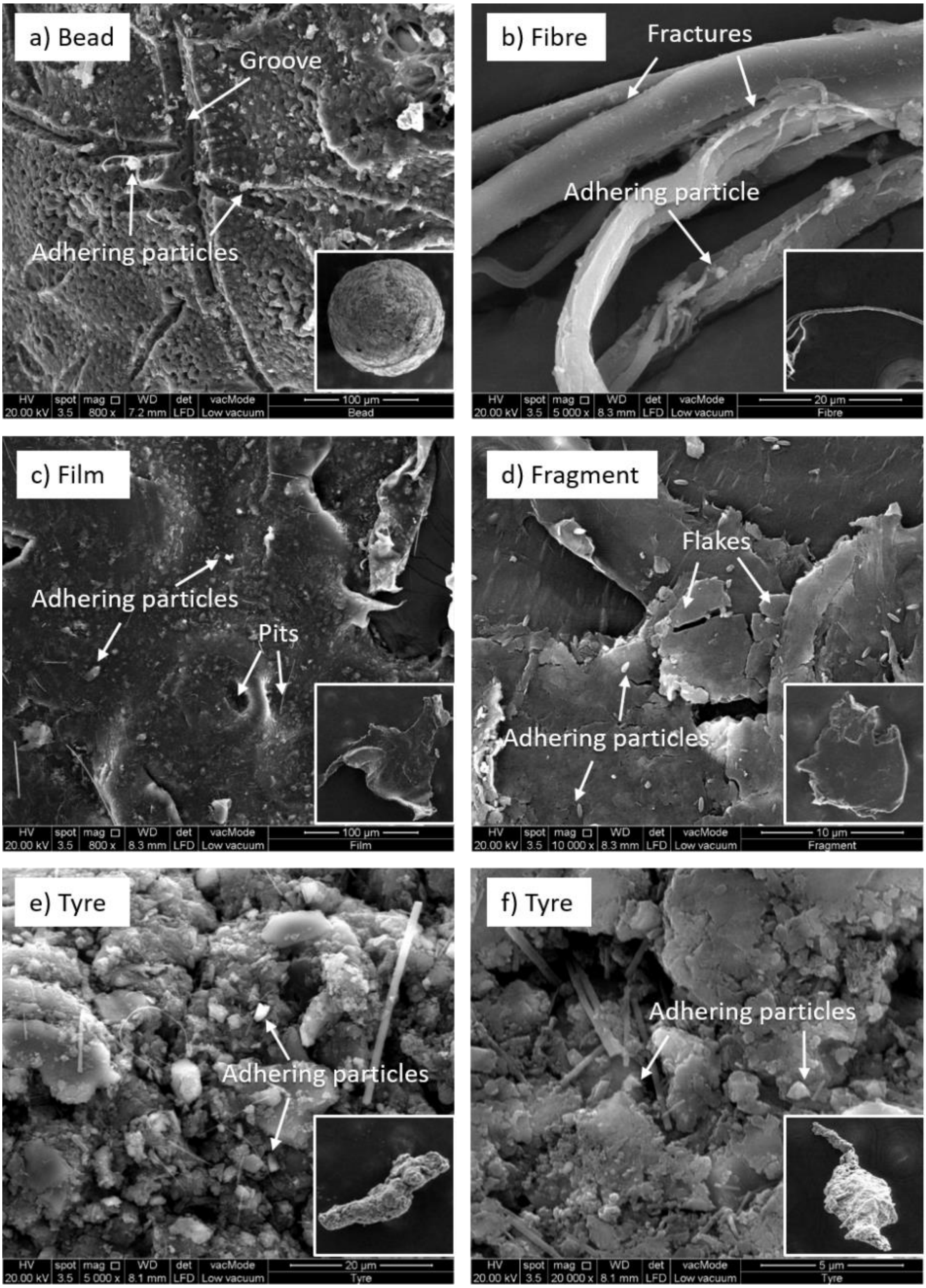

Fig 3.6 FESEM images showing the surface texture and signs of weathering on microplastic samples.

Signs of weathering were evident on the fragments inspected under SEM, including pits, long grooves and fractures. Long grooves can be seen in Fig 3.6a, fractures in Fig 3.6b, pits in Fig 3.6c, and flakes in Fig 3.6d. Uneven surfaces and adhering particles were observed on all microplastic surfaces.

3.5 Discussion

3.5.1 Local sources of microplastics

This study is among the first to look at the full range of local sources of microplastics contributing to river loads and the first of its kind in a rapidly urbanising area. The only other comparable studies were undertaken in Paris (Dris et al. 2015 and Dris et al., 2018), where the authors looked at inputs to the River Seine. They assessed a similar range of sources to the present study but had 10 sampling points spread over an area of approximately 600 km^2 and contributing to microplastics along a 60 km length of the Seine. Thus, the present study was conducted at a different scale and density, with 17 sampling points distributed across an area of approximately 20 km^2, and assessing the effects of inputs along a 5 km length of the mainstream river (the Semenyih).

Drainage from main roads was the major contributor of microplastics to the Semenyih River, delivering an average concentration of 42.20±35.29 particles/L over the six-week study period. This differs from the findings of Liu et al. (2019b) who found the highest concentrations in drainage from industrial and residential areas. However, rather than being conveyed directly to water courses, all runoff in their study area was first taken to settlement ponds. The catchment areas of these ponds were largest for industrial areas and smallest for those receiving road runoff, which likely affected the relative concentrations in respective outflows. Local differences in traffic volume and activity also play a role in influencing relative contributions from road runoff. Increased braking and acceleration lead to increased tyre wear and abrasion

(Knight et al., 2020). Samples from the main roads in the study area were mainly taken from areas with crossroads and numerous speed bumps, with associated braking, acceleration and tyre abrasion consistent with relatively high concentrations of microplastics observed in road runoff. Microplastic fragments in road runoff likely originate from TRWP, which includes tyre dust, polymer-modified bitumen and worn-out paint from road markings. SBR was abundant in the subsample of microplastics tested (Fig 3.5b), which mirrors the dominance of TRWP in road runoff reported in other studies (Järlskog et al., 2020; Rødland et al., 2020) since SBR fragments are commonly generated from vehicle tyres due to friction, heat and pressure during acceleration and braking.

Microplastics in drains from residential areas were higher than in industrial areas. This differs from other studies, where higher concentrations in stormwater runoff from industrial areas have been reported (Liu et al., 2019b; Piñon-Colin et al., 2020). Piñon-Colin et al. (2020) attributed the high concentrations to the illegal discharge of industrial wastewater into drains during rain events, while Liu et al. (2019b) attributed them to the construction of drainage pipes within the industrial area they studied. Recent work in Malaysia has shown the significance of direct discharges of domestic wastewater from textile washing into drains (Praveena et al., 2020). Thus, a complex set of factors related to (1) the controlled versus illicit discharges, (2) the exact nature of domestic washing activities (hand versus machine washing, and the prevalence of filters on machines), along with (3) how wastewater is routed to water courses (via settlement ponds or not) have a great bearing on the relative contributions from different sources. It is also likely that the relative inputs during the current study period were influenced by the COVID-19 pandemic; the period saw lockdowns that confined people to their homes for many months and the closure of many factories that halted normal industrial activities.

Microplastics in culverts draining residential areas were dominated by fragments and fibres (Fig 3.5a). The fibres, dominated by nylon (Fig 3.5b), are indicative inputs from domestic washing of synthetic textiles, where fibres are generally released due to abrasion. The fragments, films and beads (i.e. polystyrene beads) found in drains in the residential areas (Fig 3.5a) may originate from the uncontrolled disposal and subsequent breakdown of single-use plastic packaging, including items such as plastic bags, expanded polystyrene foam boxes and takeaway containers. This conclusion is supported by composition analysis, where commercial thermoplastics, including PS, PP, HDPE and LDPE particles, were recovered in moderate quantities. PP may be traced back to melt-blown disposable facemasks, while HDPE and LDPE may be associated with the use of household utilities such as detergent containers, garbage bags and kitchen films. PS, on the other hand, may be associated with foam shipping boxes, disposable clamshell containers and cutleries. In part, the preponderance of these materials in the samples from the residential areas may be attributed to the COVID-19 pandemic, which has seen a marked increase in food delivery and online shopping (Fan et al., 2021; Moon et al., 2021). Napper and Thompson (2019) studied the deterioration of various types of degradable and non-degradable plastic bags and reported the breakdown into pieces within the microplastic size range within nine months. The recurring lockdowns in Malaysia extended from March 2019 up until the study period (i.e. 14 months), long enough for the breakdown of domestic-use plastic to occur.

3.5.2 Atmospheric deposition of microplastics

Atmospheric deposition was an important source of microplastic in the study area. As atmospheric deposition rates have not been studied in Malaysia, it is not possible to give a context for the 76.07±32.85 particles/m^2/day estimated for the study area. However, these values are appreciably lower than reported elsewhere in East and South-east Asia, with Ho Chi Minh City, Vietnam, having up to 917 particles/m^2/day (Truong et al., 2021), and Dongguan city, China, having up to 313 particles/m^2/day (Cai et al., 2017). The study area was situated on the edge of Semenyih town. While the sampling points themselves were situated within built-up areas, there is much rural land in the immediate vicinity, with the upstream catchment area consisting mainly of forest and agricultural land. Thus, the nature and spatial extent of urbanisation are very different to the large Chinese and Vietnamese cities studied by Cai et al. (2017) and Truong et al. (2021) respectively. These differences most likely explain the lower atmospheric fallout in the study area.

Microplastic in the atmospheric samples was dominated by fibres (91%), which is typical (Liu et al., 2019c; Truong et al., 2021). Since airborne particles that are larger than 10 microns are more likely to be influenced by gravitational forces and deposited close to their source of origin (Kole et al., 2017), the fibres found in samples from points across the study area (>53 microns) most likely originated from adjacent residential areas and textile industries rather than more distant areas.

3.5.3 Microplastics from wastewater treatment plants

WWTPs are well known to be significant contributors of microplastic to rivers (Chen, 2021b). WWTPs in the study area delivered microplastics to the river in similar concentrations as industrial and domestic drains, although these are lower than reported in some studies (e.g. 28 particles/L in Germany [Schmidt et al., 2020] and up to 297 particles/L in Korea [Hidayaturrahman and Lee, 2019]). As in other studies (e.g. Conley et al., 2020 and Tang et al., 2020), microplastics from both WWTPs and residential areas were dominated by fibres, most likely from domestic textile washing (Alvim et al., 2020; Praveena et al., 2020). Like many other developing countries (Mara, 2004), discharges from domestic textile washing in Malaysia are either released directly into drains as greywater or channelled into WWTPs as wastewater (Praveena et al., 2020). Drainage samples collected from the residential areas were typically greywater from kitchens and washing machine discharges, while the WWTPs typically received wastewater from shower drains and toilets. This may explain the comparatively low microplastic concentrations in WWTP effluents compared to countries with WWTPs receiving both greywater and wastewater (i.e. Germany [Schmidt et al., 2020]). That said, concentrations in WWTP effluents in the study area are still lower than reported for WWTPs that mainly receive municipal wastewater (e.g. up to 30 particles/L in China [Tang et al., 2020] and up to 27 particles/L in the USA [Conley et al., 2019]), further supporting the inference that washing machine discharges are released directly into drains across the study area.

The microplastic removal efficiency of the five WWTPs ranged from 30.95% to 69.94%, with WWTP 4 being the most efficient and WWTP 2 being the least efficient (averaged across the four sampling dates). The somewhat higher efficiency of WWTP 4 is likely due to the presence of additional baffles (absent from the others) which help remove suspended solids. However, the removal efficiency of this plant was highly variable, ranging from 40% to 85% on the sampling dates. Variability in efficiency was evident for all the plants. The consistently low efficiency of WWTP 2 could, in theory, be explained by the higher capacity (2800 PE) of this plant compared to the others (ranging from 734 PE to 1987 PE). However, no significant correlation between the capacities of WWTPs and removal efficiencies was found.

The efficiencies calculated for the five WWTPs are generally lower than in other studies, with efficiencies ranging from 66.1% in Wuhan, China (Tang et al., 2020), to as high as 98.1% and 98.9% in Charleston, USA (Conley et al., 2019) and Daegu, Korea (Hidayaturrahman and Lee, 2019) respectively. Removal efficiencies of microplastics depend on the specifications of individual WWTPs (Booth and Sorensen, 2020), with WWTPs equipped with tertiary treatment processes being more efficient than those with only secondary treatment processes (Okoffo et al., 2019). Wastewater in Malaysia are released into river systems after only receiving secondary treatment, consequently contributing to higher microplastic counts in WWTP effluents. Microplastics are not listed as a parameter for WWTP effluents in Malaysia (Department of Environment Malaysia [DOE], 1979), and so the need for their removal has not influenced the design of the smallest, local WWTPs such as those studied here.

3.5.4 Microplastics in the Semenyih River

As reported by Dris et al. (2018), concentrations of microplastics in the Semenyih River were lower than those in channels draining source areas, reflecting dilution. Nevertheless, the average concentrations at the three river sites (1.93±0.84 particles/L) were high compared to other Malaysian rivers (ranging from 0.000007 particles/L to 0.3 particles/L [Hwi et al., 2020; Pariatamby et al., 2020]). Since most of the area upstream from the uppermost of the three river sampling sites remains forested, it seems that the small rural settlements and the developed area in the immediate upstream vicinity of this point are enough to result in concentrations that are higher than so far reported in other Malaysian rivers. Despite the drains, culverts and WWTP outlets sampled across the study area discharging microplastics to the Semenyih downstream from this uppermost site, they did not cause a significant increase in concentration that could be detected at the middle and lower sites (Fig 3.4a). This most likely reflects the confounding influence of a tributary that enters the Semenyih between the upper and middle sites (Fig 3.1). This tributary drains a relatively undisturbed sub-catchment area, and unlike the mainstem river does not have its flow regulated by a dam. Patterns in the data suggest that water from this tributary dilutes microplastics in the Semenyih to a degree that renders the increased load (inputs from all sources across the study area) not detectable when expressed as concentration. Further down the Semenyih River, concentrations rise to 90 particles/L (Chen et al., 2021c), paralleling increasingly urban and industrial landuses.

The data allowed us to produce a microplastic budget for the study area (Fig 3.7). Overall, runoff draining main roads was the main contributor of microplastics to the Semenyih River over the period. Dris et al. (2018) found that combined sewer overflows (CSOs) contributed most to microplastics into rivers, followed by terrestrial runoff and discharges from WWTPs. However, these authors noted the contribution of other runoff to CSOs, likely elevating concentrations in this source. Dris et al. (2018) found concentrations up to seven times higher in WWTPs and up to five times higher in atmospheric deposition than the present study, with the differences likely resulting from the differences in levels of urban development and population density (as discussed above).

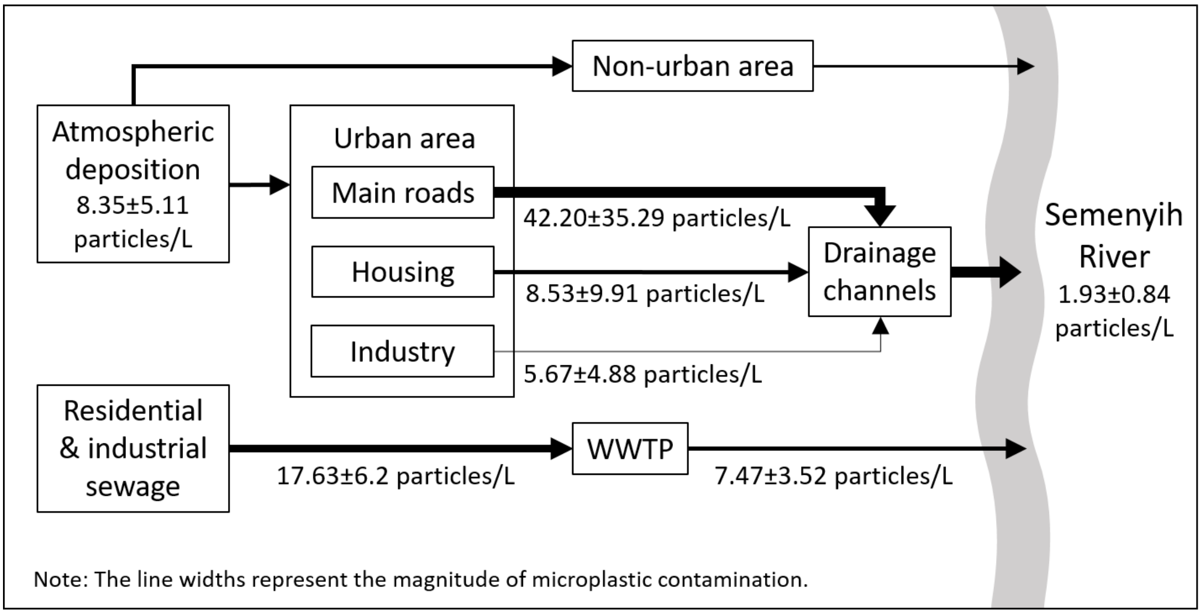

Fig 3.7 Microplastic budget of the Semenyih River.

Microplastics sampled from the study area experienced weathering processes (Fig 3.6). Grooves (Fig 3.6a), often characterised by the long indentations on particles, are likely caused by friction against tougher particles, suggesting mechanical weathering (Wu et al., 2018). Evidence of mechanical weathering can also be seen on the fractures as well as the pits formed on the surfaces of microplastics. The presence of adhering particles on the surfaces of microplastics has been reported to further enhance processes of mechanical weathering (Zbyszewski et al., 2014). The flaking of microplastic particles (Fig 3.6d) suggests oxidative weathering, likely caused by the degradation of plastic additives from the particles themselves, weakening the structure of plastics and causing the formation of flakes (Zbyszewski et al., 2014). Evidence of weathering suggests the possibility of degradation of microplastics into even smaller, nano-sized particles (Rist and Hartmann, 2018), and that higher concentrations might be found if nanoplastics were also enumerated. These smaller particles pose risks to riverine organisms since evidence points to increasing microplastic toxicity with decreasing size (Anbumani and Kakkar, 2018).

3.6 Conclusion

Our data indicate that (1) the main road is a major contributor to riverborne microplastic in the study area and that (2) due to their low efficiencies (30-70%), the WWTPs contribute as much as culverts draining domestic and industrial areas. Comparisons with published studies indicate that the absolute and relative contributions from the various sources in the study area all differed from other areas. This suggests that local-scale factors that influence the generation, treatment and routing of microplastics have a strong bearing on the nature of river contamination. Our work also shows that time-specific factors add a complicating dimension, in the present case related to lower-than-normal industrial activity and confinement of people to houses due to the COVID-19 pandemic.

The high quantities of microplastics in culverts draining the main road cutting across the study area indicate the need for road sources to be treated before their discharge into the river. As common in Malaysia and many other parts of SEA, drains in the study area receive domestic and industrial wastewater and discharge directly into the river, without treatment. However, the WWTPs do not incorporate processes designed to remove microplastics and so at present are also major sources of riverborne microplastic. Re-routing existing drains and adding additional treatment processes to WWTPs may be an unrealistic ambition currently, but drainage systems in new urban developments and WWTPs should be designed to address this problem. Water quality indices used in Malaysia do not currently include microplastics (DOE, 1979), and so they are not assessed as part of routine monitoring or included in discharge consents. A first step in tackling the microplastic problem in the country would be to include this material in routine monitoring so that the true extent and magnitude of the problem could be established.

Our work represents one of the very few studies to have assessed microplastic inputs from multiple sources within a specified area. It could usefully be extended by collecting samples over a longer period, to check whether the relative patterns reported here are representative of river inputs over the year. In particular, sampling could be repeated during rainfall to assess concentrations during times when road wash-off is occurring and drains are full. Future work could also usefully assess the discharges in drains and culverts so that the total loads being delivered to the river could be estimated. Such data would be valuable for assessing human and ecological health risks (Pang et al., 2021), and in turn, indicate the need for better management of wastewater in countries such as Malaysia where many people rely directly on rivers for potable water and food.

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3.8 Appendices

Appendix 3A

Information on sampling events.

Sampling event	Sampling date	Number of dry days before sampling event *	Total rainfall on the day of sampling event (mm) *	Data collection for atmospheric samples
1	23 March 2021	3	0	No
2	4 April 2021	3	0	No
3	12 April 2021	3	0	Yes
4	5 May 2021	0	19	Yes

* Rainfall data were obtained from the Department of Irrigation and Drainage Malaysia (DID, 2021).

Appendix 3B

Dimensions of the atmospheric deposition samplers.

Appendix 3C

P-values of Tukey's honestly significant difference test of one-way ANOVA of microplastic concentration between sources.

	Residential	Road	Industrial	WWTP
Residential		0.0001	0.9751	0.9978
Road	0.0001		0.0001	0.0001
Industrial	0.9751	0.0001		0.9919
WWTP	0.9978	0.0001	0.9919	

4

Spatio-temporal variation of microplastics in the Langat River

4.1 Abstract

Microplastic pollution is widely recognised as a global issue, posing risks to natural ecosystems and human health. The combination of rapid industrial and urban development and relatively limited environmental regulation in many tropical countries may increase the amount of microplastic entering rivers, but basic data on contamination levels are lacking. This is especially the case in tropical South-east Asian countries. In this chapter, the abundance, composition and spatio-temporal variation of microplastic in the Langat River, Malaysia, are assessed, and the relationship between microplastic concentration and river discharge is investigated. Water samples were collected over a 12-month period from 8 sampling sites on the Langat, extending from forested to heavily urbanised and industrial areas. All 508 water samples collected over this period contained microplastic; the mean concentration across all sites and times was 4.39±5.11 particles/L. Most microplastics were secondary in origin and dominated by fibres. Microplastic counts correlated directly with river discharge, and counts increased and decreased in response to changes in flow. A time-integrated assessment of the microplastic load conveyed by the Langat suggested that the river typically (50% of the time) delivers around 5 billion particles per day to the ocean. The positive correlation between the concentration of microplastics and suspended sediments in the Langat suggested that continuously logging turbidity sensors could be used to provide better estimates of microplastic loads and improve the assessment of human and ecological health risks.

4.2 Introduction

Microplastics, defined as insoluble synthetic particles smaller than 5 mm in length (Peterson and Hubbart, 2021; Rochman, 2018), are widely recognised as a global environmental problem (Zhang et al., 2020a). Literature on freshwater microplastic contamination has increased rapidly in recent years, due to concerns about threats to aquatic ecosystems and risks to potable water supplies, as well as because rivers are key conduits of microplastic transport to the oceans (Lebreton et al., 2017; Yang et al, 2021). Nevertheless, research documenting freshwater microplastic contamination remains scarce relative to marine systems; research is also geographically biased, with the majority of freshwater studies carried out in Europe (Chen et al., 2021a). Studies are needed in low and middle-income countries, such as those in tropical South East Asia (SEA), where the rapid increase in industrial and urban development and population growth, along with poor waste management and water treatment (Chen et al., 2021b) may increase freshwater microplastic contamination and heighten health risks (Dikavera and Simon, 2019). Despite the potentially high and increasing levels of microplastic in SEA, only nine papers have reported on contamination in rivers in the region (Chen et al., 2021a).

Catchment-wide studies are critical for the identification of contamination hotspots and to help understand associated ecological and human health risks. Repeat surveys are critical for the proper assessment of concentrations and risks, as well as for estimating the total loads being delivered to oceans (Stanton et al., 2019). While such work is beginning to shed light on the dynamics of microplastics in streams and rivers and the factors that influence these dynamics (Besseling et al., 2017; Fan et al., 2019), few studies have assessed both spatial and temporal variation in microplastic concentrations (Fan et al., 2019; Rodrigues et al., 2018; Wu et al., 2020; Zhang et al., 2020b) and none have done so in tropical rivers.

Modelling and empirical studies have stressed the importance of river hydrological regimes as controls on the transport and storage of microplastic. In general, microplastics carried downstream in suspension are likely to be deposited on riverbeds during low flow periods where they accumulate until being entrained by high flow periods (Hurley et al., 2018; Nizzetto et al., 2016; Waldschläger and Schüttrumpf, 2019; Watkins et al., 2019). Thus, high concentrations during periods of elevated discharge may simply reflect re-mobilised material rather than ‘new’ microplastic coming into the channel from the catchment. This parallels the situation with fine sediments, which often show very complex and variable relations with discharge (Buendia et al., 2014). For example, high suspended sediment concentrations (SSCs) can sometimes result from very small floods because of the re-mobilisation of material that has been accumulating on the bed during preceding periods of low flow. However, at times when there is little material on the bed, intense or sustained precipitation and high river flows may be needed to deliver material from the catchment to channels and increase SSC.

The complex relations between river flows and SSC reflect the inputs from multiple sources that have different levels of connectivity to watercourses, as well as the settlement, storage and entrainment dynamics of fine material within river channels. Similar complex relations might be expected between microplastic and flow, but to date, very few studies have addressed this directly. Several studies have looked at the influence of flow on microplastics, but these have focused either on assessing changes in bed concentrations after floods (Hurley et al., 2018; Ockelford et al., 2020) or the rise and settlement velocities of microplastics in water (Waldschlager and Schuttrumpf, 2019).

This chapter addresses two important gaps in fundamental knowledge: (1) the limited information on microplastic loads in rivers in rapidly developing tropical countries, and (2) the lack of knowledge of spatial and temporal variation in microplastic loads across individual catchments and how these are influenced by flow conditions. The study is based on repeat surveys undertaken at multiple sites across a mesoscale tropical catchment (the Langat River) in Peninsular Malaysia. The specific objectives of the chapter are: (1) to assess the composition and spatial variation in microplastic concentration in the Langat, (2) to assess temporal variation in microplastic concentration in the river and its correlation with river flow, and (3) to assess whether the dynamics of microplastic correspond with those of fine sediment. A broader goal of the work was to use the data to estimate the total amount of microplastic delivered by the Langat to the ocean each day.

4.3 Methods

4.3.1 Study area

The Langat River basin is the largest in Selangor State, Malaysia, and acts as an important water supply to the populations in the Kuala Langat, Klang, Sepang and Hulu Langat districts of Greater Kuala Lumpur (Selangor Water Management Authority [SWMA], 2019). The Langat has a catchment area of 2663 km^2. It comprises two main, similar-sized upper sub-catchments (the Semenyih and Langat) that merge to form the main Langat River (SWMA, 2019). The Semenyih and Langat are regulated by dams (SWMA, 2019) of 60.6 and 33.6 MCM storage capacity respectively (Saadon and Ali, 2014). The catchment extends from relatively undisturbed (forested) headwater areas to heavily urbanised and industrial centres along its middle and lower reaches, where it skirts around the southern edge of Kuala Lumpur (districts of Kajang, Putrajaya and Klang) (Fig 4.1). The Semenyih sub-catchment has a mean annual rainfall of 2309 mm, and a mean annual flood (i.e. maximum flow) of 67.3 m^3/s; the mainstem Langat (at the lowermost gauging station) has a mean annual rainfall of 2509 mm and mean annual flood of 299.9 m^3/s (Department of Irrigation and Drainage Malaysia [DID], 2018). The previous chapter has provided a more detailed understanding of the sources of river contamination, and so focused on a small area (approximately 20 km^2) within the Semenyih catchment. The current study was designed to assess microplastic contamination across the whole of the Langat, including the Semenyih, and therefore focused on a larger catchment-scale area.

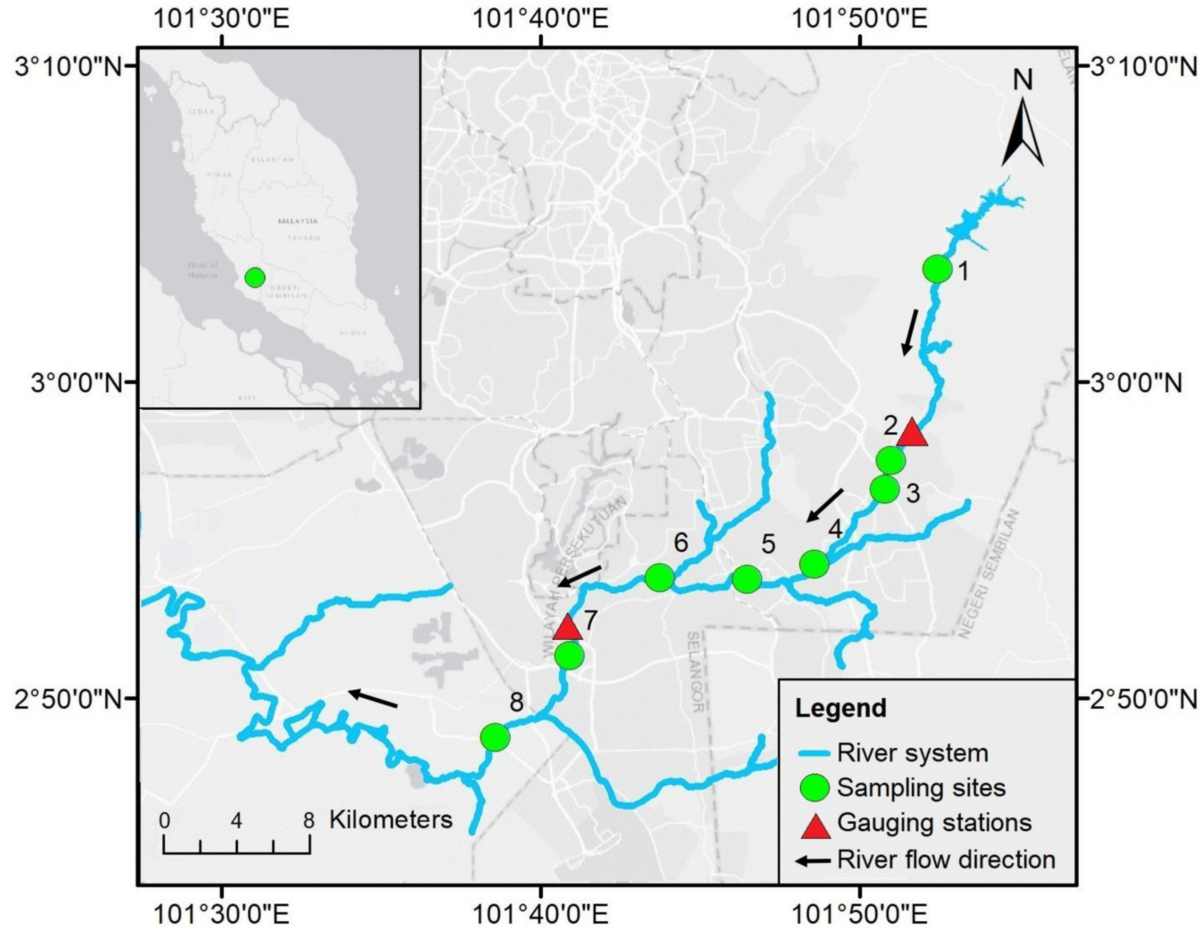

Fig 4.1 Map of the study area, showing sampling sites and gauging stations.

A total of 8 sites were distributed along the mainstem freshwater section of the river, from the Semenyih dam along the Semenyih River and down the mainstem Langat (Appendix 4A); the tidal reach was avoided due to complexities resulting from bidirectional flows. The sites were distributed in a way that reflected changes in landuse. Site 1 was approximately 1 km below the dam and had no settlements in its upstream catchment area; the land cover was almost wholly forest, with human settlement consisting of only a small number of isolated dwellings. Site 2 was on the upstream edge of Semenyih town, before the main conurbation but within an area of housing and light industry. Site 3 was immediately downstream from Semenyih, and received water from drains, culverts, tributaries and a number of wastewater treatment plants within the town area. The remaining downstream sites all represented points along a transition to less natural land cover, with increasing urban and industrial areas and fewer natural habitats in their upstream catchment areas. Site 2 and 7 were close to gauging stations (operated by the DID). Sites mostly had bridges that allowed for sampling. Repeat sampling of these 8 sites over a 12-month period allowed representation of the spatial and temporal variation along a rural to urban/industrial transition.

4.3.2 Sample collection

A pilot study was carried out to help with the sampling design. The main focus of the pilot was whether there was any variation in microplastic concentration across the channel that needed to be considered when collecting water samples. The pilot study was undertaken at site 8, the widest and least turbulent site where we expected the potential for lateral variation to be greatest. A transect from the left to the right bank was set up (30 m), with 1 L water samples collected at 2 m intervals across the channel. There was some variation in microplastic concentration across the channel (data shown in Fig 4.2), indicating that samples should be collected across the full width of the channel rather than at a single point. The pilot also allowed us to establish that due to the large numbers of microplastic particles present in the river (see results) a total sample volume of 10 L was adequate for estimating concentrations (i.e. statistical assessment of differences between sites or dates was not compromised by lots of zero or very low concentration values). Accordingly, the routine sampling consisted of 2 L of water being collected from each of the five points across the channel, yielding a 10 L sample at each site on each date.

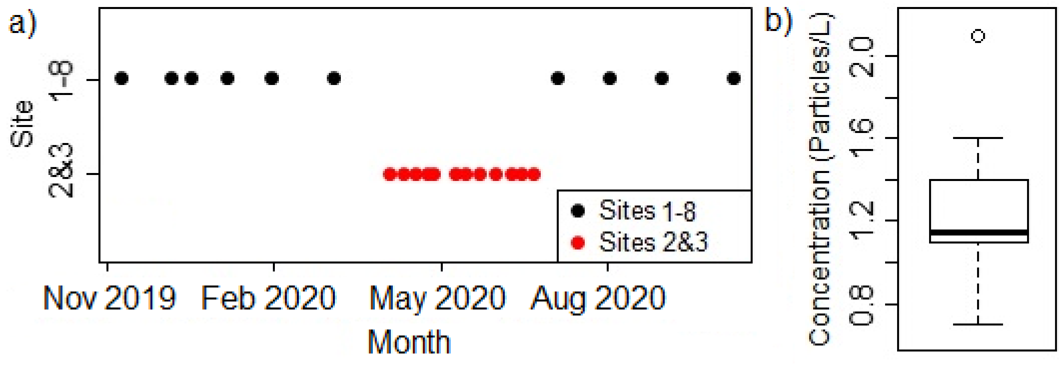

Fig 4.2 a) Sampling programme throughout the 12-month sampling period, indicating monthly sampling occasions in Sites 1 to 8 and weekly sampling occasions in Sites 2 and 3 (i.e. months April to June). b) The lateral variation of microplastic concentration across the Langat, showing mean, first quartile and third quartile (Site 8; September 2019).

The routine sampling programme is summarised in Fig 4.2a. Samples were collected from November 2019 to October 2020 inclusive. Monthly sample collection was initiated at the 8 mainstem sites (Fig 4.1) with the aim of sampling each site once per month for the 12-month period. This sampling programme was broadly based of other similar published works (e.g. However, due to the 2020 Movement Control Order implemented by the federal government of Malaysia during the COVID-19 pandemic, sample collection from April to June 2020 was restricted to Sites 2 and 3. To compensate for the reduced spatial coverage, samples were collected at weekly intervals for these two sites for the April to June period.

In addition to this routine monitoring, samples were collected before, during and after a flood event at Site 4 at hourly intervals over a 24-hour period (June 2020). The aim of this sampling was to understand changes in microplastic concentration in response to changes in discharge during an individual flood event. To understand the hydrological characteristics of the flood, data from the nearest gauge were used and adjusted according to the catchment area to produce a flood hydrograph for Site 4.

Samples were collected from bridges or, at shallower sites, by wading across the channel. For bridge sampling, weights were attached to a 2 L sampling bottle, which allowed it to be lowered through the water from the surface to the bed as it filled up with water. Thus, samples were ‘depth-integrated’ as is standard for sampling and estimating SSC (Wang and Ribberink, 1986). In the field, each 2 L sample of water was passed through a stainless steel sieve with a mesh size of 53 μm to remove suspended silt and clay particles (Stanton et al., 2019). Materials on the sieve were then washed with deionised water into glass sample bottles (with metal caps) to be transported to the laboratory. An additional 0.5 L of water sample was collected from each site on each sampling occasion to assess SSC; these 0.5 L samples were collected from the middle of the channel.

4.3.3 Sample processing

Material from each bottle was processed separately (i.e. samples were not bulked). Processing followed standard methods set out by Masura et al. (2015). To digest organic material, sample contents were treated with 20 ml each of 30% hydrogen peroxide and 0.05 M Iron (II) Sulphate solution (catalyst), and were heated to 60°C for 30 minutes. The samples were passed through the 53 μm sieve to remove silt and clay particles formed during the disaggregation of sediments during peroxide digestion (Stanton et al., 2019). Samples were then filtered through 1.2 μm glass microfiber filter papers (Whatman GF/C) attached to a vacuum pump filtration apparatus and oven-dried at 60°C for 24 hours prior to the enumeration process. Particles on the filter papers were examined and enumerated visually under a stereoscopic microscope (Leica EZ4) at 8x to 35x magnification.

To help validate counts, 85 pieces of particles identified as microplastic were picked out randomly from the processed samples and their polymer composition was verified using a Fourier transform infrared (FTIR) spectrometer (PerkinElmer Frontier). For this, each particle was scanned with 4 scans under transmission mode and a spectra range between 4000 and 400 cm^{-1} (Jung et al., 2018). The obtained spectra were then compared against known polymer references. This process provided an estimation of the accuracy of the visual identification of microplastics, and hence sample counts.

To minimise sample contamination, cotton clothing was worn during sample collection and white cotton lab coats, as well as nitrile gloves, were worn while handling the samples in the laboratory. All samples were kept covered in the laboratory. All apparatus in direct contact with the samples were rinsed thoroughly with deionised water prior to the introduction of the samples. Blank samples were collected to assess the potential contamination from atmospheric deposition from the field and the laboratory. For this, while in the field, sieves were washed with deionised water and the potential particles on the sieve were rinsed into a sample bottle. The blank samples were processed and analysed alongside the other water samples. No contamination was found.

Water samples collected for assessment of SSC were processed using the standard protocol EPA 160.2 (United States Environmental Protection Agency [USEPA], 1983). Microfiber filter papers (1.2 µm; Whatman GF/C) were first weighed, and water samples were filtered onto the respective filter papers attached to a vacuum pump. The filter papers were then oven-dried at 100°C for 24 hours and weighed again. The SSC of water samples was calculated using the following formula:

$$\mathrm{SSC} = \frac{\mathrm{M\ after} - \mathrm{M\ before}}{\mathrm{V}}$$

Where SSC = suspended sediment concentration (g/L), M = mass of filter paper (g), and V = volume of sample (L).

4.3.4 Data analysis

Microplastic concentrations are reported as particles/L. As assumptions of analysis of variance (ANOVA) were met (i.e. data were normal and homoscedastic), two-way ANOVA and Tukey's honestly significant difference (HSD) tests were used to determine whether the microplastic concentrations were significantly different between sites and sampling dates. Ordinary least squares (OLS) regression models were fitted to discharge v microplastic concentration data, and analysis of covariance (ANCOVA) was used to determine if these relations differed between sites. A locally weighted least squares regression (LOESS) was fitted to microplastic concentration data collected during the flood, to help visualise the changes during the event. Quantile regression (Koenker and Hallock, 2001) was used to understand the relations between microplastic concentration and SSC. This regression fits models to specific, user-selected quantiles. It has two main advantages over OLS regression. First, it does not have an assumption of homoscedasticity (the assumption that the error term in the dependent variable (i.e. the vertical scatter) is the same across all values of the independent variable). The microplastic and SSC data violated this assumption, so OLS could not be used. The second advantage is that quantile regression is not constrained to modelling the general (central) response of the dependent variable; it can also model the upper or lower limits of the response, as is useful in a number of environmental contexts, e.g. understanding the maximum expected value of a pollutant (dependent variable) for a given value of the independent variable. Quantiles represent lines that bound certain proportions of the data; for instance, quantile 0.9 models the line below which 90% of the sample values sit. For the Langat microplastic v SSC data, quantile regression models were fitted to the 50th quantile (0.5) to model the general trend (analogous to fitting a standard [central response] model to the data) as well as the 90th quantile to model the upper bounds of the relationship. The quantile models were used to assess whether SSC could be used as a surrogate for microplastic concentration; if so, this would suggest the possibility of using automatically logged turbidity/SSC data to produce estimates of microplastic loads in the river. All statistical analyses were carried out using RStudio (Desktop Version 1.2.5042).

4.4 Results

4.4.1 Concentration and composition of microplastics

The FTIR analysis of a subset of sample material (N=85) indicated that the accuracy of visual identification of microplastics was 96%. Microplastics were detected in every sample collected from the Langat catchment, including the uppermost site close to the dam. The mean concentration of microplastics at the mainstem sites over the 12-month period (i.e. all monthly and weekly samples) was 4.39±5.11 particles/L.

Fibres were the dominant type of microplastics in the Langat (96%), followed by fragments (3%), films (1%) and beads (<1%). Of the particles tested using FTIR, polyethylene terephthalate (PET) was predominant (43% of particles), followed by high density polyethylene (HDPE; 21%) and low density polyethylene (LDPE; 13%). Out of the 85 particles tested, 5 were non-synthetic materials, consisting of cotton, viscose and silica.

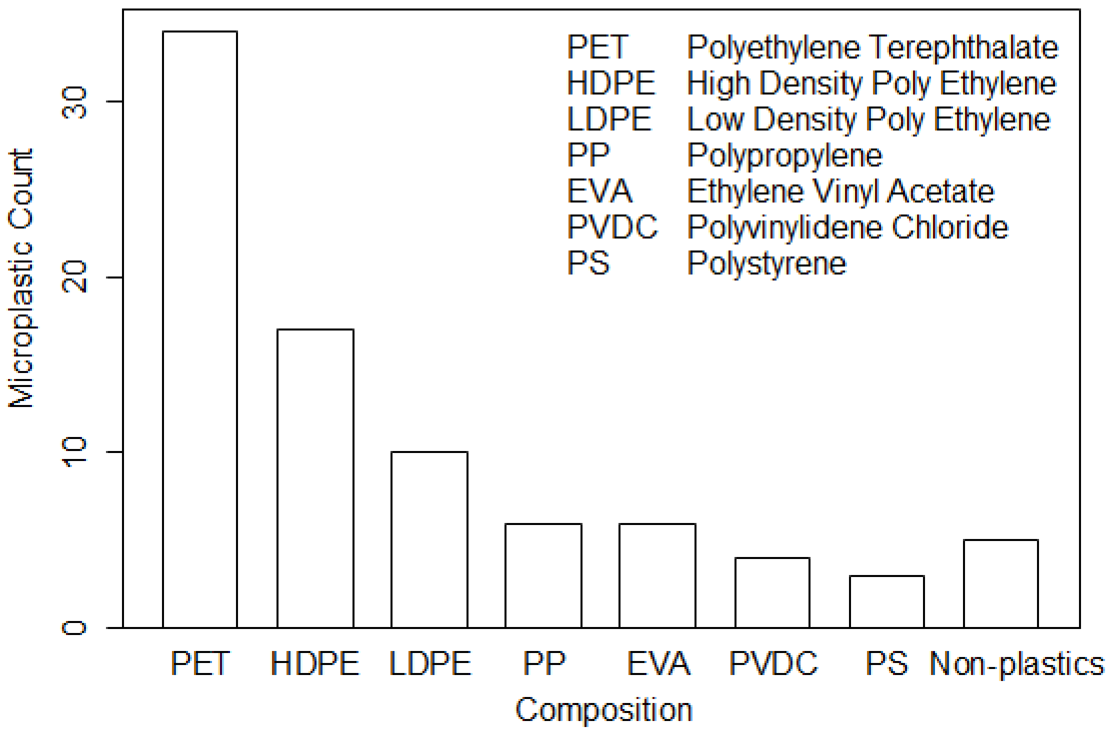

Fig 4.3 Composition of a subsample (N=85) of microplastics found in the Langat, showing polyethylene terephthalate (PET) as the most commonly found microplastic type.

4.4.2 Spatial and temporal variation of microplastics

A general increase in microplastic concentration with distance downstream was evident (Fig 4.4). For the mainstream sites that were monitored regularly, two-way ANOVA indicated significant spatial ($F=5.681$, $df=7$, $p=0.001$) and temporal ($F=25.922$, $df=11$, $p=0.001$) variation in concentrations of microplastics, although there was no interaction between site and time ($F=0.370$, $df=59$, $p=0.986$). The two most downstream mainstream sites (Sites 7 and 8) had significantly higher concentrations than all the others (Tukey HSD; $p<0.050$), while the upper-most two sites (Sites 1, 2 and 3) had significantly lower concentrations than the others (Tukey HSD; $p<0.050$; Appendix 4B); there were no significant differences between the middle sites (Tukey HSD; $p>0.050$).

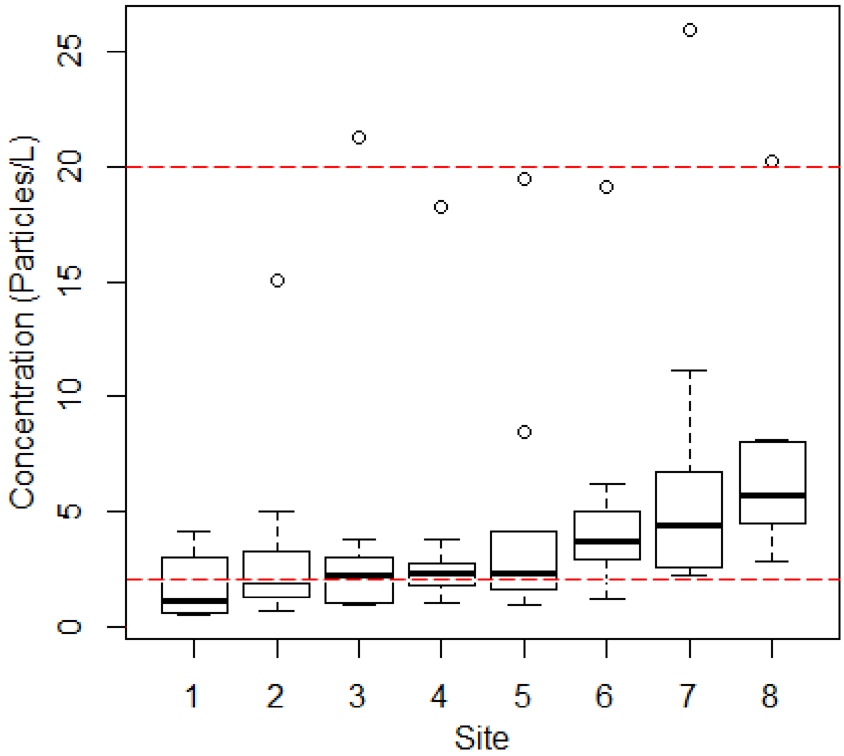

Fig 4.4 Spatial variation in microplastic concentration (particles/L). The locations of the sites are shown in Fig 4.1. The red lines indicate the minimum and maximum concentrations recorded in similar studies carried out in Asia (Yan et al., 2019; Zhang et al., 2019).

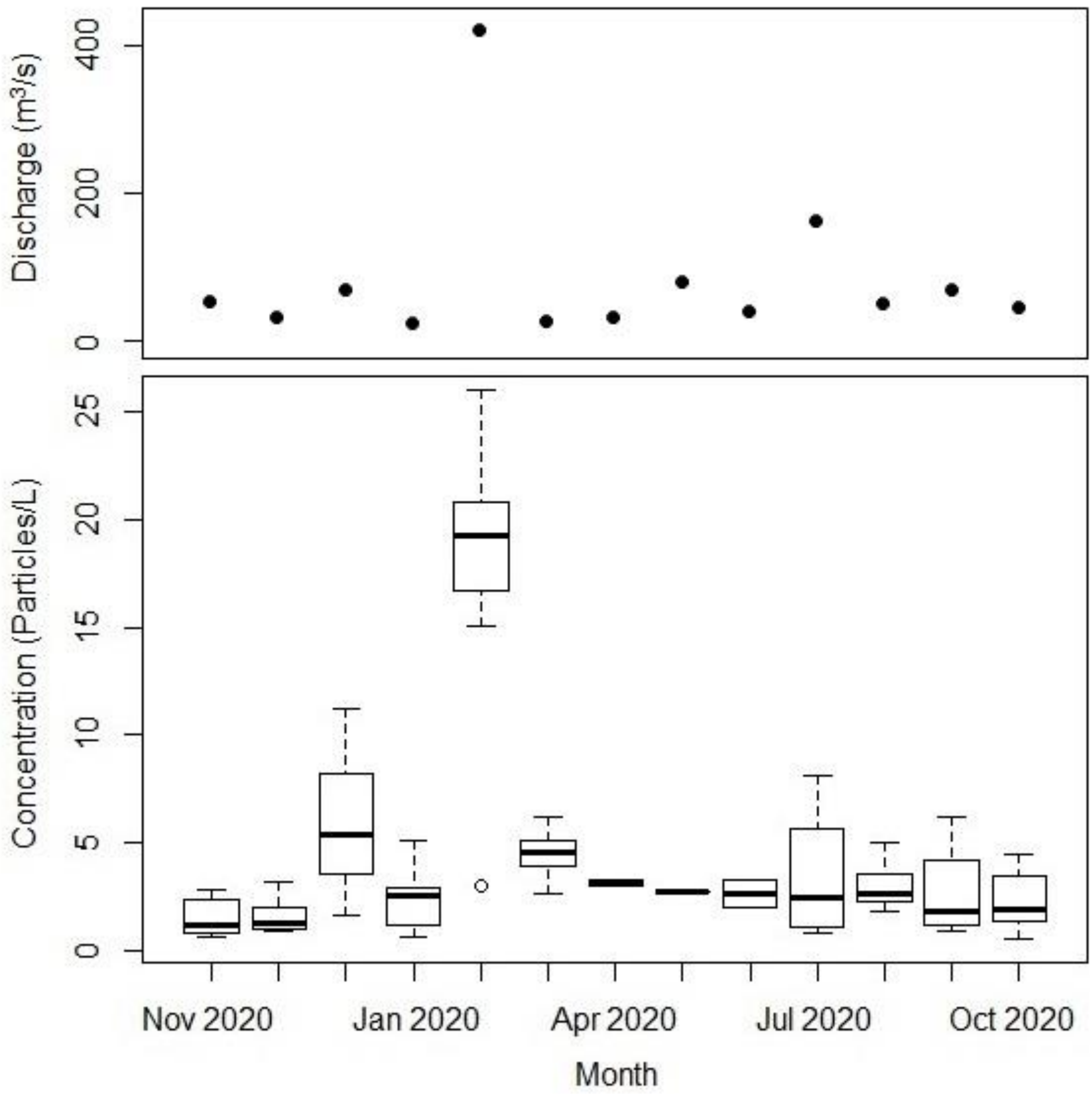

Fig 4.5 Temporal variation in microplastic concentration (particles/L) throughout the 12-month sampling period (November 2020 to October 2020) at 8 mainstream sites, along with the discharge (m^3/s) on the days of sampling (data from gauging station at Site 7). The average weekly concentration from Sites 2 and 3 was used for April to June.

For the monthly data, there were significant differences in microplastic concentration between sampling dates (ANOVA; $F=25.920$, $df=11$, $p=0.001$), but only February proved to be significantly higher than all others (Tukey HSD; $p<0.050$; Appendix 4C). Differences in microplastic concentration corresponded broadly with discharge (Fig 4.5); concentrations during lower flows averaged 1.53±0.87 particles/L but increased to 17.83±6.73 particles/L during periods of elevated discharge. No significant difference in microplastic concentration was found on a weekly timescale at Sites 2 and 3 over the April to June period (ANOVA; F=1.930, df=1, p=0.179; Appendix 4D).

4.4.3 Interactions between microplastic concentrations, discharge and suspended sediments

Fig 4.6 shows relations between discharge and microplastic concentration for the two sites for which gauged river discharge data are available. There were significant linear relationships for both sites (Site 2, p=0.001; Site 7, p=0.001) but relations differed between sites (ANCOVA; p=0.001). The discharge-concentration relationship for the lowermost mainstem site was used to produce a time-integrated assessment of microplastic loads, based on the annual discharge time series (daily flows for the one-year study period). The total loads are based on a simple conversion of particles/L to particles/m^3, and the integration of this with the gauged discharge values to show exceedance percentiles for total daily loads (Fig 4.4 and details therein). On this basis, the Langat is estimated to typically (50% of the time) transport approximately 5 billion particles per day; however, depending on discharge conditions, it can sometimes convey more than this, with approximately 30 billion particles being transported for 10% of the time (i.e. around 36 days each year).

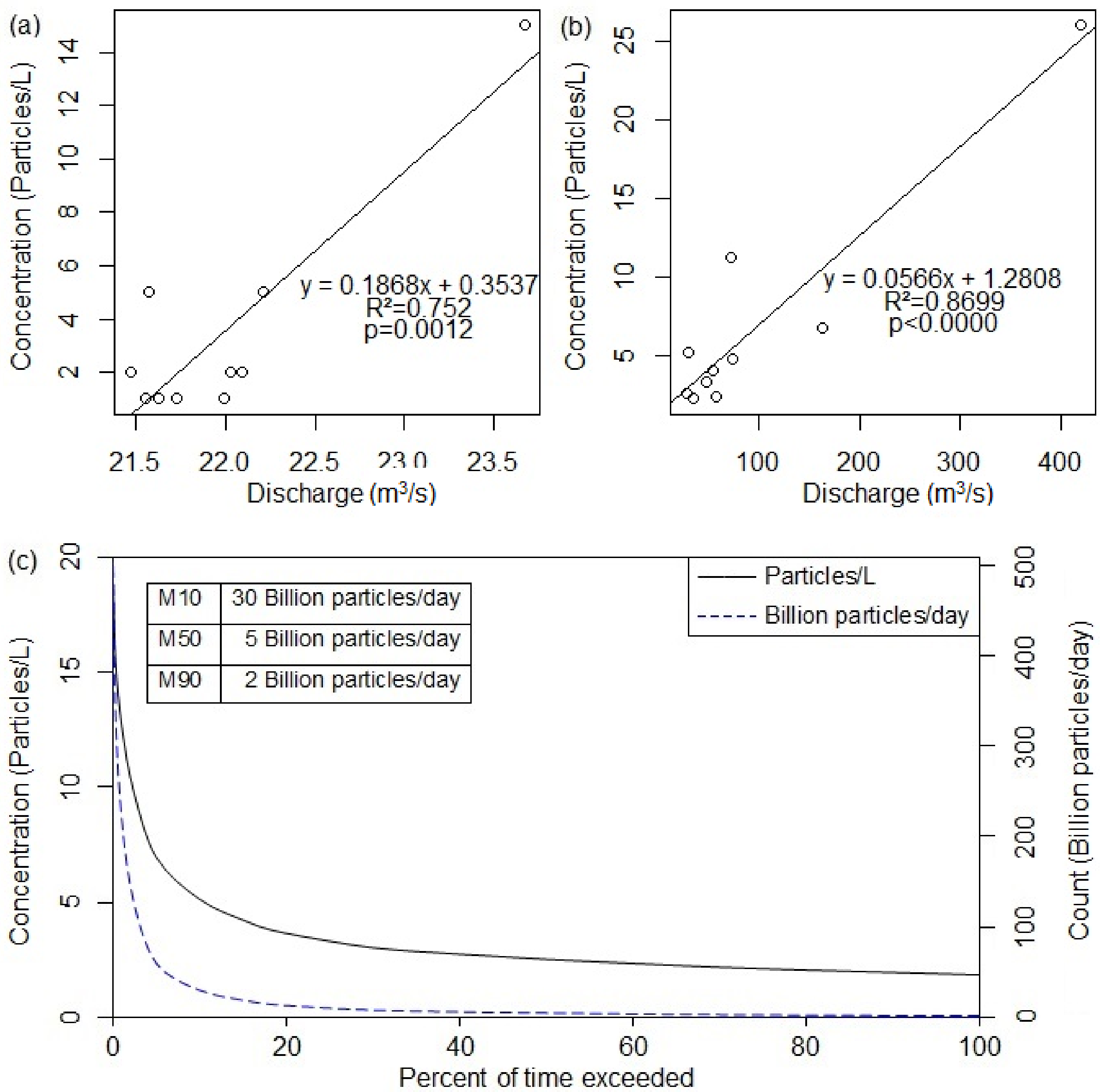

Fig 4.6 Linear models of microplastic concentration (particles/L) against discharge (m^3/s) at (a) upstream rural Site 2 and (b) downstream urban Site 7. The lower panel (c) shows time exceedance curves produced using the linear equation from (b). This equation allowed the estimation of the total number of particles in the river for a given discharge. This number was then integrated with the daily discharge data over the year to produce a times series of the total number of pieces of microplastic in the river each day. In the same way that daily discharge data are used to produce a flow duration curve that shows exceedance values for each discharge, the daily count data were used to produce exceedance curves for microplastic; they show the

percent of the time that particular concentrations or total counts are equalled or exceeded. The inset box shows some indicative microplastic exceedance percentiles; e.g. for more than 50% of the time (M50), 5 billion particles/day are being conveyed by the Langat towards the ocean.

The concentration of microplastics and discharge during the flood event at Site 4 are shown in Fig 4.7 Microplastics appeared to increase markedly in response to the flood (threefold increase), and show a broadly similar pattern to the rise and fall of discharge. A possible lag in the response was observed; this lag is discussed later (Section 4.5.2).

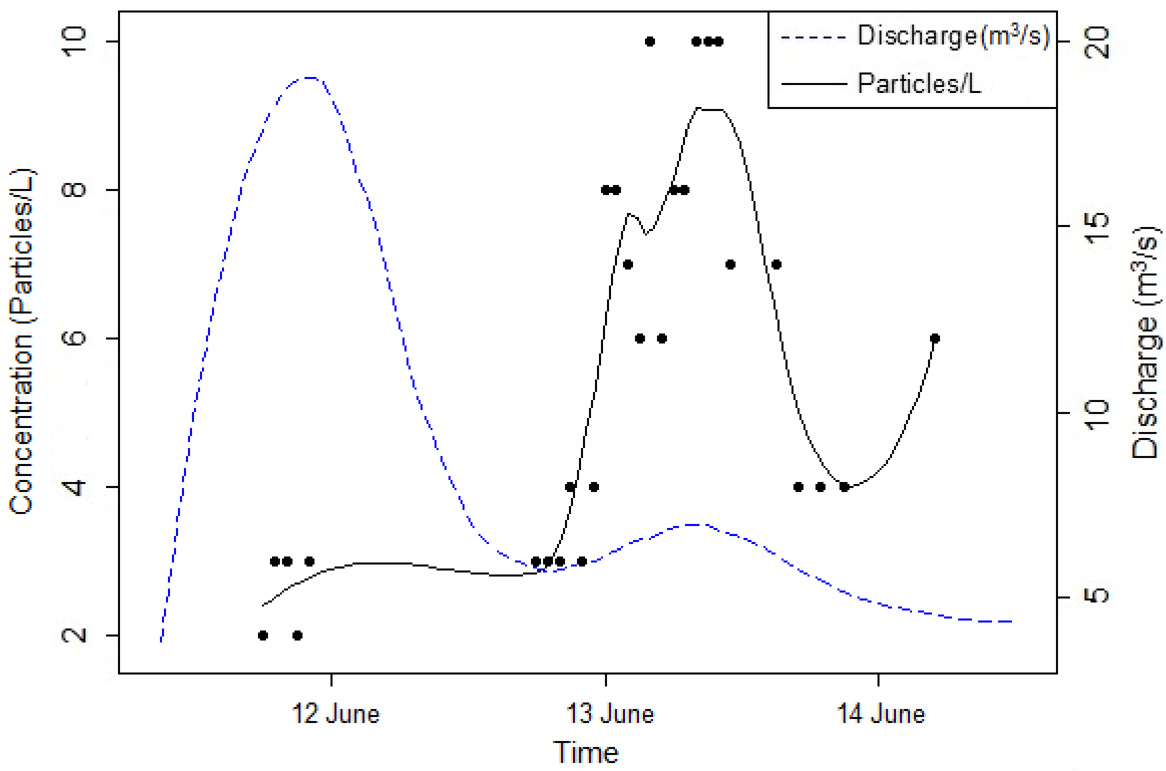

Fig 4.7 Time series of microplastic concentration (particles/L) fitted with LOESS and discharge (m^3/s) (data from gauging station at Site 7) during a flood event (Site 4; June 2020).

Integrated across the whole of the data set (i.e. all mainstem sites and dates for which flow data are available), the quantile regression indicated linear relations between the general response of microplastic to SSC (i.e. quantile 0.5; p=0.001) and the upper limit to the response (quantile 0.9; p=0.001) (Fig 4.8). The significant regression models indicate that microplastic concentrations can be predicted by SSC. However, although both were formally significant, the model for the central response had less explanatory power than that for the upper limit: model pseudo-R^2 values were 0.1 for quantile 0.5 and 0.65 for quantile 0.9. Variability in microplastic concentration was appreciable at high values of SSC, and this scatter explains the poor explanatory power of the model for quantile 0.5. The high pseudo-R^2 value for the quantile 0.9 model indicates that the maximum microplastic concentration can be predicted confidently using SSC.

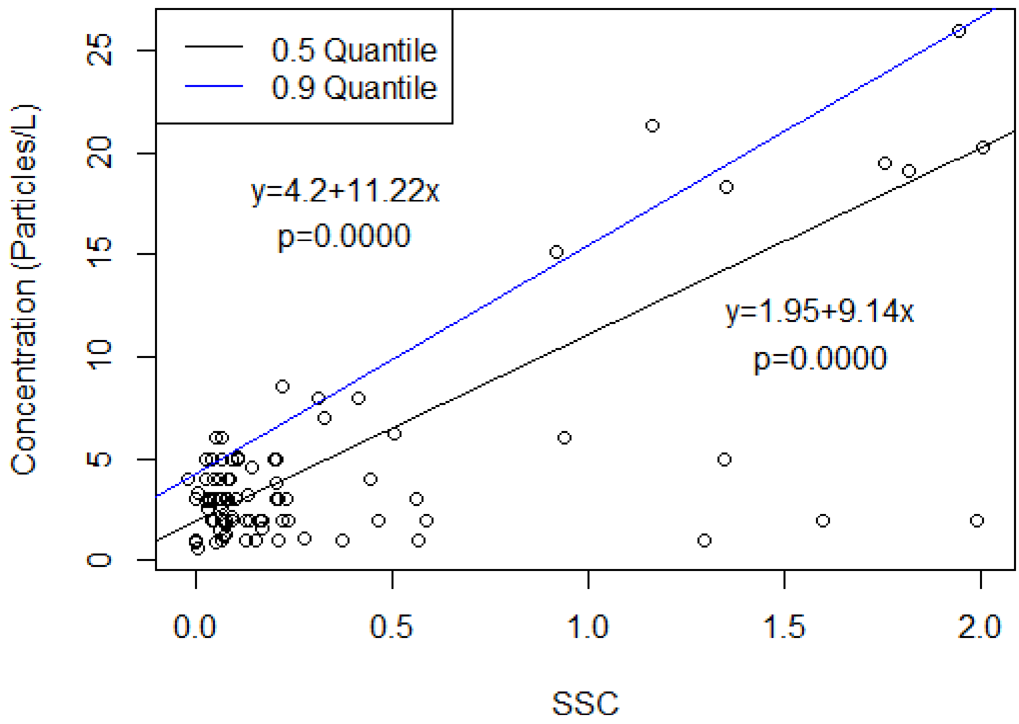

Fig 4.8 Quantile regression of the relationship between microplastic concentration (particles/L) and suspended sediment concentration (g/L), showing 0.5 and 0.9 quantiles. Data from all sampling sites and dates were used for this analysis.

4.5 Discussion

4.5.1 Concentrations and composition of microplastics

This study is one of very few detailing microplastic loads in tropical rivers, and the first looking at both spatial and temporal variation across a catchment in a tropical SEA country. The Langat is typical of many rivers draining large tropical cities in that it has experienced marked deterioration in water quality as a result of rapid and poorly controlled urban and industrial development, but with very little known about microplastics. As only three studies (Hwi et al., 2020; Pariatamby et al., 2020; Sarijan et al., 2018) have been carried out in other rivers in Malaysia to date, it is hard to give a general context for the microplastic concentrations observed in the Langat. Concentrations in the mainstem Langat appear comparatively high, at 4.39±5.11 particles/L, compared to the average concentrations of 0.000007±0.000003 particles/L (Pariatamby et al., 2020) and 0.02±0.30 particles/L (Hwi et al., 2020) reported for other Malaysian rivers, although they are less than observed in other Asian countries (e.g. compared to the Pearl River, China, where an average concentration of 19.86 particles/L has been reported; Yan et al. [2019]). These direct comparisons are possible only with caution since they are confounded by methodological differences; for instance, Pariatamby et al. (2020) used a 100 μm mesh-size sieve, while a 53 μm mesh-size sieve was used in this study. This is a perennial problem in the microplastic literature (Chen et al., 2021a).

The majority of microplastics found in the Langat were of secondary origin, i.e. material resulting from the fragmentation of larger plastic debris. They were dominated by fibres, which is consistent with other published work (Alam et al., 2019; Baldwin et al., 2016; Jiang et al., 2019; Watkins et al., 2019). The dominance of fibres may reflect the direct discharges of domestic wastewater into watercourses across the catchment, which often contain large amounts of synthetic fibres released from textile washing (Alam et al., 2019; Jiang et al., 2019). Microplastics were comprised mainly of PET, which is used in the production of fibres for textiles and the production of bottles for carbonated drinks and mineral water (Nisticò, 2020). HDPE and LDPE constituted 20% and 12%, respectively, of all the microplastics found in the Langat. HDPE and LDPE are used for the production of shampoo bottles, pipes and toys (PlasticsEurope, 2019).

4.5.2 Spatial and temporal variation of microplastics and relations with suspended sediments

Microplastic concentrations in the mainstem Langat varied spatially, increasing more than 3-fold from the relatively rural upstream parts of the catchment to heavily urbanised downstream areas. This downstream increase, along with a rural-urban transition, has been reported in other published studies (Yan et al., 2019; Zhang et al., 2020a). Changes in population density and urbanisation are recognised as contributing factors to such increases (Dikavera et al., 2019; Kataoka et al., 2019; Yan et al., 2019; Zhang et al., 2020a). Gerolin et al. (2020) is a rare example of a tropical river study, although their work focused on microplastics in sediment. They found patterns of spatial variation that reflected contributions from major urban areas as well as flow hydraulics that favoured deposition of material and its accumulation on the bed.

Interactions between flow magnitude and microplastic concentrations in water have been discussed by Rodrigues et al. (2018), Wu et al. (2020) and Chen et al. (2021a). In the Langat, temporal variation in microplastic concentrations at both monthly and hourly time steps (monthly routine and flood sampling respectively) corresponded to river discharge. The highest monthly concentration of microplastics (February) was associated with the highest discharge on days samples were collected (Fig 4.5). Similar direct relations have been reported by Hitchcock (2020) and Stanton et al. (2020). Although some authors have reported dilution of microplastic by high flows (e.g. Watkins et al., 2019), there was no evidence of this in the Langat (at the flow magnitudes included in the present analysis).

Microplastic concentrations changed markedly over the course of the flood event that occurred in June 2020; to our knowledge, this is the first time that event-scale changes in concentrations have been reported. The exact response of microplastic concentrations to rainfall and associated increases in channel discharge are likely to be location specific, reflecting the factors that influence runoff (e.g. land cover) and the rainfall itself (intensity and duration), as well as catchment size and channel dimensions. Tropical rivers are characterised by higher runoff and sediment yield per unit area than those in other climate regions (Chong et al., 2021), reflecting the intense nature of precipitation and soil characteristics. The paucity of studies means that at present, it is hard to comment on changes in microplastic observed during the flood in the Langat; this single event may not be representative of events of other magnitudes at this site, and there are few data for tropical or other rivers to allow comparison of the magnitudes and timing of changes in microplastic concentrations. The lag in the response relative to the flood peak may suggest that the material in suspension did not originate locally in the channel (it was not simply re-entrained) but came from either the catchment or upstream channel areas (Hitchcock, 2020; Ockelford et al., 2020). As the gauging station used to assess discharge was 20 km downstream from the sampling site, the time lag between discharge and microplastic concentration would be greater than indicated in Fig 4.5. As we do not have velocity data for the river during the flood, we have avoided trying to estimate the true lag time. Further studies are needed, including monitoring during flood events of different sizes, to fully understand the general responses of microplastic concentration to short-term flow change and the lag times in these responses. Until such studies are conducted, it is unwise to infer too much from the apparent lag in the response to the June 2020 event.

The significant quantile regression models for the Langat suggest that both the general trend (0.5 quantiles) and maximum (0.9 quantiles) microplastic concentrations correspond to suspended sediment. This in turn suggests that sample values of SSC or its surrogate measures (e.g. turbidity) could be used to predict microplastic concentration. The laboratory counting of microplastics in samples needed to understand temporal variation in microplastic can be prohibitive, and so may limit studies to occasional spot samples. Spot samples can miss periods of high concentration that may carry high risks, as well as introduce bias into estimates of loads delivered to oceans (Stanton et al., 2020). Smaller volumes of water are needed to estimate SSC, and samples can be processed more rapidly since only weighing is needed to determine concentration. More particularly, turbidity (an index used routinely as a measure of the amount of material in suspension) can be monitored continuously using turbidity sensors. In the same way that turbidity v SSC calibration can be used to estimate SSC and fine sediment loads (Marteau et al., 2018), relations between turbidity and microplastic could be used to produce more robust estimates of microplastic loads carried by rivers based on continuously logged turbidity values. The correspondence between SSC and microplastics reported here indicates that this is possible. The best relations were with the upper limit. This is advantageous from a risk assessment perspective because it indicates that the maximum microplastic concentration expected for a given SSC can be predicted with confidence.

4.5.3 Environmental implications

The findings of this study are useful to improve environmental risk assessments and allow the formulation of policies to reduce microplastic contamination in the Langat River. The spatial data indicate that microplastics are present in river water even in relatively undisturbed parts of the catchment (Site 1, upstream from any agriculture, significant housing developments and industrial areas). The dominance of fibres and PET in this part of the Langat, together with the absence of industries, suggest that domestic textile washing and/or aerial dispersal are key sources. In these more rural areas, control of domestic effluent is limited and many drains route water directly to small streams and culverts that flow into the Langat. It is not until further down the catchment that domestic wastewater is routed through wastewater treatment plants before being discharged to the Langat. Further down the mainstem, multiple sources likely contribute to the higher concentrations observed, notably, inputs from domestic textile washing and tributaries that drain small industrial areas where many clothing and textile manufacturers are located. These areas could be targeted for better wastewater disposal practices.

The estimates of the load conveyed by the Langat towards the coast (around 5 billion particles per day for half of the days each year) are extremely high compared to existing published estimates. For example, the Ebro River, Spain, delivers 2.2 billion microplastic particles every year to the Mediterranean (Simon-Sánchez et al., 2019) and the Pearl River, China, is estimated to deliver 39 billion microplastic particles every year to the ocean (Mai et al., 2019). However, such estimates are so scarce in the literature that it is hard to say how representative the Langat is for rivers in rapidly growing SEA cities; it may be that the river is unusually badly polluted by microplastic, or it may rather be typical of urban rivers in the region. Other sources further downstream from site 7 likely deliver more microplastic to the mainstream river and potentially increase loads coveted towards the ocean. However, because of changing flow hydraulics and bidirectional flows in the tidal section of the river and its estuary, there are likely to be complex spatio-temporal patterns of settlement (Hale et al., 2020). Thus, our estimates are best seen as providing data on daily loads conveyed to the coastal zone, rather than into the ocean.

The average concentration of 4.39±5.11 particles/L in the Langat may not pose a significant acute toxicity threat to river organisms. Although microplastics have been reported to cause effects such as disturbed energy metabolism in *Danio rerio* (Lu et al., 2016) and decreased assimilation efficiency in *Gammarus fossarum* (Blarer and Burkhardt-Holm, 2016), the microplastic concentration in which organisms were subjected to in these experiments was very high (2.90×10^8 particles/L). Nevertheless, there may be chronic risks associated with long-term exposure to concentrations observed in some parts of the Langat, as reported for *Pomatoschistus microps* and *Daphnia magna* (Fonte et al., 2016; Pacheco et al., 2018). In general, more work is needed to assess the ecological and human health risks of microplastics (Chen et al., 2021a), and a useful next step in ecotoxicological studies would be to assess the acute and chronic effects of the concentrations found in rivers such as the Langat, rather than the unrealistically high concentrations tested in most studies.

4.6 Conclusion

Microplastics were present at all sites in the Langat River on all dates sampled. The upper catchment area of the Langat is largely forested, so the most likely cause of contamination here is domestic textile washing from rural settlements and/or atmospheric fallout. Concentrations generally increased in the downstream direction, reflecting changes in land cover (increasing urban and industrial areas). The high concentrations in the lowermost sites suggest the need for management designed to better control wastewater discharges from textile and other industries located here. The marked temporal variation in concentrations indicates the need for repeat surveys when assessing loads, especially to include samples collected at different discharges. The correspondence between microplastic and suspended sediment concentrations suggests that continuously logging turbidity sensors could be used to improve load and risk assessments.

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4.8 Appendices

Appendix 4A

Key characteristics of study sites.

Site	Coordinates		Distance from dam (km)	Altitude (m)	Channel width (m)
1	3°03'34.63"N	101°52’24.48”E	2.09	69	7.94
2	2°57’31.11”N	101°50’56.64”E	13.61	35	10.94
3	2°56’35.85”N	101°50’46.62”E	18.42	29	13.86
4	2°54’14.02”N	101°48’31.74”E	22.89	21	15.19
5	2°53’46.87”N	101°46’28.82”E	29.53	15	34.06
6	2°43’48.57”N	101°43’36.01”E	35.49	10	36.87
7	2°51’19.58”N	101°40’53.17”E	44.90	6	37.24
8	2°48’47.65”N	101°38’32.22”E	52.45	3	33.67

Appendix 4B

P-values of Tukey's HSD Test of 2-way ANOVA of microplastic concentrations between sampling sites.

	1	2	3	4	5	6	7	8
1		0.809	0.639	0.551	0.175	0.053	0.003	0.001
2	0.809		1.000	0.999	0.906	0.618	0.016	0.011
3	0.639	1.000		1.000	0.975	0.792	0.036	0.026
4	0.551	0.999	1.000		0.997	0.930	0.107	0.082
5	0.175	0.906	0.975	0.997		0.999	0.406	0.340
6	0.053	0.618	0.792	0.930	0.999		0.733	0.662
7	0.003	0.016	0.036	0.107	0.406	0.733		1.000
8	0.001	0.011	0.026	0.082	0.340	0.662	1.000	

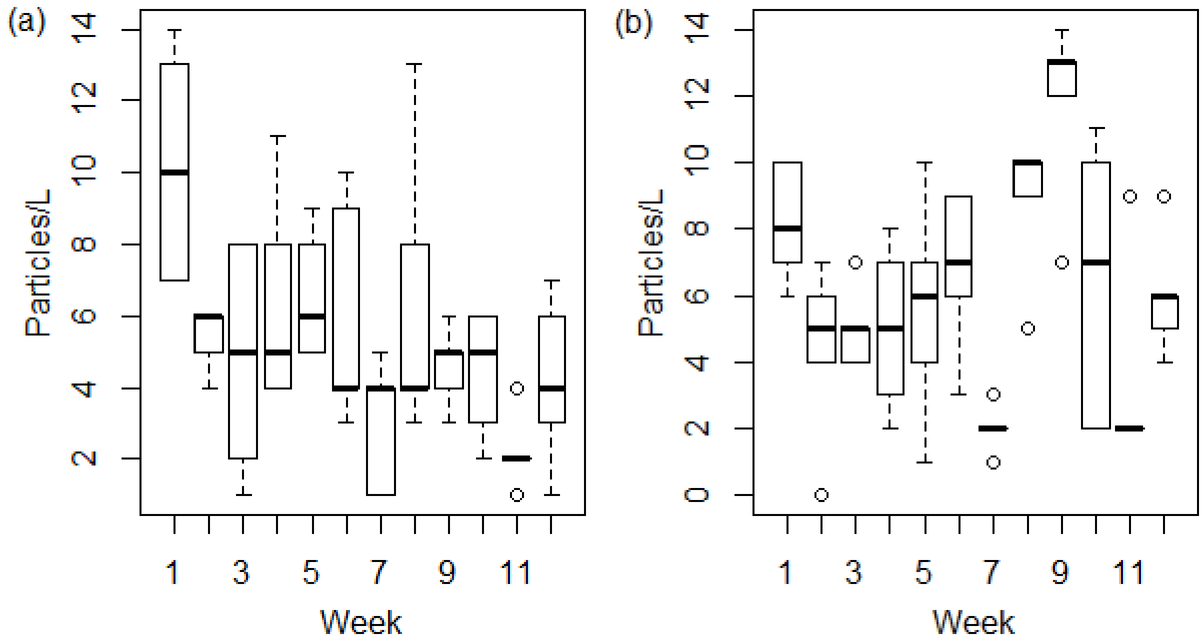
Appendix 4C

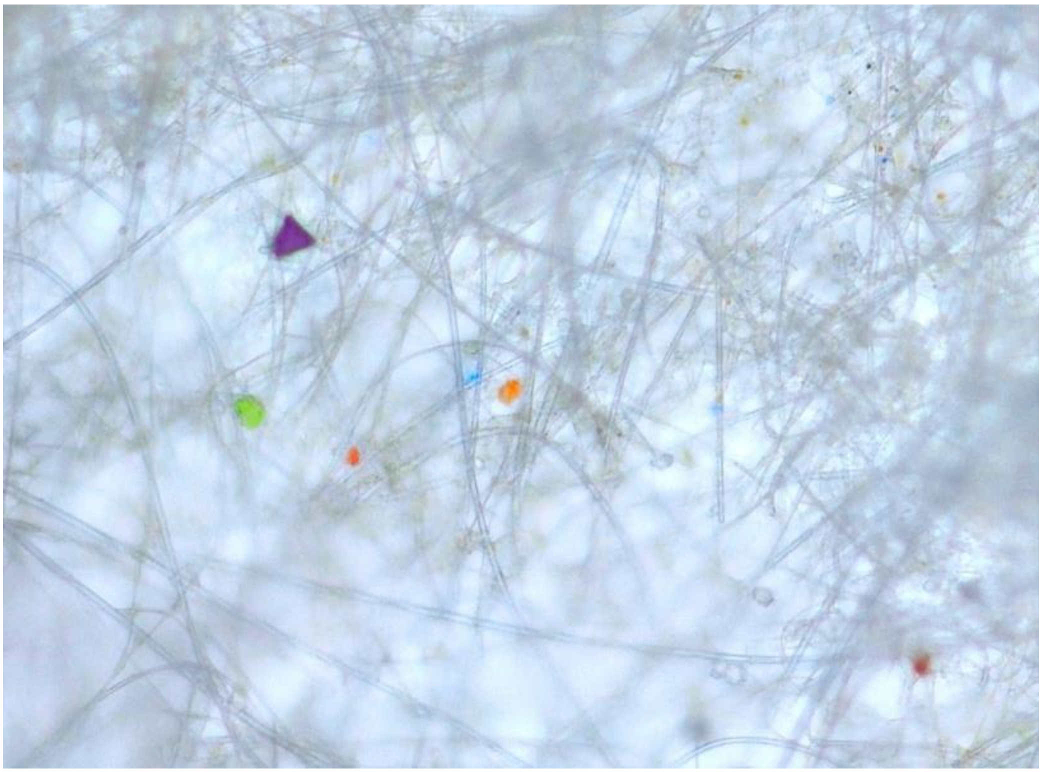
P-values of Tukey's HSD Test of 2-way ANOVA of microplastic concentrations between sampling dates.

	1	2	3	4	5	6	7	8	9	10	11	12
1		0.001	0.825	0.998	0.999	0.999	0.999	1.000	1.000	1.000	0.999	0.974
2	0.001		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
3	0.825	0.001		1.000	1.000	1.000	0.999	0.978	0.931	0.802	0.365	0.999
4	0.998	0.001	1.000		1.000	1.000	1.000	1.000	0.999	0.997	0.957	1.000
5	0.999	0.001	1.000	1.000		1.000	1.000	1.000	1.000	0.999	0.986	1.000
6	0.999	0.001	1.000	1.000	1.000		1.000	1.000	1.000	0.999	0.991	1.000
7	0.999	0.001	0.999	1.000	1.000	1.000		1.000	1.000	0.999	0.903	1.000
8	1.000	0.001	0.978	1.000	1.000	1.000	1.000		1.000	1.000	0.987	0.999
9	1.000	0.001	0.931	0.999	1.000	1.000	1.000	1.000		1.000	0.998	0.997
10	1.000	0.001	0.802	0.997	0.999	0.999	0.999	1.000	1.000		0.999	0.996
11	0.999	0.001	0.365	0.957	0.986	0.991	0.903	0.987	0.998	0.999		0.559
12	0.974	0.001	0.999	1.000	1.000	1.000	1.000	0.999	0.997	0.996	0.559	

Appendix 4D

Weekly temporal variation of microplastic concentration (particles/L) in Site 2 (a) and Site 3 (b). Weekly samples were collected from April 2020 to June 2020. No significant difference between weeks was observed (p=0.179).





5

Microplastic concentrations in river water and bed sediments in a tropical river: implications for water quality monitoring and ecological status

5.1 Abstract

The recent increase in awareness of the extent of microplastic contamination in marine and freshwaters has heightened concern over the ecological and human health risks of this material. Assessing risks posed by microplastic in freshwater systems requires sampling to establish contamination levels, but standard sampling protocols have yet to be established. An important question is whether sampling and assessment should focus on microplastic concentrations in the water or the amount of microplastic deposited on the bed. In this study, the relationship between microplastic contamination in river water and on the surface of bed sediments was assessed. On three dates, five replicated water and bed sediment samples were collected from eight sites along the upper reach of the Semenyih River, Malaysia. Microplastics were found in all 160 samples, with mean concentrations of 3.12±2.49 particles/L in water and 6027.39±16585.87 particles/m^2 deposited on the surface of riverbed sediments. Within-site variability in microplastic was high for both water and bed sediments, and very often greater than between-site variability. Integrated across sampling dates, there were significant between-site differences in the amount of plastic on the bed, but not the concentration in river water. The amount of microplastic on the bed did not correspond to the concentration in the water either at the site scale or the patch scale. Patterns suggest that microplastic accumulation on the bed is spatially variable, and single samples are therefore inadequate for assessing bed contamination levels at a site and consequently the risk posed to benthic invertebrates. Sites with the highest mean concentrations in samples of water were not those with the highest concentrations on the bed, indicating that spot sampling of water will not provide a reliable indication of the likely levels of microplastic deposited on the bed. River and water quality monitoring based only on water samples may not provide a good picture of either relative or absolute bed contamination levels.

5.2 Introduction

Many countries have water quality standards designed to protect human health and aquatic ecosystems. These standards are often then used to set consents and licences for industrial activities that discharge wastewater into rivers (e.g. EU Urban Waste Water Directive [European Commission, 2022], US Effluent Guidelines [United States Environmental Protection Agency, 2022] and Japanese National Effluent Standards [Government of Japan, 2015]). A common practice for water quality assessment is to establish some form of water quality index (WQI) which uses threshold values of several determinants to assess overall water quality (Andrade Costa et al., 2020; Uddin et al., 2021). Indices most often use levels of chemical and biological oxygen demand, dissolved oxygen, pH and temperature. Water quality assessment also embraces fine sediment, most commonly by including turbidity or suspended solids in the WQI. Some biomonitoring tools and indices incorporate sensitivity to fine sediment by linking the index to the amount of fine material on the bed rather than in the water column (e.g. the PSI Index; Turley et al., 2016).

Microplastic pollution in streams and rivers has only recently attracted attention. However, microplastics (typically defined as plastics smaller than 5 mm in size (Lenaker et al., [2019]) are now recognised as being omnipresent in the environment, recorded in all continents and all types of ecosystems (Chen et al., 2021a). In rivers, microplastics are present within the water column (suspended plastics) but are also deposited on the bed as a function of hydraulic conditions (Chen et al., 2021; Hurley et al., 2018). With the increasing observation of microplastics in the tissues and lungs of aquatic mammals, invertebrates and fish (Prokić et al., 2019), numerous studies have argued that the main ecological risk comes from microplastics deposited on the bed rather than the amount in suspension (Wang et al., 2019). This is because bed sediments are where many benthic organisms spend most of their time, and most importantly, where they feed. For example, many invertebrates ingest fine particulate organic material from the bed, and when feeding they may also ingest deposited microplastics (Wang et al., 2019). Therefore, from an ecological perspective, assessing the risks posed by microplastics requires information on the amount of material deposited on the bed. Most studies, however, focus on the contamination of river water rather than the amount of material that has settled on the bed (Lenaker et al., 2019; Yang et al., 2021).

The prevalence and significance of microplastic contamination suggest that it should be included in WQIs. Using a measure of microplastic in the water within such an index may be of limited value because both the total amount and suspended concentration depend on river flows and vary markedly over short timescales (e.g. during individual hydrological events; Chen et al., 2021b), leading to potential misrepresentation of water quality from spot sampling of water alone. Several studies have looked at microplastics in both river water and sediments (e.g. Ding et al. [2019], Huang et al. [2021] and Scherer et al. [2020]). These authors reported that microplastic concentrations in water do not correspond directly with those in sediments at the same sites (Huang et al., 2021; Ding et al., 2019), suggesting that sampling solely from water may not capture or indicate the ecologically important issue of how much microplastic is deposited on the bed. However, these studies did not assess the relationship statistically (i.e. using correlative-type analyses), but rather only compared their water and sediment concentrations with other published studies.

Developing reliable water quality monitoring programs is particularly crucial in the rapidly developing South-east Asian (SEA) region where urban expansion has outpaced the capacity of waste management systems and technologies to deal with the wastes produced. For instance, Malaysia has a WQI but this does not consider microplastic (Department of Environment Malaysia [DOE], 2021). Data for the Semenyih and mainstem Langat River presented in earlier chapters suggests that Malaysian rivers may be badly contaminated with microplastic. However, apart from the work presented in this thesis, data to assess this are scarce; only a handful of studies have focused on the occurrence of microplastics in river systems (e.g. Sarijan et al. [2018], Hwi et al. [2020] and Pariatamby et al. [2020]). Notably, only one study (Sarijan et al., 2018) quantified bed concentrations, so it is hard to assess whether the values reported by these authors for their one study site are typical of others across the country.

The objectives of this chapter are: (1) to assess levels of microplastic contamination in bed sediments at multiple sites in a typical Malaysian river, (2) to assess the correlation between microplastic concentrations on the bed and those in the water column, and (3) to provide recommendations for the most appropriate way to monitor microplastic contamination in rivers, particularly to assess ecological risks. The work was undertaken in the Semenyih River, which previous studies have shown received inputs of microplastic from multiple sources (Chen et al., 2022).

5.3 Methods

5.3.1 Study area

Located on the Southeastern border of Selangor, Malaysia, the Semenyih River serves as one of the main rivers for potable water supply in the Hulu Langat District of Greater Kuala Lumpur. The Semenyih catchment is one of the two upper sub-basins of the Langat catchment; the two join to form the mainstem Langat River on the southern edge of Greater Kuala Lumpur. The Semenyih River is regulated by the Semenyih Dam, which has a storage capacity of 33.6 MCM (Selangor Water Management Authority [SWMA], 2019), and flows from largely forested headwater areas to an increasingly urbanised town of Semenyih. The Semenyih has a mean annual rainfall of 2309 mm and a mean annual flood of 67.3 m^3/s (Sungai Rinching gauging station; Fig 5.1a) (Department of Irrigation and Drainage Malaysia [DID], 2018).

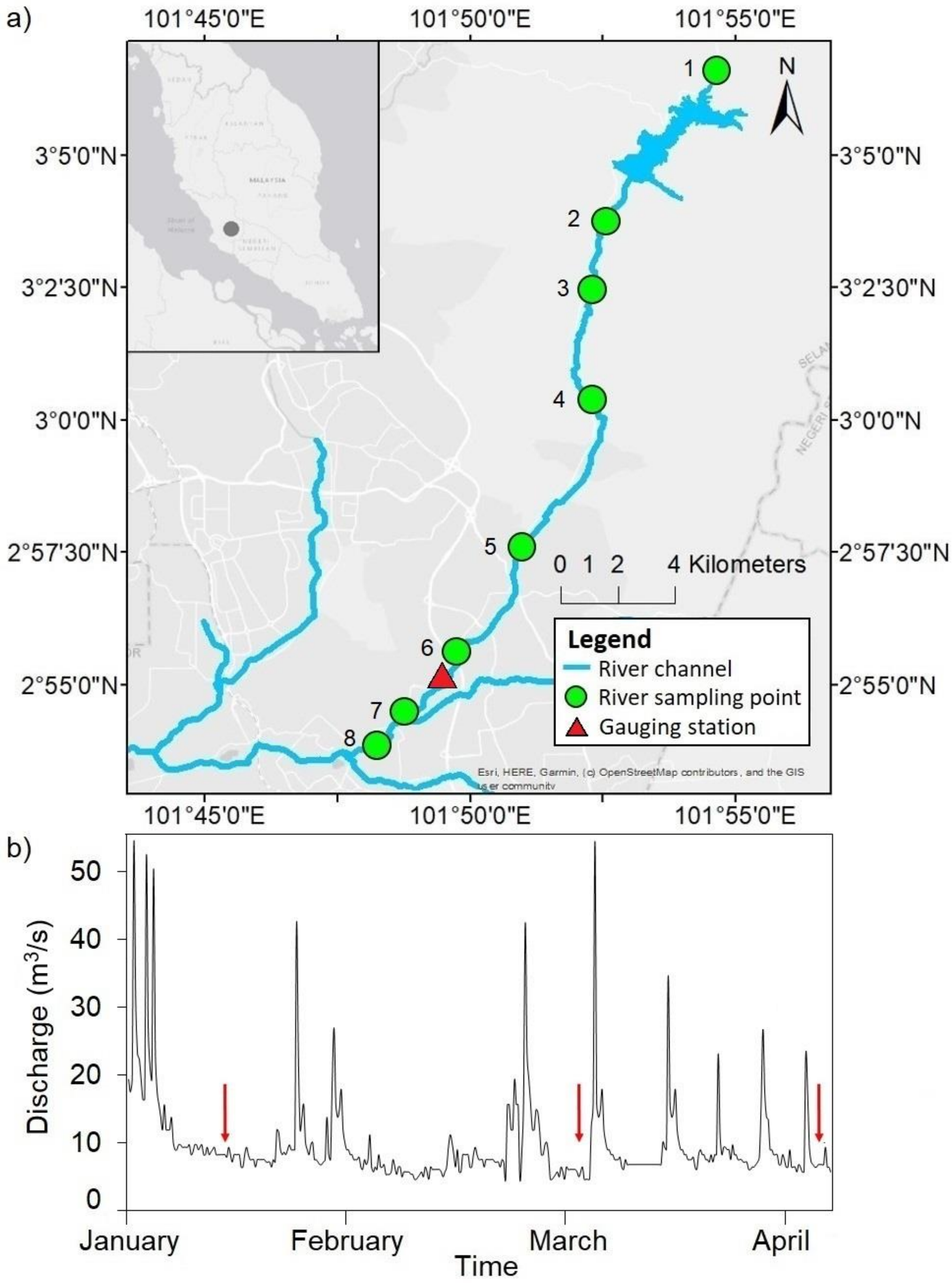


Fig 5.1 a) Map of the study area and sites and b) discharge (m^3/s) of the Semenyih River throughout the sampling period with red arrows showing the sampling occasions.

This study was carried out in the upper to mid-section of the Semenyih River (Fig 5.1a). A total of eight sites were distributed along this 30 km section. Based on the work presented in earlier chapters, this allowed the inclusion of sites with a wide range of microplastic contamination levels. The key characteristics of the sites are detailed in Appendix 5A. These sites were deliberately chosen to allow the collection of both surface water samples and sediment samples, i.e. accessibility and suitability for wading in the channel for the collection of sediment samples were key factors affecting site choice. At each site, samples were deliberately collected from points with different hydraulic conditions (i.e. from pools and riffles). At each site, samples were collected from five points, with an estimate of microplastic in the water and deposited on the bed derived for each point. In total, 80 water and 80 bed sediment samples were collected.

5.3.2 Sample collection

Samples were collected on three occasions (Fig 5.1b) between January and April 2022. Bed microplastic samples were collected using a technique that is widely used for measuring fine sediment deposited on the riverbed surface (Lambert and Walling, 1988) and has recently been applied to assess microplastic (Hurley et al., 2018). For this, a known volume of water was enclosed by pushing an open-ended cylinder (approx. 0.5 m diameter and 1 m tall) into the riverbed. The riverbed within the enclosed area was then agitated vigorously using a stick, to resuspend fine material (mineral and organic matter, and any microplastic) deposited on the bed (Appendix 5C). Once the material was suspended, a 1 L sample of water was taken from the enclosed area. A 2 L sample of water was simultaneously taken from the water column immediately outside of the enclosed area to assess the amount of plastic in the river water. All water samples were passed through a 53 μm stainless steel sieve to remove suspended silt and clay particles, and materials remaining on the sieve were washed into glass sample bottles to be transported to the laboratory.

5.3.3 Sample processing

All water samples were treated with 20 ml each of 30% hydrogen peroxide and 0.05 M Iron (II) Sulphate solution as a catalyst and heated to 60°C for 30 minutes to digest organic materials in the samples. The samples were then filtered through 0.7 μm glass microfibre filters (Whatman GF/F) and oven-dried for 24 hours at 60°C. Particles on the filters were examined and enumerated under a stereoscopic microscope (Nikon SMZ1500) at 4x to 180x magnification. All microplastic particles were classified as either fibres, fragments, films or beads.

Similar methods were used for bed samples, except for an additional step for density separation before filtering the samples onto filter papers; this step was necessary because of the high fine sediment content of these samples. For this, the contents in the beakers were sieved through the 53 μm mesh-size sieve to remove all liquid after peroxide digestion. Using saturated zinc chloride solution, contents on the sieve were then washed into 50 ml falcon tubes and placed in a centrifuge (Eppendorf 5810R) with 10,000 rpm at room temperature (25°C) for 10 minutes. The supernatant was then extracted using a micropipette and processed the same way as surface water samples.

When enumerating bed concentrations, the respective water sample also acted as a blank, and so was subtracted from the concentration recorded inside the cylinder. The number of particles of microplastic per unit area of bed was calculated using the following formulae (adapted from Lambert and Walling, 1988):

①

$$V = \frac{1}{3}\pi\,(R^2 + r^2 + Rr)\,h$$

Where V = volume of water in the cylinder (cm^3), h = depth of water in the cylinder (cm), R = radius of the upper base of the cylinder (cm), and r = radius of the lower base of the cylinder (cm).

②

$$A = \pi r^2 h$$

Where A = area of enclosed riverbed (m^2), r = radius of the lower base of the cylinder (m) and h = height of the cylinder (m).

③

$$MP_S = \frac{V\,(\text{N sediment} - \text{N water})}{A}$$

Where MP_S = microplastic count (particles/m^2), V = volume of water in cylinder (L), N = number of microplastic particles, and A = area of enclosed riverbed (m^2).

To minimise contamination of samples, field and lab blanks were collected to assess the potential contamination from atmospheric deposition from the field and the laboratory. For this, while in the field, sieves were washed with deionised water and the potential particles on the sieve were rinsed into a sample bottle. The blank samples were processed and analysed alongside the other water samples. Any microplastics found in the blanks were subtracted from the total microplastic counts.

5.3.4 Data analysis

Microplastic concentrations from surface water are reported as particles/L, while the amount deposited on the bed is reported as the number of particles/m^2. As the data were heteroscedastic and non-normal, Kruskal-Wallis tests were used to assess whether microplastic concentrations differed significantly between sites. Values of the sum of squares within (SSW) and the sum of squares between (SSB) were used to quantify within-site and between-site variability in microplastic concentrations in water and the amount of material deposited on the bed. SSW and SSB values were obtained from an analysis of variance (ANOVA). A generalised linear mixed model (GLMM) was used to examine the relationship between microplastic concentrations in surface water and bed sediments, with sampling sites used as the fixed effect and sampling dates as the random effect. Spearman's rank correlation was used to examine the relationship between microplastic concentrations in surface water and bed sediments at the site scale (microplastic concentrations were averaged within each site). Statistical analyses were carried out using RStudio (Desktop Version 2022.07.1) and IBM SPSS Statistics (Version 28.0.0.0).

5.4 Results

5.4.1 Microplastics in surface water and bed sediments

Microplastics were found in all 160 samples collected from the Semenyih River. Across all sites and dates, mean concentrations of microplastics were 3.12±2.49 particles/L in water and 6027.39±16585.87 particles/m^2 on the riverbed. Fibres were the dominant type in both water (97%) and sediment (92%) samples, followed by fragments, which were detected more frequently in sediment samples (6%) compared to water samples (3%). Films and beads were scarce in both water and sediment samples (<1%). At Sites 1 and 4, 100% of the microplastics recovered from surface water were fibres.

5.4.2 Spatial variation of microplastics

Although the medians differed, there were no significant between-site differences in microplastic concentration in the water (Kruskal-Wallis; $H=10.076$, $df=7$, $p=0.184$; Fig 5.2a). The highest median values were found at the uppermost site (4.9±6.74 particles/L) and the most downstream one (4.9±3.75 particles/L). There was a clearer pattern in the amount of microplastic deposited on the bed, with a general downstream increase (Fig 5.2b) and significant Kruskal-Wallis test results (H=29.708, df=7, p=0.001). Unlike in water, the amount of material on the bed at Site 2 (7091.38±9078.82 particles/m^2) was significantly greater than at Site 1 (357.13±376.41 particles/m^2) (Appendix 5B). Sites 6 and 8 had significantly greater amounts of microplastic on the bed than site 1, while Site 6 is also significantly different from Site 3 (Appendix 5B). All others are not significantly different from each other.

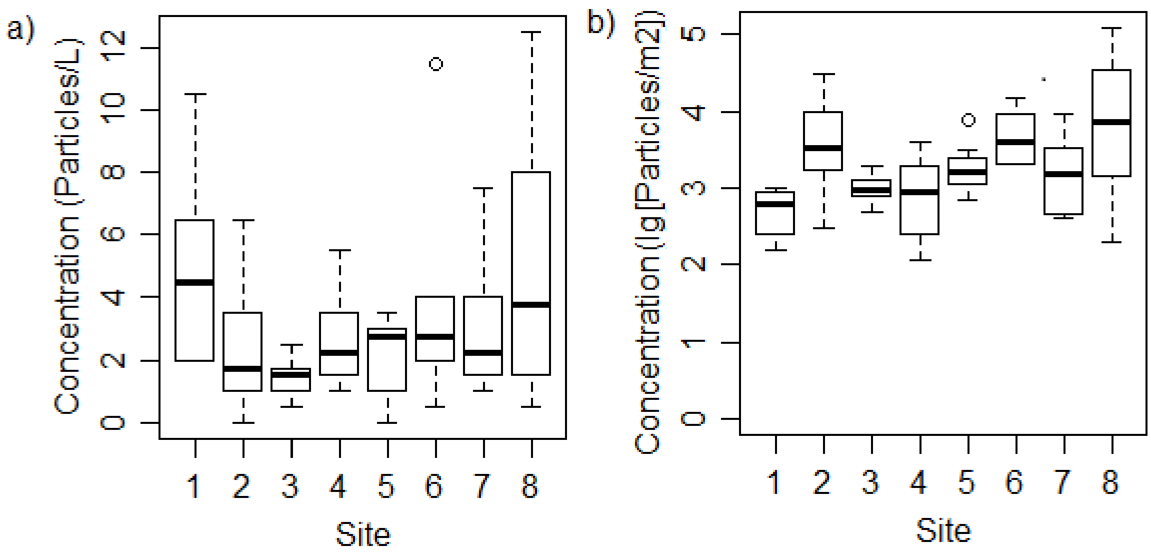


Fig 5.2 a) Spatial variation in microplastic concentrations in river water (particles/L) and b) the amount of material deposited on the bed at study sites in the Semenyih River. Note that bed data are shown on the $\log_{10}$ scale (lg [particles/m^2]).

5.4.3 Variability in microplastic concentrations

For sampling occasions 1 and 3, most of the variability in microplastic concentrations in the water resulted from within-site differences (Fig 5.3); for sampling occasion 2, most variability resulted from between-site differences, but within-site variability was appreciable (39%). Most of the variability in the amount of microplastic deposited on the bed resulted from differences between the patches (within-site variability) sampled on two of the three occasions, with within- and between-site variability apportioned equally on the first sampling occasion.

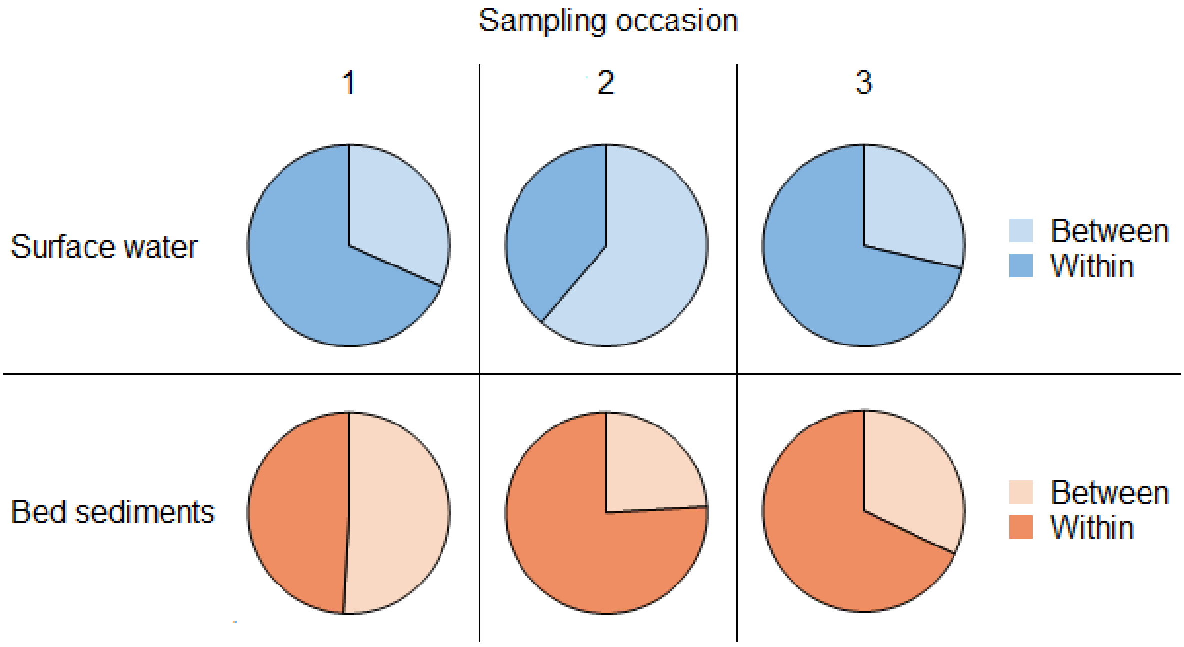


Fig 5.3 Variability of microplastic concentrations between and within sites during individual sampling occasions. Each pie chart shows the variability apportioned between within and between sites. Charts are based on SSW and SSB values derived from the analysis of variance.

Fig 5.4 shows the water concentrations and values for corresponding bed locations for all three sampling occasions; the inset shows the bed values on a log scale, to help visualisation of between-sample differences at lower values. Overall, the GLMM fitted to the raw data was not significant (p=0.941), indicating that at the scale of individual sampling patches, there was no relationship between the amount of material deposited on the bed and the concentration in the water column above. The intercept was significant (p=0.006), indicating that microplastics can be expected to be present on the bed when concentrations in water are zero.

Using site-averaged values, there was no relationship between water and bed concentrations (Spearman's rank correlation; ρ=0.310, p=0.456). Thus, sites with higher concentrations in water were not always those with higher amounts of microplastic deposited on the bed.

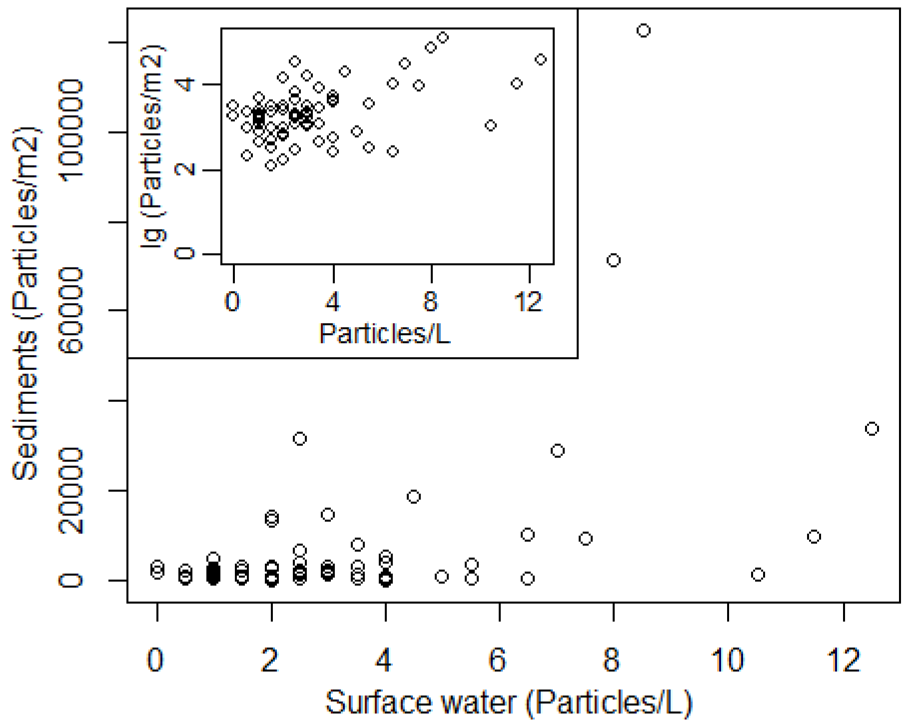


Fig 5.4 Microplastic concentration in sediments (particles/m^2) against surface water (particles/L). The inset shows a plot with log values of sediment concentrations to help with visualising the patterns.

5.5 Discussion

5.5.1 Concentrations and types of microplastics

This study is one of the first to elucidate the relationship between microplastics in river water and sediments in a direct statistical way. Chen et al. (2022) reported higher average concentrations for the Semenyih River (4.39±5.11 particles/L) than reported in the present chapter, but their work extended to more downstream areas that included much more urban and industrialised parts of the catchment. Only Sarijan et al., (2018) have assessed bed contamination in a Malaysian river, but they collected bulk samples of sediment to estimate the weight of microplastic per unit volume of bed material. Thus, direct comparisons are not possible. In other geographical regions, authors have reported concentrations ranging from 1800 to 30000 particles/m^2 in sediments of the Rhine River, Germany (Klein et al., 2015), 0 to 517142 particles/m^2 in sediments of the Mersey River, England (Hurley et al., 2018) and an average of 37311 particles/m^2 in sediments of the Nakdong River, South Korea (Eo et al., 2019). The average microplastic concentration on the bed of the Semenyih was 6027.39±16585.87 particles/m^2, but this rose to 122,668 particles/m^2 in places. The differences likely reflect the relatively rural setting of many of the Semenyih sites, as well as the fact that for much of the study section (Sites 1 to 4), the Semenyih is an upland river. In particular, the river has a very flashy hydrological regime, and high flow events occurred shortly before each of the sampling occasions. These flows may have caused a washout of microplastics deposited on the bed (as par with Hurley et al, 2019); the amount of material deposited on the bed may be elevated during protracted periods of lower and more stable flows.

In both river water and bed sediments, microfibres were the dominant type of microplastic found, consistent with other published studies (Alam et al., 2019; Peng et al., 2017). These inputs can be traced back to the domestic washing of synthetic textiles, where domestic greywater is discharged directly into drains, which, in turn, are channelled directly into the Semenyih River (Chen et al., 2022). This finding is also consistent with other published studies, where authors have traced microfibres back to domestic textile washing (Blair et al., 2019; Jiang et al., 2019). The higher proportion of fragments in bed sediments compared to surface water can be explained by their relatively larger sizes and higher densities, as larger and denser microplastics have a greater tendency to settle out from suspension (Nizzetto et al., 2016).

5.5.2 Spatial variation of microplastics

The average microplastic concentration in water was high at Site 1 (4.6±2.59 particles/L), and second only to Site 8 (4.9±3.75 particles/L). This high concentration for Site 1 was unexpected, as this is the most upstream and remote of the locations sampled. Nevertheless, some small informal dwellings are located close to the river channel immediately upstream from Site 1 and our field observations indicate that greywater from domestic washing of clothes flows from here directly to the river. Fibres dominated the water samples collected at Site 1, suggesting domestic washing as a source. Similar evidence of microplastic inputs from rural communities has been observed by Tibbetts et al. (2018) and Klein et al. (2015). In contrast, of all 8 sites, Site 1 had the lowest number of microplastics on the bed (357.13±376.41 particles/$m^{2)}$. The channel here is straight and narrow (5 m wide), and has a transitional step-pool to plane-bed morphology and a predominantly coarse (gravel to cobble) bed (Appendix 5D). These attributes suggest a high-energy system where relatively light materials such as microplastic fibres are likely to remain in suspension.

Concentrations in water dropped markedly from Site 1 to Site 2. Site 2 is approximately 1 km downstream from the Semenyih dam, and the lower concentration in the water here may indicate trapping by the dam, just in the way that dams trap most of the fine sediment transported by rivers (Watkins et al., 2019; Zhang et al., 2015). Interestingly, the amount of microplastic deposited on the bed at Site 2 was higher than at Site 1. This may reflect the influence of the dam on flow and geomorphic conditions here (i.e. the site was dominated by pool habitat; Appendix 5E), with the low compensation flow which predominates for much of the time resulting in deposition and accumulation of micropalstic on the bed at this site.

There was a general trend of increasing contamination by microplastics downstream from Site 2. As detailed in previous chapters, this corresponds to changes in urbanisation and population density with distance downstream in the Semenyih (Chen et al., 2021b; Chen et al.,

2022) as has been reported elsewhere (e.g. Huang et al. [2021] and Wen et al. [2018]). The slight drop in water and bed contamination at Site 7 most likely reflects dilution from a less-contaminated tributary.

5.5.3 Variability in microplastic concentrations and implications on monitoring

To our knowledge, this study is the first to examine the relationship between microplastic concentrations in river water and sediments in a direct statistical way. At a site scale (between sites), Spearman's rank correlation shows that the sites with higher concentrations in water were not always those with higher amounts of microplastic deposited on the bed. At a patch scale (within sites), the GLMM also indicates that the amount of microplastic on the bed cannot be predicted from microplastics in the water column. This means that solely sampling water might miss ecologically important hotspots of bed contamination.

Whilst other similar published papers (Ding et al., 2019; Huang et al., 2021; Scherer et al., 2020) have not analysed and presented their results in a direct way that statistically shows the relationship between concentrations in water and sediments. They also reported no clear relationship between microplastic concentrations in water and sediment. For example, it is clear in Figure 2 of both Ding et al. (2019) and Huang et al. (2021) that the sites with high microplastic concentrations in the water column are not those with high microplastic concentrations in sediments, and vice versa. This suggests that along the course of a river, overall microplastic loads change to reflect inputs from different sources, but at smaller scales (i.e. within sites), the contamination of riverbed sediments is driven by local factors such as flow hydraulics. Just as how the deposition and accumulation of fine sediments are influenced by flow velocities (Buendia et al., 2016), microplastic may be influenced by localised differences in velocities (e.g. between pools and riffles), resulting in a higher deposition in pools (lower velocity) compared to riffles (higher velocity).

In general, water quality monitoring is designed to identify sites that do not meet required standards, and to differentiate between sites with good and poor water quality. However, our data show that using water concentrations to indicate which sites are good or bad is problematic; using only water may yield different rankings to that based on bed contamination, and accordingly different site prioritisation for management. Proper assessment of ecological risks, therefore, requires multiple samples being collected per site, from both water and sediments. This is discussed further in Chapter 6.

5.6 Conclusion

Results indicate little correspondence between the amount of microplastics in the water column and that deposited on the riverbed, either at the patch or site scales. In turn, this means that sampling designed to assess risks posed by microplastic should assess both concentrations in the water and the amount deposited on the bed. Marked within-site variability in bed accumulation suggests that local factors related to flow hydraulics and channel morphology mediate the effects of point sources of microplastic. Marked within-site variability in concentrations in water suggests limited mixing and/or spatially variable deposition or suspension, leading to patchiness across sites. This indicates that replicated sampling is critical for quantifying microplastic concentrations at sites or times, especially where data are used to underpin decisions related to prioritising management. The high spatial variability of bed accumulation suggests that risks to aquatic organisms of microplastic on the bed may vary considerably, depending on their microhabitat use and mobility.

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5.8 Appendices

Appendix 5A

Key characteristics of study sites.

Site	Coordinates		Distance from dam (km)	Altitude (m)	Channel width (m)
1	3°06'33.95"N	101°54’46.63”E	0.34	121	5
2	3°03'35.44"N	101°52’26.19”E	2.09	63	7.94
3	3°02'33.67"N	101°52’24.07”E	4.38	58	8.21
4	3°00'19.92"N	101°52’17.76”E	9.86	46	10.23
5	2°57’31.11”N	101°50’56.64”E	13.61	35	10.94
6	2°55’11.11”N	101°49’37.78”E	20.09	28	17.33
7	2°54’19.18”N	101°48’41.20”E	22.59	19	13.03
8	2°54’14.02”N	101°48’31.74”E	22.89	21	15.19

Appendix 5B

P-values of Bonferroni Test of microplastic concentrations between sampling sites.

	1	2	3	4	5	6	7	8
1		0.008	1.000	1.000	0.177	0.002	0.621	0.004
2	0.008		0.140	1.000	1.000	1.000	1.000	1.000
3	1.000	0.140		1.000	1.000	0.047	1.000	0.088
4	1.000	1.000	1.000		1.000	0.532	1.000	0.867
5	0.177	1.000	1.000	1.000		1.000	1.000	1.000
6	0.002	1.000	0.047	0.532	1.000		1.000	1.000
7	0.621	1.000	1.000	1.000	1.000	1.000		1.000
8	0.004	1.000	0.088	0.867	1.000	1.000	1.000	

Appendix 5C

Cylinder sampling method for microplastics in bed sediments.

Appendix 5D

Image of Site 1, showing a transitional step-pool to plane-bed morphology.

Appendix 5E

Image of Site 2, showing pool morphology.

6

General conclusions and future perspectives

6.1 Introduction

Despite the rise in microplastic contamination studies globally, studies in Malaysia remain scarce. Empirical field studies on the occurrence and transport of microplastics within river channels are lacking in Malaysia, as indeed in South-east Asian (SEA) countries generally, and nothing was known about the spatial and temporal distribution of microplastics in Malaysian rivers prior to this thesis. Understanding loads is crucial to comprehend the fluxes of microplastics and improving assessments of risks. In addition, information related to the point and non-point sources of microplastics in Malaysian rivers remained unknown prior to the commencement of this work, and as is the case globally, there remains a paucity of information related to the concentration of microplastics in riverbeds and how best to monitor the contamination levels of microplastics in rivers. This thesis helped to address some of these gaps, by focusing on microplastic contamination in the Langat River Basin, which is typical of many in Malaysia (as detailed in Chapter 3).

This final chapter is structured around the main findings of this thesis. The research approach for each objective is first discussed in Section 6.2, and a summary of the main findings for each one, along with the wider research implications, will be discussed in Section 6.3. Finally, Section 6.4 provides recommendations for future work.

6.2 Research approach

For the first objective, the <u>contribution of different sources of microplastic contamination into river water in the Semenyih subcatchment</u> (a main tributary of the Langat) was assessed by collecting water samples from the river and several potential sources. These included culverts draining main roads, residential and industrial areas, wastewater treatment plants (WWTPs), as well as inputs from atmospheric deposition. Comparison of concentrations between different sources helped assess their relative contributions and this information can potentially be used for the development of geographically targeted remediation. Water samples collected from both the inflows and outflows of WWTPs also provided information on the removal efficiency of microplastics by these plants.

For the second objective, the spatial and temporal variation in the concentration of microplastics along the Langat River was assessed by collecting water samples over a 12-month period. These sites extended from relatively forested upstream areas to heavily urbanised downstream areas. The work was interrupted by COVID-19 because of the recurring travel restrictions imposed during the pandemic (March 2020 – October 2020). The restrictions meant that not all 8 sites were visited each month, as originally planned. Instead, over the main period of the restrictions, a subset of sites (2, both close to campus) was visited more frequently. Over this study period, the relationship between microplastic concentration and river discharge on both daily and flood scales provided insights into hydrological controls on the transport and storage of microplastic as well as enabled the estimation of microplastic conveyance from the Langat to the ocean. Additionally, the relationship between microplastics and SSC was used to understand if the transport of microplastics in rivers parallels that of fine sediments.

For the third objective, the relationship between microplastic concentrations in surface water and sediments of the Semenyih River was assessed by collecting river water and bed sediment samples from 8 sampling sites along the upper reaches of the Semenyih River (a tributary of the Langat). The within- and between-site variability of microplastic concentrations in samples from both water and sediment helped in the assessment of the challenges to quantifying microplastic loads, and in turn, evaluating the magnitude of ecological risks at different sites.

6.3 Summary of main findings and research implications

The aim of this thesis was to assess the sources and levels of microplastic contamination in rivers in the Langat River Basin, Malaysia. There were multiple sources of microplastics, where road runoff was the key contributor, with significant contributions from WWTPs. The levels in river water were higher than so far reported in other Malaysian rivers, but this may either reflect the dearth of studies or that the Langat is indeed badly contaminated.

The first objective of this thesis was to assess the contribution of different sources of microplastic contamination in river water. Of all source types, drains adjacent to main roads were observed to be the highest contributor of microplastics, contributing an average of 42.20±35.29 particles/L to the Semenyih River. The composition analysis showed an abundance of styrene butadiene rubber, indicating sources from tyre and road wear particles. Contributions from residential (8.53±9.91 particles/L) and industrial (5.67±4.88 particles/L) areas into the Semenyih River were also significant. These inputs were observed to be influenced by: (i) controlled and/or uncontrolled discharges from residential and/or industrial plastic and textile manufacturing and use as well as (ii) the watercourse to which wastewater is routed (i.e. settlement ponds, WWTPs or directly into rivers).

The significant contribution of microplastics from culverts draining main roads and residential and industrial areas shows the need for drainage discharges to be treated before their discharge into the river. However, the WWTPs sampled in this study contributed as much microplastics (7.47±3.52 particles/L) to the Semenyih River as urban and industrial drainage, with removal efficiencies ranging from 31% to 70%. This calls for microplastics to be included in the routine water quality monitoring and incorporated as a parameter for WWTP treatment processes, at least in new urban developments.

Considering that most of the area upstream from the uppermost river sampling point remains forested, the average microplastic concentration of 1.93±0.84 particles/L at this sampling point demonstrates that even rural settlements are enough to cause concentrations higher than so far reported in other Malaysian rivers. However, the highly contaminated inputs from drains, culverts and WWTP outlets did not result in detectable changes in microplastic concentrations between all of the sites in this part of the Semenyih. Nevertheless, the complex inputs from multiple sources suggested that across the Langat basin, there may be variation in river loads, as well as variation that relates to flow.

The second objective assessed the spatial and temporal variability as well as the influence of hydrological conditions on microplastic in surface water. As with Objective 1, this work has been reported in a paper which represents a baseline assessment of microplastic concentrations in the Langat River. The work indicated an average microplastic concentration of 4.39±5.11 particles/L. Although this concentration is comparatively higher than in other Malaysian Rivers (e.g. the Dungun and Cherating Rivers [Hwi et al., 2020; Pariatamby et al., 2020]), the small number of studies overall means that it is hard to say whether the Langat has unusually high concentrations or not. Further studies and monitoring of microplastics in other Malaysian rivers are therefore needed to fully understand the magnitude of the microplastic problem in Malaysia and give a context for the results reported for the Langat.

Microplastic concentrations in the Langat River were observed to vary both spatially and temporally. A general increase in microplastic concentrations in the downstream direction was observed, which reflected changes in land cover and landuse. In general, the spatial variation of microplastics paralleled the increasingly urbanised and industrialised areas as the river flows seaward. The observed temporal variation in microplastics was largely influenced by changes in flows, with higher concentrations on sampling dates with high flow. On the scale of an individual flood event, microplastic concentrations rose and fell in response to flow, though with a lagged response. This demonstrates the complex fluxes of microplastics and emphasises the need for more extensive research to fully understand the general responses of microplastic concentrations to short-term changes in flow, notably the interacting effects of dilution and supply exhaustion that may occur depending on the magnitude and duration of high flows.

The time-integrated assessment suggested that Langat River generally delivers 5 billion particles of microplastics to the ocean per day. These concentrations are extremely high compared to the Ebro River, Spain and the Pearl River, China, for example, which have estimated ocean delivery of 2.2 and 39 billion particles per year respectively (Mai et al., 2019; Simon-Sánchez et al., 2019). However, since existing published estimates are scarce, and often are based on different methods of assessment, comparisons are hard to draw. Thus, it remains difficult to know if the delivery of microplastics to the ocean estimated for the Langat is typical or different to other rivers in rapidly urbanising SEA cities.

The work for Objective 2 confirmed the positive correspondence between microplastics and SSC (direct linear relationship). In turn, this may suggest that following calibration and validation tests, it may be possible to use continuous recordings of turbidity as a surrogate for microplastics. This would ease monitoring because traditional microplastic sampling is time-consuming. However, assessing the ecological risks posed by microplastics may require information on the amount of microplastic deposited on the riverbed, not just the open water. The third and final objective of this thesis addressed this point.

The third objective of this thesis was to evaluate the degree of correspondence between microplastics suspended in surface water and deposited on bed sediments and make recommendations for monitoring and assessment. Like the work related to the second objective of this thesis, this provided baseline data on microplastic concentrations in bed sediments, something which was largely lacking in Malaysia. The sampling yielded estimates of a mean microplastic concentration of 6027.39±16585.87 particles/m^2 of the riverbed, across the 8 sites. This is considerably less contaminated than bed sediments of the Rhine, Nakdong and Mersey

Rivers (Eo et al., 2019; Hurley et al., 2018; Klein et al., 2015) which were sampled using the same technique.

Variability of microplastics within sites (i.e. differences between the bed patches sampled) was high, but interestingly, between-site variability was formally significant only for the bed data and not the concentrations in the water. This might suggest that assessments based on bed accumulation are more sensitive than those based on samples obtained from the water. Microplastic concentrations on the bed show little correspondence to those in the water column directly above the sampled patches, nor when averaged across sites to look at whether those with high bed accumulations were those with higher water concentrations. To some extent, these results are to be expected, because of spatial variability in settlement (linked to flow hydraulics) and the stochastic nature of sediment transport when measured over short timescales. Nevertheless, the results show that single samples of water, especially if volumes are small, are unlikely to give a good indication of what is present on the bed. Sampling programmes designed to assess risks posed by microplastic should therefore assess both concentrations in the water and the amount deposited on the bed.

6.4 Recommendations and future research needs

This thesis has provided some new insights but raised many questions that need further work. The study of sources (Chapter 3) was only carried out over a 3-month period in a sub-catchment of the Semenyih River. This work could be scaled up, to look at the whole basin, but also integrated over longer and different periods. Further studies carried out over a longer period will be beneficial for assessing the long-term inputs of microplastics into rivers, allowing for improved assessments of ecological and human health risks. The sampling should also take into account rainfall periods to assess concentrations when road particles are being washed off and drains are full. This is important for the management of urban wastewater and allows for more comprehensive mitigation strategies. Moreover, it is important to note that the study of microplastic sources was undertaken during COVID-19 when many residents were confined to their homes and industries were closed or operating at a low level. Thus, further studies are needed to assess whether the return to more normal conditions alters the relative inputs from the various sources detailed in Chapter 3.

Future research should also be directed towards understanding the degradation processes of microplastics into nanoplastics in the environment. The deterioration of microplastics has been recorded in Chapter 3, where evidence of mechanical and oxidative weathering was observed in material collected from the Langat. This finding suggests that higher microplastic concentrations might be present in the Semenyih River than reported here if many fine particles are present in addition to those retained in the sieves used here. Though several published works have tried to elucidate the degradation processes of microplastics into nanoplastics, the majority of studies were carried out using simulations (Bianco et al., 2020; Lambert and Wagner, 2016; Naik et al., 2020; Song et al., 2017), instead of actual empirical field studies. Future research should take advantage of newly developed techniques to assess nano-sized materials, and include these smaller size ranges in environmental contamination studies. This is particularly important from the human and ecological perspectives, because of the different risks posed by nanomaterials.

Flood events are often used to help elucidate the transport dynamics of suspended sediments in rivers (Krajewski et al., 2018; Vale and Dymond, 2020). As, in general, the transport and settlement of microplastics parallels that of fine sediments, studies of microplastics during flood events would greatly benefit knowledge of the general responses of microplastic concentration to short-term changes in flow. In general, only single flood events have been assessed (e.g. Hitchcock, 2020; Treilles et al., 2022), which may be inadequate to elucidate this relationship. Hence, there is a need for studies relating microplastic concentrations with discharge, primarily those assessing changes in microplastic concentrations during individual flood events. This is important as fluvial bed sediments are areas of intense biological activity. Understanding the fluxes of microplastics in and out of the river system will therefore provide information on the exposure of riverine organisms (primarily benthic organisms) to microplastics, and how this is affected by changing flow conditions.

The findings of Chapters 3, 4 and 5 have emphasised the need for including microplastics as a parameter in the routine monitoring of river quality. Malaysia has a WQI, which monitors as many as 35 parameters (Department of Environment Malaysia, 2022), but microplastics are not included. A first step in tackling the microplastic problem would be to include this material in routine monitoring so that the true extent and magnitude of the problem could be established.

The correspondence between microplastics and suspended sediments has the potential to improve assessments of microplastic loads. As field sampling and laboratory processing of samples are tedious, constant monitoring of microplastics is not feasible, which, in turn, may lead to biases in data (i.e. data are not integrated spatially and/or temporally). Although a general trend between microplastics and suspended sediments has been identified in Chapter 4, this trend may differ according to rivers and can be affected by varying hydrological controls. Additional data, primarily in rivers from different geographic regions, are therefore needed to fine-tune the relationship and help with the development of a generalised model that estimates microplastic concentrations based on geographic information, discharge as well as continuously logged turbidity or SSC values.

Finally, a standardised sampling protocol for future microplastic research and WQI monitoring should be endorsed to facilitate data transfer between different studies, which is crucial to help direct mitigation efforts. For example, although research on microplastics in

Malaysian rivers has seen a gradual increase, comparison of the extent of microplastic contamination is compromised by the differences in (1) the sampling programme, i.e. studies ranged from one-off spot sampling to comprehensive spatio-temporal studies, (2) the type of samples collected, i.e. collecting water samples and/or sediment samples, (3) the units of measurements used, primarily caused by differences in methods of sample collection, and (4) the size ranges of reported microplastics, which are influenced by the sizes of the microscopes and sieve used. The following points should therefore be taken into account to help develop a sampling protocol for microplastic and/or WQI monitoring:

1. As using only water may yield different rankings to that based on bed contamination (Chapter 5), monitoring of microplastics in rivers should therefore include both surface water and bed sediments.
2. As microplastic concentrations have been shown to vary within sites, replicate samples should be taken from multiple points. Water samples should be taken at five points laterally across the channel (Chapter 4), while sediment samples should be integrated across different geomorphic units (Chapter 5) to help produce more representative estimates of mean site concentrations.
3. As sampling programmes for water quality monitoring need to be cost-effective yet efficient, the cylinder method used in Chapter 5 is recommended for future monitoring of microplastics on bed sediments.
4. The volume of water to be collected should depend on the concentration of microplastics present in the water column. Based on the findings of Chapters 4 and 5, at least 5 L of bulk water samples and 2 L of water from within the cylinder used for sediment samples should be collected.

5. In order to determine the ecological risks of microplastics, a range of microplastic sizes should be reported, which can be done by stacking sieves of different sizes on top of each other.

6.5 Future perspectives

Plastic is, without doubt, one of the most useful inventions of humankind, replacing traditional materials such as metal and wood in the manufacturing, automotive, textile and packaging industries (Chen et al., 2021). While the production of plastics has provided numerous benefits, it has also become one of the worst pollutants on Earth. The mass production of plastic only started to increase exponentially around the 1950s, yet only a few decades later, it has already polluted every part of the Earth, either in the form of plastics or microplastics (Ostle et al., 2019).

The complete removal of microplastics from the natural environment is virtually impossible, but several measures can be used to address the microplastic problem. The first step is to create awareness by presenting scientifically validated data on the sources and extent of microplastic pollution in the environment. This thesis has contributed to this goal and has found substantial microplastic contamination in the Langat River, which is shown to be governed by changing hydrological conditions as well as inputs from local sources (i.e. roads, residential and industrial areas and drains). Studies such as this are especially important in rapidly developing countries, which often have some of the worst plastic pollution problems, thanks to limited awareness, behaviours and controls.

Whilst recovering microplastics from the environment may reduce potential threats to organisms and human health, such efforts are often not feasible, both economically and logistically (Eriksen et al., 2018). Hence, effort needs to be focused on upstream intervention. This includes (1) proper management of point and non-point sources of microplastics by mandating the monitoring of microplastics through WQI assessments and upgrading existing WWTPs, (2) minimising the loss of plastics to the environment by adopting a plastic management model that supports the circular economy (Chen et al., 2021), (3) enforcement of legislation to minimise single-use plastics through plastic bans and levies, and (4) changing public attitudes towards plastics by promoting recycling habits and the zero waste lifestyle. All in all, addressing the microplastic problem requires the collective intervention of policymakers, organisations, industries and the public. It remains a major challenge, but a challenge we must address.

6.6 References

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