OCCURRENCE AND ECOLOGICAL RISK ASSESSMENT OF MICROPLASTICS AND ENDOCRINE DISRUPTING COMPOUNDS IN RIVERINE ENVIRONMENT

By

MUHAMMAD ROZAIMI BIN MOHD ZAKI

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the degree of Doctor of Philosophy

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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February 2025

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The presence of microplastics and endocrine disrupting compounds (EDCs) in aquatic environments raises concerns owing to their adverse effects on ecosystems and human health. Predominantly originating from urban sources and anthropogenic activities, these contaminants enter rivers, accumulate in riverine environments, and contribute to habitat degradation, thereby posing risks to aquatic organisms and humans. Moreover, an understanding of the ecological risk, pollution loads and land-use influence associated with these pollutants is lacking, necessitating comprehensive assessment. The aim of this study was to investigate the occurrence and sources of microplastics and EDCs in the riverine environment of the Selangor River Basin. This study also included an ecological risk assessment, estimation of daily pollution loads, and investigation of the influence of different land-use activities on the distribution of microplastics and EDCs in the Selangor River Basin. Thus, this study involved the optimization of a developed method for determining the concentrations of both microplastics and EDCs. Microplastic samples underwent an extraction process

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involving wet peroxide oxidation and density separation, followed by microscopic identification and Fourier-transform infrared spectroscopy (FTIR). Targeted multiclass EDCs were quantified using solid-phase extraction coupled with liquid chromatography mass spectrometry-mass spectrometry (SPE-LC-MS/MS). Prevalent microplastic particles were detected in the Selangor River Basin up to 3.4 particles/L and 55 particles/kg in surface water and sediment, respectively. The most prevalent polymer compositions of the detected microplastics in the riverine samples included polyethylene (PE), polypropylene (PP), and polyester. This study also showed the predominant abundance of bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), 17α-ethynylestradiol (EE2), and 17β-estradiol (E2) in both surface water and sediment of the Selangor River Basin. Other compounds such as progesterone, testosterone and propranolol were below the method detection limit (MDL) in surface water, while only testosterone was below the MDL in sediment. Phenolic compounds such as BPF and BPA were observed at the highest concentration in surface water (up to 1098.40 ng/L) and sediment (up to 117.20 ng/g), respectively. The ecological risk assessment of microplastics in the Selangor River Basin revealed a low risk with a risk quotient (RQ) of 0.06, indicating that exposure to microplastics is considered lower than the threshold level of concern. This also suggests that the potential adverse effects on the ecosystem and aquatic organisms are anticipated to be minimal. Meanwhile, all EDCs indicated negligible risk with $RQ < 0.01$, except for EE2 and E2, which had RQ values ≥ 1 (high risk). This emphasizes the need for immediate regulatory attention and further detailed studies to understand the implications, as these emerging contaminants could disrupt ecosystems and human health even at trace concentrations. Despite the low ecological risk associated with microplastics ($RQ = 0.06$), these findings underscore the

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significance of implementing measures to mitigate the risk of both microplastic and EDCs contamination in the environment. Based on daily pollution loads and land-use influence, the primary sources of microplastics and EDCs are urban areas, where various anthropogenic activities including industrial operations, residential development, agriculture, and commercial activities contribute to their release. These contaminants enter riverine systems primarily through wastewater discharge, surface runoff, and improper waste disposal, leading to their accumulation and potential ecological impacts. Therefore, this study provides essential baseline data, identifies key contaminant sources, and supports the development of effective strategies for managing and mitigating these emerging pollutants.

Keywords: Microplastics; Endocrine disrupting compounds; Emerging contaminants; Riverine water; Risk assessment

SDG: GOAL 6: Clean Water and Sanitation; GOAL 14: Life Below Water

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Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KEWUJUDAN DAN PENILAIAN RISIKO EKOLOGI MIKROPLASTIK DAN SEBATIAN PENGENDALA ENDOKRIN DALAM PERSEKITARAN SUNGAI

Oleh

MUHAMMAD ROZAIMI BIN MOHD ZAKI

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Kehadiran mikroplastik dan sebatian pengendala endokrin (EDC) dalam persekitaran akuatik telah menimbulkan kebimbangan akibat kesan buruknya terhadap ekosistem dan kesihatan manusia. Kebanyakannya berasal daripada sumber bandar dan aktiviti manusia, bahan tercemar ini dilepaskan ke dalam sungai, terkumpul di dalam persekitaran sungai, dan menyumbang kepada kemusnahan habitat, sekali gus menimbulkan risiko kepada hidupan akuatik dan manusia. Selain itu, pemahaman mengenai risiko ekologi, kemasukan pencemaran harian, dan pengaruh guna tanah yang berkaitan dengan pencemar-pencemar ini masih tidak mencukupi dan memerlukan penilaian menyeluruh. Matlamat kajian ini adalah untuk menyiasat kehadiran dan sumber mikroplastik dan EDC dalam persekitaran Lembangan Sungai Selangor. Kajian ini juga mengkaji penilaian risiko ekologi, anggaran kemasukan pencemaran harian, dan pengaruh pelbagai aktiviti guna tanah terhadap taburan mikroplastik dan EDC di Lembangan Sungai Selangor. Dengan itu, kajian ini melibatkan pengoptimuman kaedah yang telah dibangunkan untuk menentukan

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kepekatan kedua-dua mikroplastik dan EDC. Sampel mikroplastik menjalani proses pengekstrakan yang melibatkan pengoksidaan peroksida basah dan pemisahan ketumpatan, diikuti dengan pengenalpastian mikroskopik dan spektroskopi inframerah transformasi-Fourier (FTIR). Sementara itu, kepelbagaian kelas sebatian pengendala yang disasarkan dikira menggunakan pengekstrakan fasa pepejal digandingkan dengan kromatografi cecair spektrometri jisim (SPE-LC-MS/MS). Partikel mikroplastik tersebar luas dikesan di Lembangan Sungai Selangor sehingga 3.4 partikel/L dan 55 partikel/kg dalam permukaan air dan sedimen. Komposisi polimer yang paling lazim dikesan bagi mikroplastik dalam sampel sungai adalah termasuk polietilena (PE), polipropilena (PP), dan poliester. Kajian ini juga menunjukkan kehadiran dominan bisfenol A (BPA), bisfenol S (BPS), bisfenol F (BPF), sulfonat perfluorooktana (PFOS), asid perfluorooktanoik (PFOA), 17α-etinilestradiol (EE2), dan 17β-estradiol (E2) dalam kedua-dua permukaan air dan sedimen Lembangan Sungai Selangor. Sebatian lain seperti progesteron, testosteron, dan propranolol berada di bawah had pengesanan kaedah (MDL) dalam permukaan air, manakala hanya testosteron yang berada di bawah MDL dalam sedimen. Sebatian fenolik seperti BPF dan BPA masing-masing ditemui mempunyai kepekatan yang paling tinggi dalam permukaan air (sehingga 1098.40 ng/L) dan sedimen (sehingga 117.20 ng/g). Penilaian risiko ekologi mikroplastik di Lembangan Sungai Selangor mendapati menunjukkan risiko rendah dengan nilai kuotien risiko (RQ) sebanyak 0.06, menandakan bahawa pendedahan kepada mikroplastik dianggap lebih rendah daripada tahap ambang kebimbangan. Ini juga menunjukkan bahawa potensi kesan buruk terhadap ekosistem dan organisma akuatik dijangkakan adalah minimum. Sementara itu, semua EDC menunjukkan risiko yang boleh diabaikan dengan $RQ < 0.01$, kecuali EE2 dan E2, yang mempunyai nilai $RQ \geq 1$ (risiko tinggi). Ini menunjukkan keperluan segera untuk perhatian pihak

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pentadbiran dan penyelidikan lanjut yang terperinci bagi memahami implikasi, kerana pencemaran yang hadir ini boleh menyebabkan gangguan kepada ekosistem dan kesihatan manusia walaupun pada kepekatan yang sangat rendah. Walaupun risiko ekologi yang rendah dikaitkan dengan mikroplastik (RQ = 0.06), penemuan ini menekankan kepentingan melaksanakan langkah-langkah mitigasi untuk mengurangkan risiko kedua-dua pencemaran mikroplastik dan EDC dalam alam sekitar. Berdasarkan anggaran kemasukan pencemaran harian dan pengaruh guna tanah, sumber utama mikroplastik dan EDC adalah kawasan bandar, di mana pelbagai aktiviti antropogenik termasuk operasi industri, pembangunan kediaman, pertanian, dan aktiviti komersial menyumbang kepada pelepasan bahan tersebut. Pencemar ini memasuki sistem sungai terutamanya melalui pelepasan air sisa, larian permukaan, dan pembuangan sisa yang tidak terkawal, menyebabkan pengumpulan serta berpotensi memberi kesan ekologi. Justeru, penyelidikan ini menyumbang kepada penyediaan data asas yang penting, mengenal pasti sumber pencemar utama, serta menyokong pembangunan strategi yang berkesan untuk mengurus dan mengurangkan bahan pencemar yang semakin meningkat.

Kata Kunci: Mikroplastik; Sebatian pengendala endokrin; Kemunculan bahan tercemar; Air sungai; Penilaian risiko

SDG: MATLAMAT 6: Air Bersih dan Sanitasi; MATLAMAT 14: Kehidupan dibawah Air

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TABLE OF CONTENTS

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LIST OF TABLES

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LIST OF FIGURES

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LIST OF ABBREVIATIONS

ACN	Acetonitrile
AF	Assessment factor
Al_2O_3	Aluminum oxide
BPA	Bisphenol A
BPF	Bisphenol F
BPS	Bisphenol S
$CaCl_2$	Calcium chloride
COVID-19	Coronavirus disease 2019
CV	Coefficients of variation
DBP	Dibutyl phthalate
DEHP	Bis(2-ethylhexyl) phthalate
DIBP	Diisobutyl phthalate
di-PAEs	Phthalate diesters
DL	Daily load of contaminants
E2	17β-estradiol
EC_{50}	Effective concentration of 50%
EDCs	Endocrine disrupting compounds
EE2	17α-ethynylestradiol
Eq.	Equation
$FeSO_4$	Ferrous sulphate
FTIR	Fourier-transform infrared spectroscopy
FTIR-ATR	Fourier-transform infrared spectroscopy with attenuated total reflectance
H_2O_2	Hydrogen peroxide

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HC_5	Hazardous concentration for 5%
HCl	Hydrochloric acid
K_{oc}	Soil sorption coefficient
log K_{ow}	Octanol–water partition coefficient
LC_{50}	Lethal concentration of 50%
LC-MS/MS	Liquid chromatography mass spectrometry-mass spectrometry
LDPE	Low-density polyethylene
LOD	Limit of detection
MDL	Method detection limit
ME	Matrix effect
MEC	Measured environmental concentration (mean)
MeOH	Methanol
MPs	Microplastics
MTBE	Methyl-tributyl ether
NA	Not available
Na_2EDTA	Tetrasodium ethylenediamine-tetraacetate dihydrate
NaCl	Sodium chloride
NaI	Sodium iodide
ND	Not detected
NH_4OH	Ammonium hydroxide
NOAA	National Oceanic and Atmospheric Administration
NOEC	No observed effect concentration
NP	Nonylphenol
OP	Octylphenol
ORP	Oxidation reduction potential

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PA	Polyamide
PAEs	Phthalates
PAHs	Polycyclic aromatic hydrocarbons
PC	Polycarbonate
PCBs	Polychlorinated biphenyls
PE	Polyethylene
PET/PETE	Polyethylene terephthalate
PFAS	Perfluoroalkyl substances
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PMMA	Polymethyl methacrylate
PNEC	Predicted no-effect concentration
POPs	Persistent organic pollutants
PP	Polypropylene
PS	Polystyrene
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl chloride
QAQC	Quality assurance and quality control
r	Linearity
ROS	Reactive oxygen species
RQ	Risk quotient
RSD	Relative standard deviation
S/N	Signal-to-noise ratio
SD	Standard deviation
SP	Sampling point

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SPE	Solid-phase extraction
SPE-LC-MS/MS	Solid-phase extraction coupled with liquid chromatography mass spectrometry-mass spectrometry
SPRHiN	National Hydrological Network Management System
SSD	Species sensitivity distribution
SSP1	Selangor River Phase 1
SSP2	Selangor River Phase 2
SSP3	Selangor River Phase 3
TBBPA	Tetrabromobisphenol A
TDS	Total dissolved solids
TPA	Terephthalic acid
UPW	Ultrapure water
WD	Water discharge
WTP	Wastewater treatment plants
$ZnCl_2$	Zinc chloride

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Aquatic environments have been exposed to a wide range of emerging contaminants since the beginning of the Industrial Revolution in the 18th century. The significant impacts on the aquatic environment persist to this day, owing to the fact that environmental awareness and regulations concerning pollution and resource management were not fully developed during the early stages of industrialization. Rapid industrialization and urbanization have resulted in the introduction of numerous persistent and emerging contaminants, such as microplastics and endocrine disrupting compounds (EDCs), into the aquatic environment. As a result, these contaminants have permeated aquatic organisms, presenting potential health hazards to humans via routes such as consumption of contaminated drinking water and food, inhalation, and direct contact with the skin (Wee and Aris, 2017b; Zaki and Aris, 2022).

Plastics have become widespread and mass-produced owing to their convenience, affordability, and non-biodegradable nature (Cole et al., 2011; Alimi et al., 2018; Oliveira and Almeida, 2019). However, the mass production of plastics without sufficient consideration of ethical practices and environmental values has resulted in severe environmental contamination. This is exemplified by the staggering statistic that over twelve million tons of plastic waste have been introduced into the marine environment (Jambeck et al., 2015). Moreover, plastics may fragment into small particles, referred to as microplastics, which are smaller than 5000 μm. EDCs are substances that are ubiquitously present in the environment and commonly used in

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various aspects of daily life, including food and consumer products. EDCs encompass a wide range of natural and synthetic compounds, including steroidal hormones, phenolic compounds, polyhalogenated compounds, phthalates (PAEs), pharmaceuticals, and personal care products. The production of these EDCs has escalated to meet the demands of various sectors such as healthcare, agriculture, industries, and personal care products. Consequently, these compounds are often discharged into sewage water, ultimately reaching aquatic environments including rivers, estuaries, and oceans (Aris et al., 2014; Wee and Aris, 2017b; Biel-Maeso et al., 2018).

Recently, there has been increasing concern regarding the ubiquity and widespread distribution of microplastics and EDCs in aquatic environments. Microplastics and EDCs have been detected in various water sources including riverine surface water, groundwater, drinking water supplies, and wastewater (Koelmans et al., 2019). The presence of microplastics and EDCs in riverine environments raises concerns about their potential for human exposure. Rivers act as major transport pathways for microplastics and EDCs, carrying pollutants from various sources, including urban runoff, wastewater effluents, industrial discharges, and agricultural activities. Due to continuous hydrodynamic processes, microplastics and EDCs are dispersed throughout the river system, interacting with sediment and aquatic organisms. Numerous studies have revealed that a wide range of aquatic organisms, from planktonic species to whales, have been found to ingest microplastics (Fossi et al., 2012; Setälä et al., 2014; Abreo et al., 2019). Moreover, these aquatic organisms have been found to be contaminated with multiclass EDCs (Lee et al., 2015a; Salgueiro-González et al., 2015; Ismail et al., 2020). Once these contaminants enter riverine

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environments and are subsequently ingested by aquatic organisms, there is the possibility of associated human health risks. Their persistence in riverine environments is particularly concerning, as rivers serve as a key link between terrestrial sources and downstream ecosystems, including estuaries and coastal waters. The potential for exposure to these risks is also evident through various pathways, including the intake of contaminated food (Cho et al., 2019; Zaki et al., 2021a; Zainuddin et al., 2022), intake of other substances such as salt (Yang et al., 2015; Lee et al., 2019a), and exposure to microplastics through atmospheric fallout (Liu et al., 2019a; Vianello et al., 2019).

However, few studies have comprehensively addressed the co-occurrence of microplastics and EDCs in riverine environments and their associated risks to aquatic ecosystems and human health (**Figure 1.1**). This is particularly concerning, given the diverse range of exposure pathways identified in scientific research. Microplastics have been found to act as carriers and accumulators of EDCs in the environment. These EDCs can adsorb onto the surface of microplastics, potentially concentrating their concentrations (Lu et al., 2021b; Wu et al., 2022). Once aquatic organisms ingest microplastics, they are simultaneously exposed to the associated EDCs. The co-occurrence of microplastics and EDCs can enhance the toxicity, synergistic effects, bioaccumulation, and biomagnification of these chemicals, leading to more significant adverse effects on organisms (da Costa et al., 2023; Ullah et al., 2023).

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1.2 Problem statement

The occurrence and distribution of microplastics and EDCs extends beyond marine environments, encompassing freshwater ecosystems, including rivers, lakes, and groundwater. Microplastics and EDCs originate from various anthropogenic sources, such as industrial effluents, agricultural runoff, and wastewater discharge, leading to widespread pollution in freshwater systems (Wee et al., 2019; Campanale et al., 2020; Li et al., 2020c; Wee et al., 2021). The presence of these contaminants in freshwater ecosystems poses significant threats to various aquatic species, such as amphibians, mollusks, and fish, by disrupting endocrine functions, leading to reproductive disorders, skewed sex ratios, and population declines (Huang et al., 2021c; Ismail et al., 2021; Santos et al., 2021; Ullah et al., 2023). Furthermore, these pollutants can bioaccumulate and biomagnify within freshwater food webs, increasing the cascading effects on aquatic ecosystems and human health, as these pollutants can enter the food chain and eventually reach humans through the consumption of contaminated water or aquatic organisms.

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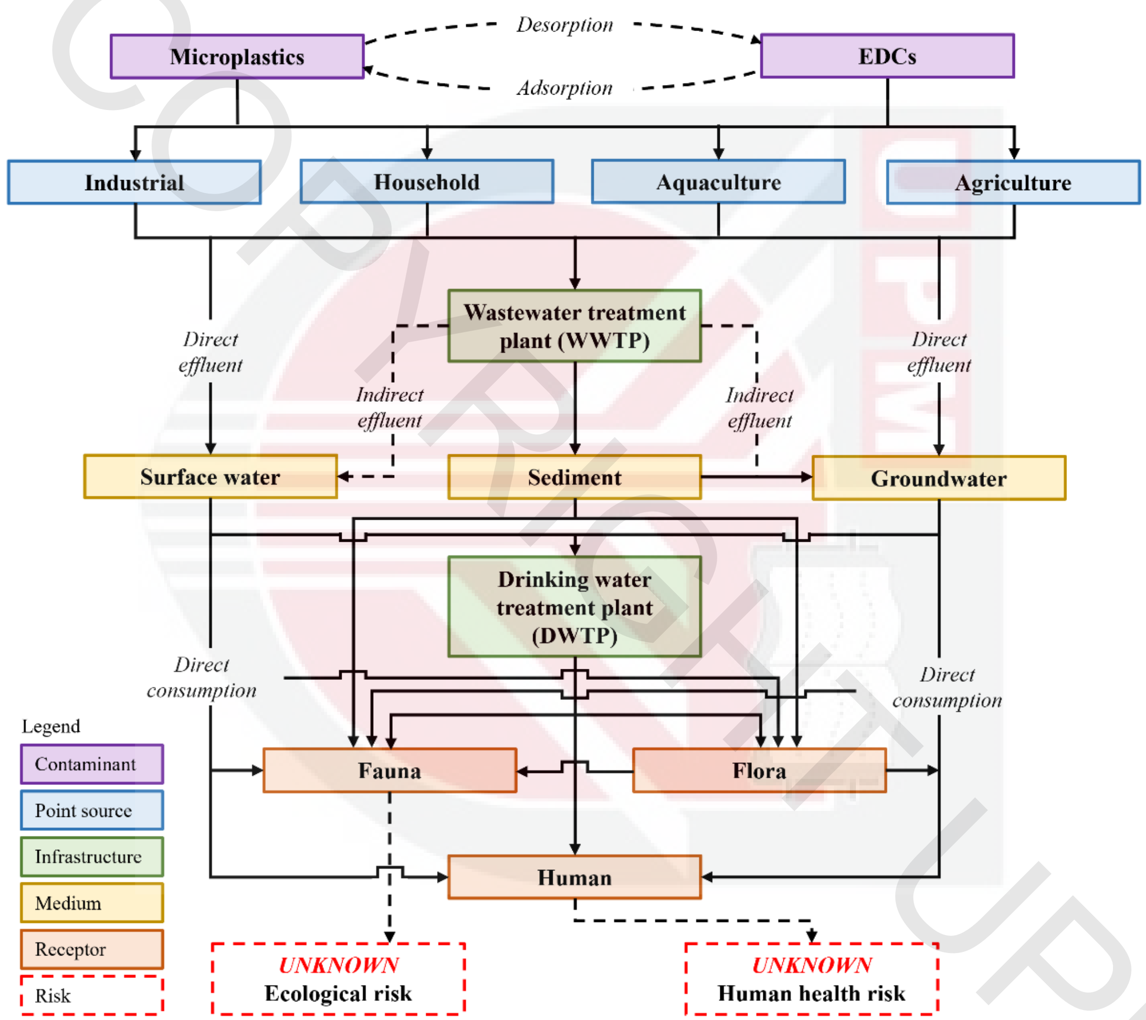

Figure 1.1: The overview sources, pathways, and fate of the microplastics and EDCs in the environment

These contaminants originate from various anthropogenic activities, including industrial processes, wastewater treatment plant effluents, agricultural practices, recreational activities, and landfill leachates (Aris et al., 2014; Zaki et al., 2021a). These sources contribute to the emission of microplastics and EDCs into riverine ecosystems. Once released, these contaminants are transported through water bodies, such as rivers, surface runoff, river currents, and tidal movements. This leads to the pervasive prevalence of microplastics and EDCs throughout riverine ecosystems, as these contaminants are transported downstream and accumulate in the open sea. Changes in land-use patterns driven by urbanization, agriculture, and deforestation play a crucial role in exacerbating pollution and degradation of aquatic environments (Wee and Aris, 2017b; Koelmans et al., 2019; Zaki and Aris, 2022). Urban expansion increases impervious surfaces, reduces natural filtration, and increases runoff, whereas agricultural practices contribute to sedimentation and nutrient runoff (Aris et al., 2014; Sakai et al., 2017b). Additionally, deforestation and land-use changes disrupt ecosystems, leading to habitat loss and fragmentation, amplifying the spread of pollutants, and threatening aquatic biodiversity.

Microplastics primarily exert physical effects on the environment and organisms, whereas EDCs interfere with the endocrine system, disrupting hormonal functions in organisms. Owing to their small size, microplastics are easily ingested by diverse aquatic species, from plankton to fish (Wright et al., 2013; Zaki et al., 2021b). Microplastics can obstruct digestive systems, causing blockage or reduction in nutrient absorption (Ryan, 2018; Jepsen and de Bruyn, 2019; Wang et al., 2020d). Moreover, microplastics can accumulate in tissues, potentially leading to inflammation or damage (Hu and Palić, 2020; Kim et al., 2021). Additionally, microplastics can act as vectors

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for other pollutants, including EDCs and heavy metals, thereby increasing their potential for bioaccumulation and biomagnification (Zaki and Aris, 2022; da Costa et al., 2023). The widespread presence of microplastics in freshwater ecosystems raises concerns regarding their role in exacerbating chemical pollution, with potential long-term consequences for aquatic biodiversity and human health.

EDCs are chemical substances that can be found in numerous products, including plasticizers, synthetic hormones, pesticides, personal care products, and pharmaceuticals. EDCs can interfere with the endocrine system, which regulates hormone production and signalling in organisms (Aris et al., 2014; Wee et al., 2019). EDCs can also mimic, block, or alter hormone functions, disrupting normal physiological processes (Frye et al., 2012; Lee et al., 2013; Balaguer et al., 2017). Ultimately, EDCs can affect reproductive systems, growth and development, metabolism, and immune functions in both wildlife and humans. Despite the detrimental effects of microplastics and EDCs on aquatic organisms and human health, the ubiquity of these contaminants can contribute to habitat degradation, leading to higher remediation costs and ultimately diminishing water quality for human consumption.

The pervasive occurrence of microplastics and EDCs in aquatic environments has received worldwide attention, and exposure to these contaminants has been linked to various ecological and human health risks. Despite increasing awareness of these pollutants, the co-occurrence and extent of their distribution, as well as their impact on aquatic ecosystems and potential risks to human populations, remain poorly understood. **Figure 1.2** illustrates the primary factors, encompassing the causes and

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effects of microplastics and EDCs in aquatic environments. Therefore, there is an urgent need for comprehensive research, awareness, and implementation of effective mitigation measures to address and mitigate the detrimental effects of microplastics and EDCs. The implementation of robust mitigation strategies and regulatory measures is imperative to effectively minimize the input and accumulation of microplastics and EDCs in aquatic ecosystems. These proactive measures are essential for preserving the health, integrity, and sustainability of these critical environments while ensuring the well-being of human populations that depend on them.

Therefore, this study aimed to investigate and address the following research questions to enhance our comprehension of the issue and provide valuable insights for the implementation of effective interventions:

1. What are the concentrations, spatial distributions, and influencing factors of microplastics in the surface water and sediments of tropical riverine environments?
2. What are the levels of contamination, spatial patterns, and influencing factors of multiclass EDCs in the surface water and sediment of tropical riverine environments?
3. What is the potential ecological risk associated with the occurrence and distribution of microplastics and EDCs in riverine environments?
4. How do daily pollution loads and different land-use activities influence the abundance and spatial distribution of microplastics and EDCs in riverine environments?

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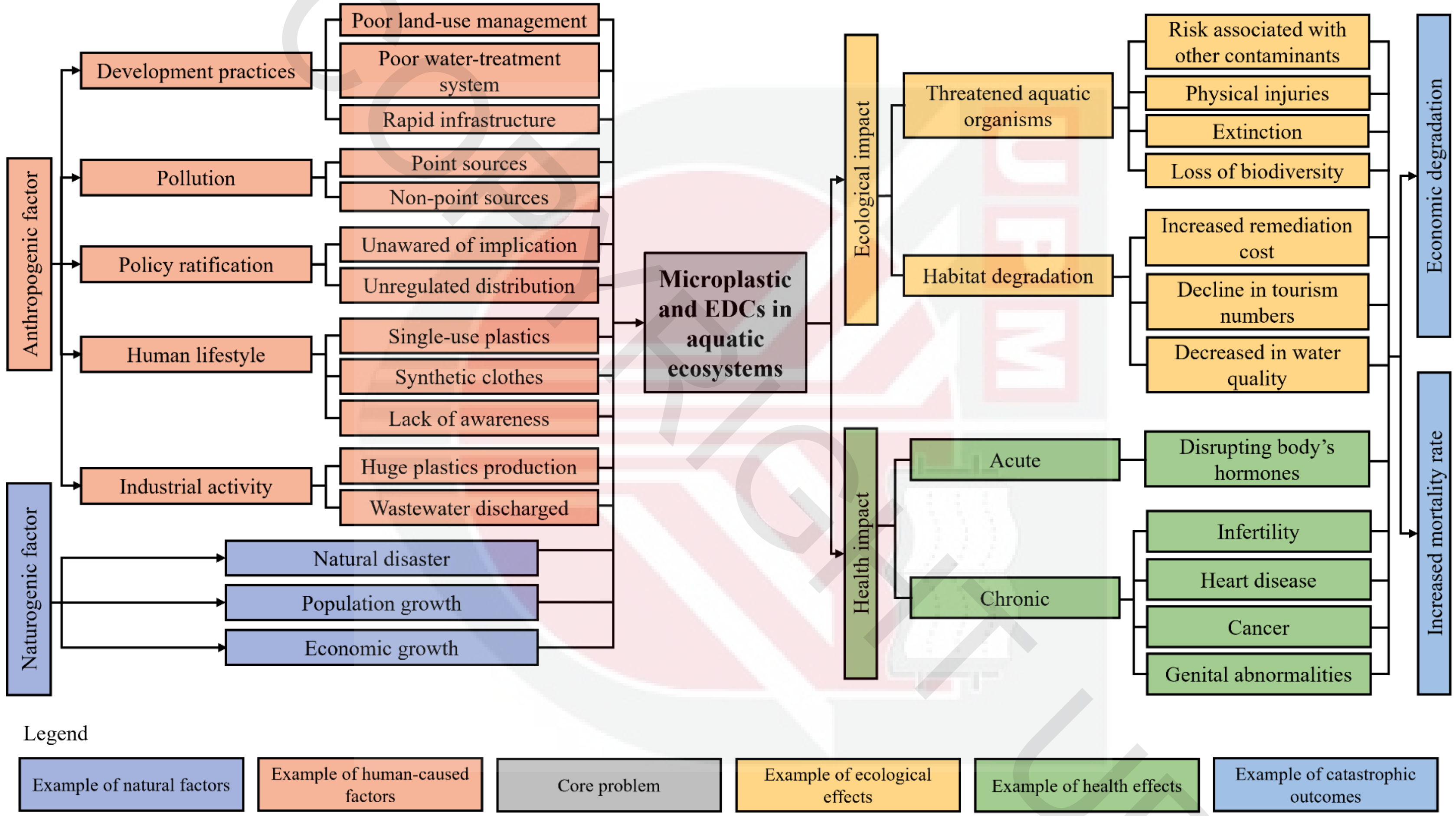

Figure 1.2: Primary issues of microplastics and endocrine disrupting compounds in aquatic environments

1.3 Objectives of the study

This study aimed to assess the occurrence and spatial distribution of microplastics and EDCs in the riverine environment of the Selangor River Basin. For microplastic analysis, an optimized analytical method based on wet peroxide oxidation and density separation was utilized. Additionally, this study employed optimized solid-phase extraction coupled with liquid chromatography mass spectrometry-mass spectrometry (SPE-LC-MS/MS) for the quantification of multi-class EDCs. This study also undertakes a thorough and comprehensive assessment aimed at elucidating the multifaceted potential ecological risks posed by microplastics and endocrine-disrupting chemicals (EDCs) within the intricate ecosystem of the Selangor River Basin. Furthermore, this study will involve the estimation of daily pollution loads and a comprehensive analysis of how diverse land-use activities affect the spatial distribution of microplastics and EDCs in the riverine environment.

The specific objectives of this study were as follows:

1. To ascertain the concentration, spatial distribution, and compare the occurrence of microplastics in samples of surface water and sediment collected from the Selangor River Basin.
2. To elucidate and compare the occurrence and spatial distribution of EDCs in surface water and sediment samples collected from the Selangor River Basin.
3. To assess the potential ecological risk associated with the occurrence of microplastics and EDCs in the Selangor River Basin.

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4. To estimate the daily pollution loads and elucidate the influence of different land-use activities on the distribution of microplastics and EDCs within the Selangor River Basin.

1.4 Scope of the study

This study covers the following areas:

1. Optimization of the analytical method for microplastics in riverine samples (surface water and sediment) by employing wet peroxide oxidation and density separation.
2. Optimization of the simultaneous analysis of multi-class EDCs (plasticizers, pharmaceuticals, perfluoroalkyl substances (PFAS), and hormones) in riverine samples (surface water and sediment) by utilizing an optimized SPE-LC-MS/MS.
3. Establishing baseline data on the occurrence, characterization, and potential sources of microplastics and EDCs in the riverine environment.
4. Understanding the presence, co-occurrence, and influencing factors of microplastics and EDCs in the aquatic environment.
5. The potential correlation and relationship between the occurrence of microplastics and EDCs in the aquatic environment were explored.
6. Assessment of potential ecological risks posed by microplastics and EDCs in riverine environments.
7. Estimating the daily pollution loads and influence of different land-use activities on the distribution of microplastics and EDCs in riverine environments.

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1.5 Significance of the study

Microplastic and EDCs contamination in various environmental compartments, including surface water, sediment, soil, biota, and atmosphere, has become a major environmental issue in recent years. The occurrence of these contaminants in water resources, such as raw water and treated water (e.g., drinking water), has attracted considerable attention worldwide owing to the potential risks posed to human consumption. However, despite growing environmental concerns regarding the occurrence of microplastics and EDCs in the environment, there is still a substantial knowledge gap in our understanding of their co-occurrence, relationship, and impacts on aquatic ecosystems. Moreover, research in the field of aquatic pollution has predominantly focused on studying individual contaminants, overlooking the complex interactions and relationships between microplastics and EDCs in aquatic environments.

The main research objective of this study is to advance our understanding of the occurrence and spatial distribution of microplastics and EDCs in riverine environments. Additionally, this study aimed to investigate the potential ecological impacts on freshwater organisms. By investigating the occurrence, potential synergistic effects, and interactions between these contaminants, this study sought to provide a comprehensive assessment of their distribution, extent, and associated risks to aquatic organisms and ecosystems. Moreover, this will contribute to identifying potential sources, pathways, and evaluating the ecological and physiological effects on key species within freshwater ecosystems. The findings of this study are crucial for enhancing our knowledge of the complex dynamics and risks associated with microplastics and EDCs in aquatic environments.

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The findings and outcomes of this study can establish a valuable baseline dataset regarding the occurrence of microplastics and EDCs in riverine ecosystems. This is particularly significant in the context of developing countries such as Malaysia, where high urbanization and extensive anthropogenic activities exert considerable impacts. This study holds significant importance in the context of environmental sustainability and human health, as it reveals the potential risks posed by microplastics and EDCs not only to aquatic ecosystems but also to human populations that rely on these ecosystems as a primary source of natural resources. Moreover, this study plays a vital role in safeguarding both environmental integrity and public health. Furthermore, this study can contribute to raising public understanding of the pressing issue of microplastics and the need for responsible waste management practices.

The findings of this study can serve as a fundamental basis and invaluable tool for enhancing legislation and policy ratification to mitigate the risks linked to microplastic and EDCs pollution in aquatic environments. This study goes beyond providing information on the occurrence of microplastics and EDCs in the environment, as it holds immense value for environmental monitoring and management purposes. Utilizing the insights gained from this study, effective measures can be implemented to protect the health and integrity of ecosystems and human populations. Ultimately, this study contributes to the broader goal of ensuring sustainable and healthy coexistence with our natural surroundings.

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1.6 Research framework of the study

The research framework, research matrix, and brief methodology (preparation, measurement, and data analysis) for the study on the occurrence and ecological risk assessment of microplastics and EDCs in the Selangor River Basin are depicted in **Figure 1.3**, **Table 1.1,** and **Figure 1.4**, respectively.

1.7 Thesis outline

This thesis comprises seven chapters dedicated to ascertaining microplastics and EDCs in the riverine environment of the Selangor River Basin. It encompasses a comprehensive assessment of the associated ecological risks, daily pollution loads, and diverse factors influencing the contamination of microplastics and EDCs through various land-use activities.

The chapters, from the introduction to conclusion, are structured as follows:

i. Chapter 1 serves as an introduction, providing a concise background of the study, presenting the problem statements, outlining the objectives, and defining the scope and significance of the study.
ii. Chapter 2 encompasses a comprehensive literature review that explores the occurrence, contamination level, sources, pathways, fate, potential effects, and associated risks of microplastics and EDCs in aquatic environments.
iii. Chapter 3 comprises the concentration and comparison of microplastics in both the surface water and sediment within the Selangor River Basin, to answer Objective 1.

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iv. Chapter 4 delves into the occurrence, spatial distribution, and comparative analysis of EDCs in the surface water and sediment of the Selangor River Basin, to answer Objective 2.

v. Chapter 5 explores the potential ecological risks associated with microplastics and EDCs in the Selangor River Basin, to answer Objective 3.

vi. Chapter 6 ascertains daily pollution loads and investigate the influence of different land-use patterns on the distribution of microplastics and EDCs in Selangor River Basin, to answer Objective 4.

vii. Chapter 7 provides a summary and conclusion of the findings presented in the thesis, as well as offers recommendations for future research.

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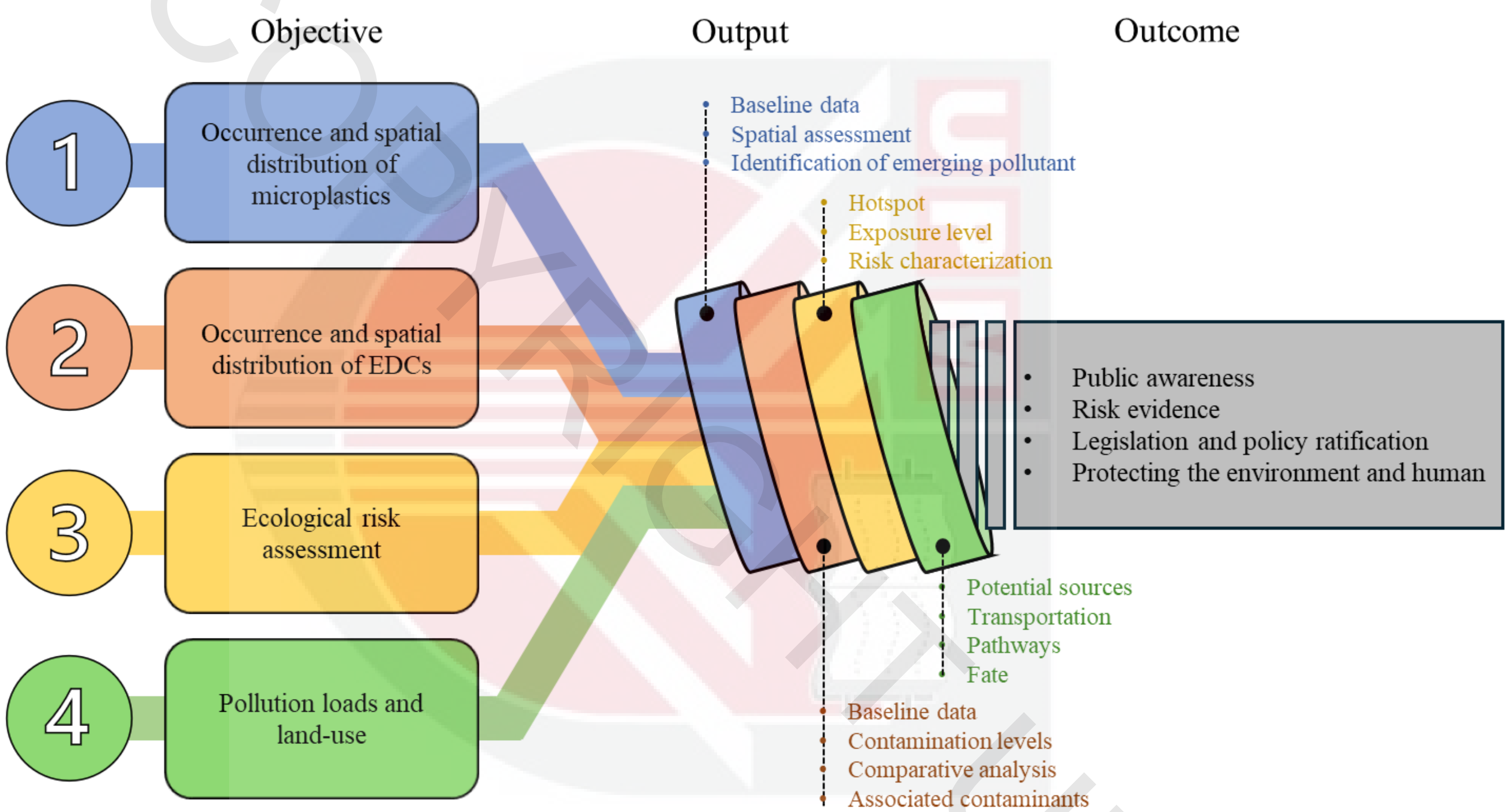

Figure 1.3: Research framework related to the occurrence and distribution of microplastics and EDCs in aquatic environments, risk assessment, daily pollution loads, and the influence of different land-use activities

Table 1.1: Research matrix related to the occurrence and distribution of microplastics and EDCs in aquatic environments, risk assessment, daily pollution loads, and the influence of different land-use activities

Justification			
○ The ubiquity of microplastics and EDCs raises concerns due to their potential adverse effects on aquatic ecosystems and human health. ○ Microplastics and EDCs exhibits characteristics of persistence, bioaccumulation, and biomagnification in the environment. ○ Riverine environments are a critical pathway for ecological and human exposure to the health risks of microplastics and EDCs. ○ Anthropogenic waste discharges and human activities heavily influence the occurrence and distribution of microplastics and EDCs.			
Research problems			
○ Lack of monitoring assessment on the co-occurrence of microplastic and EDCs in riverine environments. ○ Investigating the relationships between microplastics and EDCs. ○ Microplastics and EDCs can enter the food chain, leading to exposure for aquatic organisms and humans through the consumption of contaminated food and water sources. ○ Limited assessment of ecological risks associated with microplastics and EDCs. ○ Existing regulatory framework is ineffective in adequately addressing the sources and pathways of pollution caused by microplastics and EDCs. ○ The lack of political prioritization and effective regulation regarding the usage and discharge of plastic and EDCs in the environment directly causes continuous contamination by microplastics and EDCs.			
Objective 1	Objective 2	Objective 3	Objective 4
To ascertain the concentration, spatial distribution, and compare the occurrence of microplastics in samples of surface water and sediment collected from the Selangor River Basin.	To elucidate and compare the occurrence and spatial distribution of EDCs in surface water and sediment samples collected from the Selangor River Basin.	To assess the potential ecological risk associated with the occurrence of microplastics and EDCs in the Selangor River Basin.	To estimate the daily pollution loads and elucidate the influence of different land-use activities on the distribution of microplastics and EDCs within the Selangor River Basin.
Activities 1	Activities 2	Activities 3	Activities 4
○ Comprehensive examination of the occurrence and distribution	○ Comprehensive examination of the occurrence and distribution of	○ Risk quotient calculation and interpretation for potential ecological risk.	○ Riverine input calculations and their interpretation to

Table 1.1: Continued

of microplastics in surface water and sediment. ○ Surface water (5 L) and sediment (100 g) sampling and extraction analysis. ○ Physicochemical properties for descriptive analysis. ○ Statistical analysis: - One-way analysis of variance (ANOVA) to examine the statistical differences in microplastic concentrations among the three sampled regions (i.e., upstream, tributaries, and downstream). - Pearson correlation coefficients to evaluate the correlation between the abundance of microplastics and their characteristics or physicochemical parameters. - Pearson correlation analysis to ascertain the correlation between microplastics in both matrices (e.g., surface	EDCs in surface water and sediment. ○ Surface water (500 mL) and sediment (5 g) sampling. ○ Extraction and quantification 10 compounds of EDCs. ○ Physicochemical properties for descriptive analysis. ○ Statistical analysis: - One-way analysis of variance (ANOVA) to ascertain the statistical significance of differences in EDCs concentration across the three regions of the sampling sites (upper stream, tributaries, and lower stream). - Pearson correlation coefficients to assess the correlations between EDC concentrations and physicochemical parameters. - Pearson correlation analysis to ascertain the correlation between EDCs in both matrices (e.g., surface water	○ Data and descriptive analysis.	identify potential emissions, pathways, and routes of microplastics and EDCs in Selangor River Basin. - Daily load of microplastics and EDCs. - Mean environmental concentration of microplastics and EDCs. - Water discharge of Selangor River Basin. ○ Sample collection. ○ Land-use mapping. ○ Descriptive and comparative analysis. ○ Statistical analysis: - An independent sample *t*-test to elucidate the influences of different land-uses (e.g., rural areas and urban areas) on microplastics and EDCs concentrations in both matrices.

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Table 1.1: Continued

	water and sediment) of Selangor River Basin.	and sediment) of Selangor River Basin.

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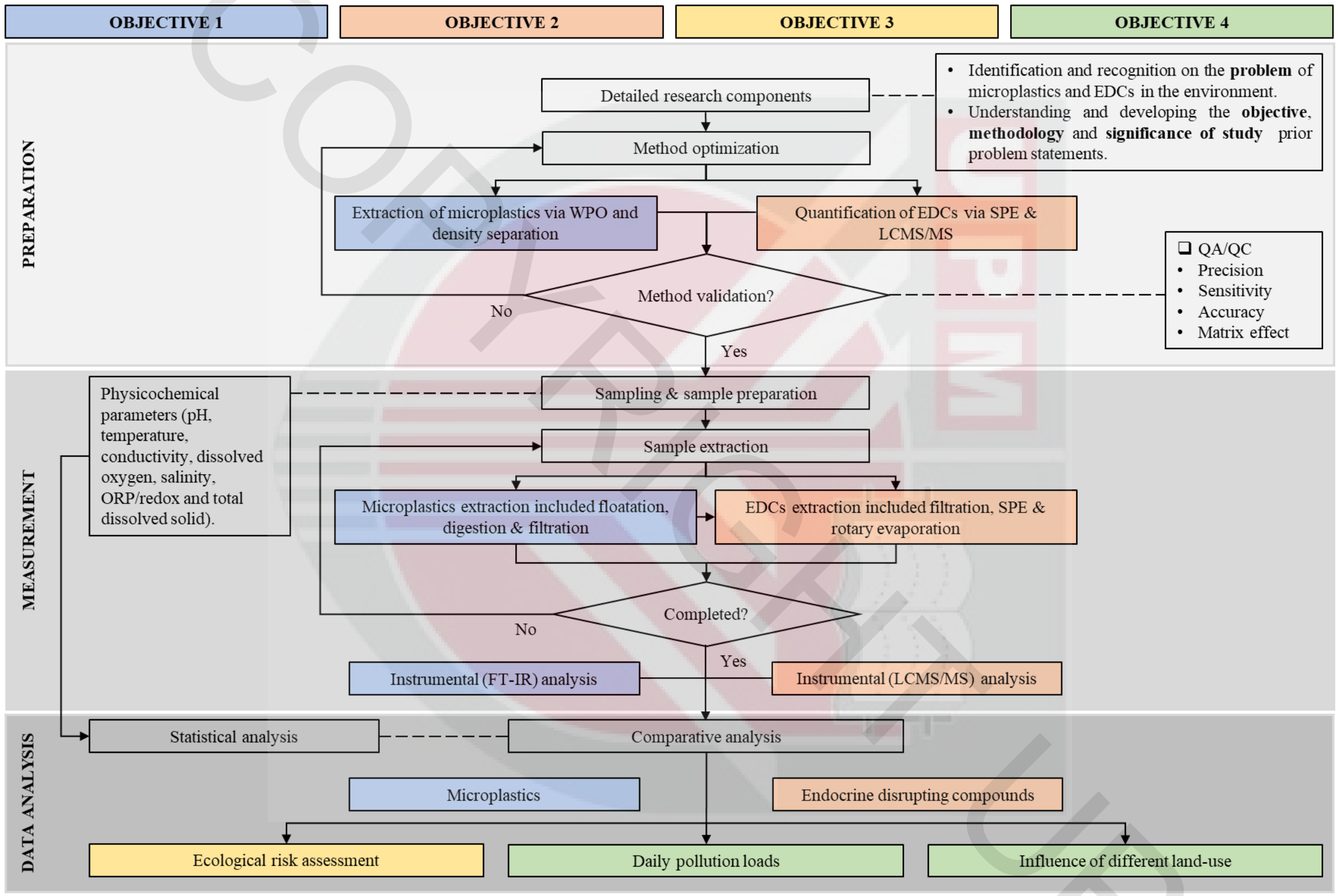

Figure 1.4: Methodology related to the occurrence and distribution of microplastics and EDCs in aquatic environments, risk assessment, daily pollution loads, and the influence of different land-use activities

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The mass production of plastics and industrial chemicals, which is associated with unethical practices, rapid urbanization, industrialization, overpopulation, and poor waste management, has led to various emerging pollutants, including microplastics and EDCs, in aquatic environments. Microplastics and EDCs are ubiquitous in aquatic environments and have various adverse effects on aquatic organisms and humans. Microplastics are ingested by various aquatic organisms and have been shown to transport toxic compounds into the environment (Teuten et al. 2009; Peng et al., 2017). EDCs can bind to and interfere with endocrine nuclear receptors, which could affect the endocrine system (Ning et al., 2007; Rubin, 2011). Subsequently, this disruption and exposure to microplastics and EDCs may be associated with various detrimental consequences for aquatic organisms, such as reproduction, immune disorders, growth, metabolic, and behavioral abnormalities. Additionally, these contaminants can accumulate in the food chain, leading to further exposure and potential harm to humans consuming contaminated seafood (Pironti et al., 2021).

This chapter is based on the papers:

a) Zaki, M.R.M., & Aris, A.Z. (2022). An overview of the effects of nanoplastics on marine organisms. Science of the Total Environment, 831, 154757. [Q1, IF: 9.8, Top 10%]
b) Zaki, M.R.M., Razak, M.R., & Aris, A.Z. Microplastics and plasticisers in the freshwater environments: A perspective on the distribution and associated risk [Science of the Total Environment – Under review, Q1, IF: 9.8, Top 10%].

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The existing literature has evaluated these contaminants in freshwater ecosystems from different perspectives, including environmental monitoring, ecological risk assessment, and ecotoxicological assessment. However, research on microplastics and EDCs as co-contaminants is lacking, contributing to a significant knowledge gap regarding the extent and fate of microplastics and EDCs in aquatic environments.

Microplastics have been detected in freshwater environments worldwide, floating in the water column and often mistakenly ingested by aquatic organisms as food. The ingestion of microplastics by a wide range of aquatic organisms has detrimental health impacts on ecosystems and humans. Abreo et al. (2019) indicated that the ingestion of microplastics has resulted in the fatality of the stranded whale shark (*Rhincodon typus*) in Tagum City, Davao del Norte, Philippines. In addition to the direct interaction (e.g., ingestion, adherence, and entanglement) of microplastics with aquatic organisms (Avio et al., 2015; Cole et al., 2015), microplastics are also capable of desorbing and leaching harmful chemicals into the environment (Batel et al., 2016; Ziccardi et al., 2016). These harmful chemicals, including plasticizers (e.g., phenolics, PAEs, and polyhalogenated compounds) and EDCs (e.g., hormones, pesticides, drugs, pharmaceuticals, and personal care products), have been linked to cancer, reproductive dysfunction, and endocrine disruption (Browne et al., 2007; Teuten et al., 2009; Wee and Aris, 2017b).

EDCs are emerging environmental contaminants comprising natural and man-made (synthetic) compounds that have been regarded to disrupt the endocrine systems of aquatic organisms and humans (Tamschick et al., 2016; Wee and Aris, 2017b). Contamination of freshwater by EDCs is a tremendous global concern because of the

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exposure of aquatic organisms and humans via drinking water consumption. Similar to microplastics, exposure to a wide diversity of EDCs has been associated with endocrine system dysfunction, including the nervous system (Aguirre-Martínez et al., 2016; Capolupo et al., 2016), reproductive capacity (Säfholm et al., 2014; Forte et al., 2016), metabolic syndromes (Préau et al., 2015), and growth (Witorsch, 2016; Yang et al., 2021).

Microplastics and EDCs have been widely detected in various environments, including aquatic organisms, and are thus emerging as an environmental concern. These contaminants can enter the environment via nonpoint sources (e.g., domestic waste and urban and agricultural runoff) and point sources (e.g., sewage, industrial, and municipal waste). In addition, various land-use types (e.g., residential, agricultural, recreation, transportation, and commercial) in terrestrial areas indicate variations in the abundance and distribution of microplastics and EDCs throughout the freshwater environment. All of these wastes may directly enter the freshwater environment and/or through discharge from wastewater and drinking water treatment plants. Therefore, it is concerning that the heavy pollution load of microplastics and EDCs from various types of land use into the freshwater environment could disrupt the ecosystem. Szalińska et al. (2021) estimated that up to 75% increment of metal loads in the suspended sediments based on the long-term predictions. However, the estimation of different types of land-use for microplastic and EDCs loads in freshwater environments remains limited.

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2.1.1 Microplastics

Plastics have been extensively used in daily human activities and industrial applications. The widespread use of plastics can be attributed to their convenience, affordability, light weight, and durability, which make them an ideal material for a vast array of consumer products such as food packaging, textiles, transportation, electronics, agriculture, healthcare, sports, and toys (**Table 2.1**). Extensive global plastic production has subsequently caused the ubiquity of plastics in the environment and microplastic pollution in the aquatic environment. Lebreton et al. (2017) estimated that over 2 million tons of plastic waste enters the ocean each year through rivers, with Asia contributing 67% of the global plastic input. Microplastics are particles smaller than 5000 µm that can be classified as primary and secondary microplastics.

Primary microplastics are intentionally manufactured plastics that are extensively used in a variety of industrial and commercial applications, including cosmetics and personal care products (e.g., facial cleansers, toothpaste, shampoo, and soap) (Gouin et al., 2015), as well as raw material plastic pellets (e.g., nurdles or mermaid tears) (Sundt et al., 2014). The predominant types of primary microplastics detected in cosmetics and other personal care products are spherical beads employed as scrubbers and abrasives. Secondary microplastics, on the other hand, are small pieces of plastic that are made when larger pieces of plastic break down in the environment owing to chemical oxidation, breakdown of microorganisms, mechanical abrasion, and weathering (Andrady, 2017). Secondary microplastics are often reported as the most prevalent microplastics, which mainly originate from the shedding of clothes during laundry (Haap et al., 2019; Kelly et al., 2019), degradation of nets or fishing gear

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(Wright et al., 2021; Zaki et al., 2021a), and fragmentation of single-use plastics (Lambert and Wagner, 2016).

Microplastics are synthetic polymers that are produced by multiple chemical reactions and additives to enhance the mechanical, chemical, and thermal properties of microplastics. The common types of synthetic polymers are polyethylene (PE), polystyrene (PS), polypropylene (PP), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polycarbonate (PC), polymethyl methacrylate (PMMA), and polyamide (PA). Synthetic polymers have a wide range of uses and applications. For instance, PE, PET, PP, PS, and PA are mainly used as food packaging materials (e.g., plastic bags, containers, bottles, and plastic films), textiles (e.g., clothes, carpets, ropes, and nets), and personal care products. Based on PlasticsEurope (2021), the highest global demand for plastic materials is packaging (40.5%), followed by construction and building (20.4%), automotive sector (8.8%), electronics and electrical (6.2%), sports and household (4.3%), and agriculture (3.2%).

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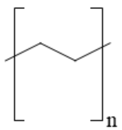
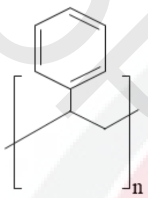
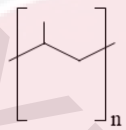
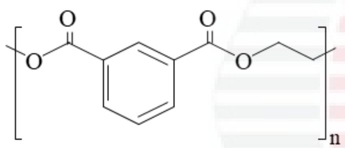
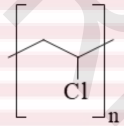
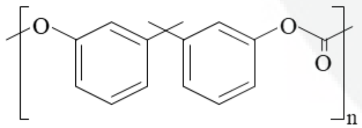
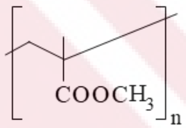
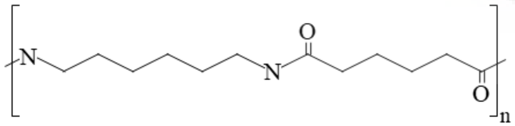
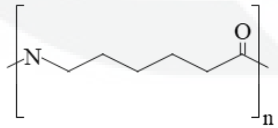
Table 2.1: The respective example, structure and application of synthetic polymers (microplastics) in the environment

Example and structure of synthetic polymer	Application
 Polyethylene (PE)  Polystyrene (PS)  Polypropylene (PP)  Polyethylene terephthalate (PET/PETE)  Polyvinyl chloride (PVC)  Polycarbonate (PC)  Poly(methyl methacrylate)  Polyamide (nylon) 6,6  Polyamide (nylon) 6	Automotive - Dashboard - Door panel - Seatbelt - Tyre Construction - Pipe - Wire and cable insulation Consumer products - Cosmetics - Housewares - Personal care products - Toys Electronics - Fuse-boxes - Plugs and sockets - Wire and cables Medical - Blood bags - Surgical eyewear and gloves - X-ray film Packaging - Bottle and container - Plastic bags Textiles - Clothes - Fishing net and ropes - Furniture

Moreover, packaging materials are predominantly found as secondary microplastics in the environment, which mostly originate from inland waters and pollute the entire aquatic ecosystem. Meanwhile, polyester (also known as PET) is commonly used for fibers and fabrics in the textile industry. Despite polyester being introduced by the textile industry, domestic laundry discharges also contribute to the contamination of polyester. As reported by Napper and Thompson (2016), a single washing cycle, involving a 6 kg load of acrylic fabric, could potentially release over 700,000 fibers into the aquatic environment. Other types of microplastics, such as PVC, PC, and PMMA, are mainly used for building and construction (e.g., pipes and cable insulation), electronics, and automotive (e.g., headlamp lenses, sun visors, and door panels). This indicates that microplastics are an emerging global environmental concern considering their wide range of applications and usage by human activities. In Europe, almost 70% of the plastic market demand is represented by European countries, including Germany, France, Italy, Poland, the United Kingdom, and Spain (PlasticsEurope, 2021).

2.1.2 Endocrine disrupting compounds (EDCs)

EDCs have been considered emerging pollutants owing to their widespread presence in the environment and severe consequences for the ecosystem. The aquatic environment constantly receives tons of EDCs, which mainly originate from untreated and treated wastewater, thus posing a potential threat and adverse effects to the water quality and endocrine systems of aquatic organisms (Aris et al., 2014; Zhang et al., 2017). EDCs are classified into two types based on their origin: natural and synthetic. Natural EDCs consist of steroidal hormones (progestogen, androgen, and estrogen) and nonsteroidal hormones (phytoestrogen), which are commonly used as growth

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stimulants and hormonal therapies (Wee et al., 2019). Synthetic compounds are chemicals that have been used in various industrial, agricultural, aquaculture, and pharmaceutical industries.

A plethora of applications and extensive utilization have contributed to the introduction of emerging organic contaminants through sewage systems (Blum et al., 2018). For instance, alkylphenols have been widely used for more than half a century in multiple applications across several sectors, including industry, households, and commercial sectors, despite their interference with estrogenic activities in organisms (Bergé et al., 2014). Synthetic compounds of EDCs, such as PFAS, phenolic compounds, and PAEs, are commonly used as additives in plastic manufacturing (Wee and Aris, 2017b). Considering that plastics, microplastics, and nanoplastics are ubiquitously present in the environment (Zaki and Aris, 2022), it is possible that EDCs could leach out and intensify their concentration.

EDCs are highly persistent, bioaccumulative, and toxic to the environment (Ismail et al., 2018), posing a potential threat to aquatic organisms and human health once released into the environment. EDCs include a wide spectrum of environmental persistent organic pollutants (POPs), such as polyhalogenated chemicals, phenolic compounds, PAEs, pesticides, hormones, and medicines. For instance, typical plasticizer compounds such as bisphenol A (BPA), nonylphenol (NP), octylphenol (OP), bis(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), and diisobutyl phthalate (DIBP) have been classified as substances of high concern by the Registration, Evaluation, and Authorization of Chemicals (1907/2006) of European Union regulation. At low concentrations or trace levels (ng/L or µg/L), EDCs can

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modify and imitate the effects of hormones during a wide range of developmental stages (Ismail et al., 2018). Moreover, EDCs are linked to endocrine system dysfunction, which causes carcinogenic, mutagenic, and neurotoxic effects in organisms and humans (Aris et al., 2014). For instance, contamination poses a wide range of severe health risks, such as the induction of an immune response, metabolic disorders, cancers, obesity, diabetes, reproductive problems, and reduced growth rate (Wee et al., 2022).

2.2 Occurrence and levels of microplastics and endocrine disrupting compounds in aquatic environments

The presence of microplastics and EDCs in various environmental compartments worldwide is now widely recognized as a significant concern and has been extensively studied. These pollutants heavily contaminate aquatic ecosystems, including rivers and estuaries, owing to the discharge of anthropogenic waste from both point and nonpoint sources. Among these environmental compartments, rivers and estuaries are particularly vulnerable to the harmful effects of microplastics and EDCs. Rivers, as freshwater environments, play a critical role as the primary recipients of anthropogenic waste containing a diverse range of emerging contaminants, including microplastics and EDCs. This influx of pollutants disrupts the delicate balance of aquatic ecosystems and poses risks to both aquatic life and human health. It is imperative to emphasize that the translocation of these contaminants is not exclusive to freshwater environments. Instead, these pollutants are carried downstream towards estuarine and marine environments. This translocation significantly amplifies the potential for adverse effects on a broader scale, impacting not only local ecosystems but also broader marine biodiversity and food webs.

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Microplastics have been studied in a variety of environmental compartments in surface water, sediments, and biota from rivers (Wong et al., 2020; Kabir et al., 2022), estuaries (Firdaus et al., 2020; Zaki et al., 2021a), coastal areas (Kim et al., 2015; Zainuddin et al., 2022), and the ocean (Zhu et al., 2019; Taha et al., 2021). Both freshwater and marine ecosystems pose analogous risk factors capable of disrupting their intricate ecological balance. For instance, estuaries receive an abundance of marine and terrestrial litter because of the strategic location linking land and sea (Yan et al., 2019; Zhang et al., 2019b). Estuaries consist of various habitats, including mangroves, and are important for reducing coastal erosion by slowing down water flows and encouraging sediment deposition. Mangroves act as nursery grounds and provide habitats for marine birds, mammals, fish, and crustaceans. Numerous studies have revealed microplastic ingestion by various organisms, such as plankton (Setälä et al., 2014), fishes (Schmid et al., 2018; Garnier et al., 2019; Su et al., 2019; Yuan et al., 2019), crustaceans (Devriese et al., 2015; Hossain et al., 2020), and mollusks (Li et al., 2015; Abidli et al., 2019; Cho et al., 2019; Domogalla-Urbansky et al., 2019).

Ingestion of microplastics causes adverse effects to organisms. For instance, research has found growth energy depletion in crabs (*Carcinus maenas*) due to the ingestion of microfibers of 1000 to 5000 μm (Watts et al., 2015). In addition, mortality and deficient reproduction of *Daphnia magna* were caused by chronic exposure to 1–5 μm microplastics (Pacheco et al., 2018). Estuary areas have been proposed as a pathway for microplastics to enter marine ecosystems. An estuary is a semi-closed ecosystem with water input from the land and ocean, where various contaminants can potentially accumulate. An estuary can act as a sink for pollutants (Haris and Aris, 2015; Looi et al., 2015). This unique ecosystem provides shelter for organisms such as fishes,

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crustaceans, and mollusks. Furthermore, estuaries act as buffer zones that protect ecosystems and habitats from natural processes, such as tsunamis and coastal erosion.

EDCs have been discovered in various environmental compartments, including surface water, sediment, and biota. Different groups of EDCs, such as phenolic compounds (plasticizers), pharmaceuticals, PFAS, hormones, PAEs, and pesticides, are frequently detected in marine and estuarine environments (Ismail et al., 2018; Omar et al., 2018; Ismail et al., 2020; Ismail et al., 2021; Zainuddin et al., 2023). In fact, DEHP has been detected in bowhead whales (*Balaena mysticetus*) at concentrations of up to 398 ng/g wet weight (Routti et al., 2021). Although freshwater environments are frequently detected and linked to the origin of EDCs and serve as a pathway for EDCs to reach the oceans, the occurrence and distribution of EDCs in river basins remain scarce (Gorga et al., 2015; Ismanto et al., 2022a). River basins have different and more complex dynamics than other environments in terms of transportation, retention, and dispersion, along with multiple sources of contamination that contribute to pollution. Rivers are often used as essential sources of drinking water, despite the fact that rivers are considered major routes for the transportation of EDCs to aquatic organisms.

Previous studies have mainly investigated the occurrence of EDCs in the surface water from the Pearl River (Gong et al., 2016), Danube River (Milanović et al., 2016), and Langat River Basin (Wee and Aris, 2017a; Wee et al., 2019). Therefore, it is crucial to study the occurrence and distribution of EDCs in river basins, which encompass multiple land uses in both rural (e.g., agricultural and forest) and urban (e.g., industrial and residential) areas. The presence of EDCs in riverine environments is primarily

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associated with anthropogenic activities, which have gradually received worldwide attention. The contamination and accumulation of EDCs in aquatic organisms may be attributed to sediment contamination (Casatta et al., 2015; Liu et al., 2017b). This indicates that humans are highly exposed to EDCs contamination through drinking water and consumption of contaminated food. Although various studies have reported the occurrence of microplastics and plasticizers in riverine and estuarine environments, few have evaluated their co-occurrence in the environment (**Table 2.2**).

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Table 2.2: The occurrence of microplastic and endocrine disrupting compound (plasticizer) in the environmental compartment across regions and countries

Sample	Location	Microplastic		Plasticizer		Reference
		Polymer	Concentration	Compound	Concentration	
Water	Faafu Atoll, Maldives	$\sum_5$MPs	0.03–0.65 items/m^3	$\sum_6$PAEs	22–313 ng/g	Saliu et al. (2019)
	Marseille Bay, France		0.0–0.3 items/m^3	$\sum_7$PAEs	100–527 ng/L	Schmidt et al. (2021)
	Bushehr port, Iran	$\sum_4$MPs	63–92 items/L	$\sum_7$PAEs	0.86–6.16 mg/L	Mohammadi et al. (2022)
	Pearl River Delta, China	$\sum_7$MPs	0.003–2.090 items/m^3	$\sum_{21}$PFASs	3.11–9.07 × 10^3 ng/g	Cheng et al. (2021)
	Jiaozhou Bay, China	$\sum_8$MPs	24.44–180.23 items/m^3	$\sum_6$PAEs	129.96–921.22 ng/L	Liu et al. (2020)
	Persian Gulf, Iran		1.2–3.0 items/L	$\sum_6$PAEs	25.32–137.54 µg/L	Hajiouni et al. (2022)
	East Lake, China	$\sum_8$MPs	952.38–10285.71 items/m^3	$\sum_{16}$PAHs	22.87–73.65 ng/L	Shi et al. (2023)
Sediment	Shandong coastal, China	$\sum_3$MPs	5–1090 items/kg	$\sum_9$PAEs	<LOD–69.9 ng/g	Zhang et al. (2018)
	Hong Kong coastal, Hong Kong	$\sum_8$MPs		$\sum_3$Bisphenols	ND–4961 ng/g	Lo et al. (2021)
	Gulf of Guinea, Nigeria	$\sum_9$MPs	36–1730 items/m^2	$\sum_6$PAEs	<MDL–164.09 mg/kg	Benson and Fred-Ahmadu (2020)
	Renuka Lake, India	$\sum_3$MPs	15–632 items/kg	$\sum_4$PAEs	6–357 ng/g	Ajay et al. (2021)
	Zhejiang coastal, China	$\sum_3$MPs	666.1 items/kg	$\sum_7$PCBs	0.15–38.40 µg/kg	Su et al. (2023)
	East Lake, China	$\sum_8$MPs	735.43–5021.78 items/kg	$\sum_{16}$PAHs	445.12–1370.33 ng/g	Shi et al. (2023)
	Lagos shoreline, Nigeria	$\sum_9$MPs	0.01–0.77 items/m^2	$\sum_{13}$PAHs	0.00–0.32 mg/kg	Fred-Ahmadu et al. (2022)
				$\sum_{17}$PCBs	0.00–0.53 mg/kg	
	Lagos lagoon, Nigeria			$\sum_{13}$PAHs	0.04–0.26 mg/kg	
				$\sum_{17}$PCBs	0.11–0.34 mg/kg	
Soil	Shihezi, China	$\sum_6$MPs	650–36450 items/kg	$\sum_{14}$di-PAEs	55.60–1236.64 µg/kg	Xu et al. (2022b)
	Jiangsu Province, China	$\sum_8$MPs	380–3786 items/kg	$\sum_6$PAEs	0.30–1.58 mg/kg	Li et al. (2021c)
Sewage sludge	USA	PET	28–12000 µg/g	TPA	<0.085–75 µg/g	Zhang et al. (2019c)
		PC	0.7–840.0 µg/g	BPA	0.0083–2.5 µg/g	
	Taiwan		0–7 items/g	$\sum_{10}$PAEs	2062–32414 µg/kg	Wang et al. (2022b)

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Table 2.2: Continued

Cetaceans	Macaronesian region, NE Atlantic	$\sum_3$MPs	59.08 fibers/animal 3 fragments/animal	$\sum_3$Bisphenols DEHP DEP	4–984 ng/g 102–1533 ng/g 13–225 ng/g	Montoto-martínez et al. (2021)
Holothurians	Cabrera Island, Spain	$\sum_3$MPs	12.7 ± 7.3 items/ind.	BPA $\sum_3$PAEs	0.45 ± 0.04 µg/g 0.49–1.48 µg/g	Rios-Fuster et al. (2022)
Bivalves	Cabrera Island, Spain	$\sum_4$MPs	4.8 ± 5.4 items/ind.	$\sum_3$Bisphenols $\sum_3$PAEs	0.25–0.29 µg/g 0.54–2.58 µg/g	Rios-Fuster et al. (2022)
	Channel coastal, France	$\sum_6$MPs	0.56–2.46 items/ind.	$\sum_{15}$PAHs $\sum_6$PAEs	0.06 ± 0.02 ng/g 29.18–75.53 ng/g	Hermabessiere et al. (2019)
			0.76–0.78 items/ind.	$\sum_{15}$PAHs $\sum_6$PCBs $\sum_6$PAEs	5.48 ± 3.09 ng/g 1.00 ± 0.59 ng/g 26.36 ± 18.16 ng/g	
Fish	Estuaries of northern Taiwan		0.185 ± 0.338 mg/g	BPA NP $\sum_6$PAEs	1.5 ± 2.2 ng/g 17.4 ± 29.1 ng/g 2.90–52.90 ng/g	Lu et al. (2021a)
	Cabrera Island, Spain	$\sum_3$MPs	3 ± 4.4 items/ind.	BPA $\sum_3$PAEs	0.32 ± 0.06 µg/g 0.17–0.88 µg/g	Rios-Fuster et al. (2022)
	Fengshan River, Taiwan	$\sum_{16}$MPs	14–94 items/fish	$\sum_{18}$PAHs	ND–1.001 mg/kg	Tien et al. (2020)

MPs: polymer composition of microplastic; PET: polyethylene terephthalate; PC: polycarbonate; PAEs: phthalates; di-PAEs: phthalate diesters; PFASs: poly- and perfluoroalkyl substances; PAHs: polycyclic aromatic hydrocarbons; PCBs: polychlorinated biphenyls; TPA: terephthalic acid; BPA: bisphenol A; DEHP: bis (2-ethylhexyl) phthalate; DEP: diethyl phthalate; NP: nonylphenol; <LOD: below limit of detection; ND: not detected; <MDL: below method detection limit

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2.3 Sources, pathways, and fate of microplastics and endocrine disrupting compounds in the ecosystem

The widespread presence of microplastics and EDCs in aquatic environments has garnered considerable attention, highlighting the need to better understand their origins, sources, pathways, and fate in the environment. Microplastics can be characterized based on their sources as primary microplastics and secondary microplastics arising from various anthropogenic activities. Primary microplastics are microsized plastic particles that are intentionally manufactured and engineered for specific purposes. Examples of primary microplastics include microbeads used in personal care products for exfoliation, plastic pellets employed as raw materials in manufacturing plastic products, and synthetic fibers used in textiles, such as polyester and nylon. Secondary microplastics include the degradation and fragmentation of larger plastics (e.g., bottles, bags, and containers) into particles.

Generally, plastics undergo processes such as photodegradation, mechanical abrasion, and weathering, causing them to fragment into smaller pieces and microplastics. Nevertheless, these particles deliberately produce microplastics that directly enter the environment through various pathways, contributing to the widespread issue of microplastic pollution. The ubiquity of microplastics mainly originates from terrestrial sources, which are subsequently transported through river runoff. Domestic and industrial sewage (wastewater and drinking water treatment plants) have been identified as major sources of microplastic pollution, receiving tons of anthropogenic waste from industrial, agricultural, aquaculture, recreational, and residential areas (Eriksen et al., 2014; Zaki and Aris, 2022).

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Sources of EDCs in the environment mainly include various industrial products (e.g., plastics, pesticides, herbicides, and flame retardants), agricultural use, pharmaceuticals, and personal care products (Wee et al., 2019). For instance, PVC and BPA are known to leach EDCs into the environment. These chemicals are often used in the production of plastic bottles, food containers, and other everyday products. When exposed to heat or other environmental factors, these plastics can release EDCs, which can enter food and water supplies (Le et al., 2008; Chen et al., 2019; Zaki and Aris, 2022). Moreover, EDCs can be byproducts or components of various industrial processes, including the production of plastics, solvents, and dyes. These chemicals can enter the environment through industrial discharge and waste streams.

The main derivation of microplastics and EDCs arises from a variety of human-induced activities, collectively referred to as anthropogenic activities. These activities are primarily concentrated in urbanized regions, which subsequently interact with and influence various environmental media, including surface water, groundwater, sediment, and plant and aquatic organisms. Therefore, anthropogenic activities are the major contributors to microplastic and EDCs pollution in the environment. Yu et al. (2016) indicated that the input of microplastics into the coastal area of China was attributed to the prevalence of heavy industries and urbanization from terrestrial sources. Langat River surface water was quantified to contain 14 multiclass EDCs, including plasticizers, pesticides, pharmaceuticals, and hormones, which were attributed to the discharge of domestic and industrial waste into the Langat River Basin (Wee et al., 2019). Similarly, Shi et al. (2023) indicated that the input of microplastics and $\sum_{16}$PAHs in the water and sediment of East Lake, China, was heavily influenced by anthropogenic activities rather than the interaction of pollutants.

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Microplastics and EDCs share some common routes of transport, including transport through rivers and streams. However, their dispersion in the environment is complex and multifaceted. Rivers and streams are significant pathways for both microplastics and EDCs to enter larger water bodies, such as lakes and oceans. Stormwater, rainwater, and surface runoff can carry these pollutants from urban areas, agricultural lands, and industrial sites, eventually depositing them into water bodies. Once in the water bodies, these contaminants can be transported downstream by the flow of the river, spreading the pollutants to different regions, including estuarine and marine environments.

In aquatic environments, both microplastics and EDCs can settle and accumulate in sediments. Microplastics, particularly denser ones, can sink and become buried in sediments, where these particles may remain for long periods (Chubarenko et al., 2016; Kooi et al., 2017; Berezina et al., 2021). In the environmental compartment of surface water, low-density microplastics such as PE and PP float, whereas high-density microplastics such as PVC sink into the sediment. However, Huang et al. (2021a) identified the occurrence of low-density microplastics in sediments, suggesting that the density of microplastics may have been increasing. Microplastics in the environment may be exposed to various environmental conditions and organic matter, which could influence their density (Galloway et al., 2017). Therefore, exposed microplastics gradually sink into the sediment over time. Microplastics have been demonstrated to transport various emerging pollutants, including heavy metals (Brennecke et al., 2016; Selvam et al., 2021) and hydrophobic organic pollutants (Liu et al., 2019b), thus increasing the exposure and contamination of aquatic organisms.

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EDCs can also adsorb to sediments and be stored there, potentially releasing back into the water column under physical disturbances, changes in water flow, temperature fluctuations, biological activity, and chemical interactions (Tran et al., 2013; Aris et al., 2014; Wilkinson et al., 2017; Mohapatra et al., 2021; Tong et al., 2022). Ultimately, these contaminants can be ingested by various aquatic organisms, including fish, shellfish, and plankton, leading to their potential bioaccumulation in the food chain. EDCs can bioaccumulate in the tissues of organisms, and their transfer through the food web can result in higher concentrations at higher trophic levels.

Microplastics and EDCs are highly persistent in the environment. Owing to their synthetic nature, these contaminants are resistant to biodegradation by natural processes. As a result, once released, microplastics and EDCs can persist in the environment for an extended period, ranging from decades to centuries. Because of their small size, microplastics can be passed through sewage treatment without posing a threat to aquatic organisms. For instance, Mason et al. (2016) indicated that wastewater treatment in the United States released more than four million microparticles per day, consisting of fibers and fragments. Therefore, these tiny particles exposed to the environment can be ingested by various aquatic organisms from lower to higher trophic levels (Chae et al., 2018; Walkinshaw et al., 2020). This enhances the bioavailability of microplastics to a wide range of aquatic organisms, indicating an emerging concern for the environment.

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In addition, the greater surface area and hydrophobic properties of microplastics make them relatively prone to the adsorption of heavy metals and organic contaminants in the environment (Andrady, 2011). The adsorption capacities of plastics, such as PE, PS, and PP, have been tested with hydrophobic organic chemicals, including polycyclic aromatic hydrocarbons (PAHs), hexachlorocyclohexanes, and chlorinated benzenes (Lee et al., 2014). The study reported the high sorption of plastics to HOCs and indicated that adsorption could reduce the concentration of organic chemicals in the environment. Plastics are generally manufactured with additive plasticizers, such as polybrominated diphenyl ethers, PAEs, and BPA, to enhance their durability and abrasion resistance. These additives eventually leach into aquatic environments, posing an emerging environmental concern. Plasticizers commonly leach out from the surface of plastics after being exposed to environmental conditions, while EDCs have been found to adhere to the surface of microplastics. The interaction between hydrophobic contaminants and microplastics may influence their occurrence in the aquatic environment by electrostatic forces, hydrophobic partitioning, and van der Waals forces (Wang et al., 2020c; Prajapati et al., 2022). These forces facilitate the adsorption and desorption of contaminants on the surface of microplastics, potentially leading to harmful effects on aquatic life and human health.

Research has demonstrated that microplastics possess unique features such as strong hydrophobicity, porous structure, and large surface area. These properties allow microplastics to absorb and collect a wide range of pollutants, including PAHs, flame retardants, antibiotics, and pesticides, in a process known as sorption (Koelmans et al., 2016; Alimi et al., 2018). Microplastics can significantly impact the environmental behavior, bioavailability, and toxicity of organic pollutants in aquatic environments.

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As carriers and releasers of these pollutants, microplastics can modify their fate and become more available to various aquatic organisms, thus altering their effects. Ultimately, the co-occurrence of microplastics and EDCs or plasticizers could represent a preliminary indication that microplastics may transport EDCs or plasticizers into a wide range of environmental compartments.

Ultimately, microplastics and EDCs in the environment present complex and intertwined challenges. The detailed sources, pathways, exposure media, routes, and fates of microplastics and EDCs in the freshwater environment are depicted in **Figure 2.1**. Microplastics, being highly persistent, can accumulate and spread throughout ecosystems, impacting aquatic life and even entering terrestrial environments. The processes of fragmentation and trophic transfer magnify their presence, posing potential threats to wildlife and human health. Similarly, EDCs exhibit diverse fates, with some being persistent pollutants that disperse widely through air, water, and soil. Their ability to bioaccumulate and bio-magnify further amplifies their impact on various organisms, disrupting hormonal systems and potentially leading to adverse effects on reproduction and development. The interplay between these pollutants in the environment underscores the need for urgent action and comprehensive strategies. Mitigating the fate of microplastics and EDCs requires a multi-pronged approach, encompassing global efforts to reduce plastic waste, adopt sustainable practices in agriculture and industry, enhance wastewater treatment methods, and enforce stricter regulations on the use and disposal of EDC-containing products.

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2.4 Associated risks and combined effects

The ubiquity of microplastics and EDCs in freshwater environments has emerged as a major global environmental issue with a wide range of health consequences on aquatic organisms and humans. Recent studies have raised concerns about the occurrence, effects, and risk of microplastics in freshwater environments, while the co-occurrence, combined effects, and associated risk of microplastics and EDCs have scarcely been reported (Huang et al., 2021b). As microplastics are relatively small particles that float abundantly in the water column, they are easily ingested, adhered, and entangled by various aquatic organisms. The ingestion of microplastics mainly causes physical injuries, such as gut and digestive system blockage, which may impair their feeding behavior, survival, and reproduction (**Figure 2.2**). Furthermore, microplastics exhibit various physicochemical properties, including surface area, hydrophobicity, size, shape, chemical stability, and density, which can contribute to the occurrence of other contaminants, such as plasticizers and EDCs, in aquatic environments. Specifically, the hydrophobic nature of microplastics enables them to adsorb emerging organic chemicals, including EDCs, thereby amplifying their distribution and potential exposure to aquatic organisms. The combined effects of microplastics with plasticizers, EDCs, and heavy metals have been reported in previous studies (Huang et al., 2021b).

Because microplastics are not limited to physical exposure, they may also possess chemical effects. This is because of the unique characteristics of microplastics in the environment, which enable them to adsorb and desorb emerging contaminants. Therefore, exposure can increase the potential of various detrimental effects, especially at the molecular and cellular levels of organisms. The occurrence of

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microplastics and EDCs in the environment has also been reported to induce toxicity at the molecular and cellular levels in aquatic organisms. For instance, Yu et al. (2020) suggested that co-exposure of zebrafish (*Danio rerio*) to microplastics and Tetrabromobisphenol A (TBBPA) significantly induces an antioxidative stress response. Moreover, the co-exposure of green algae (*Chlorella pyrenoidosa*) and freshwater snails (*Cipangopaludian cathayensis*) to microplastics and methamphetamine resulted in higher oxidative damage and apoptosis (Qu et al., 2020). These stress responses are responsible for maintaining cellular homeostasis and preventing oxidative damage.

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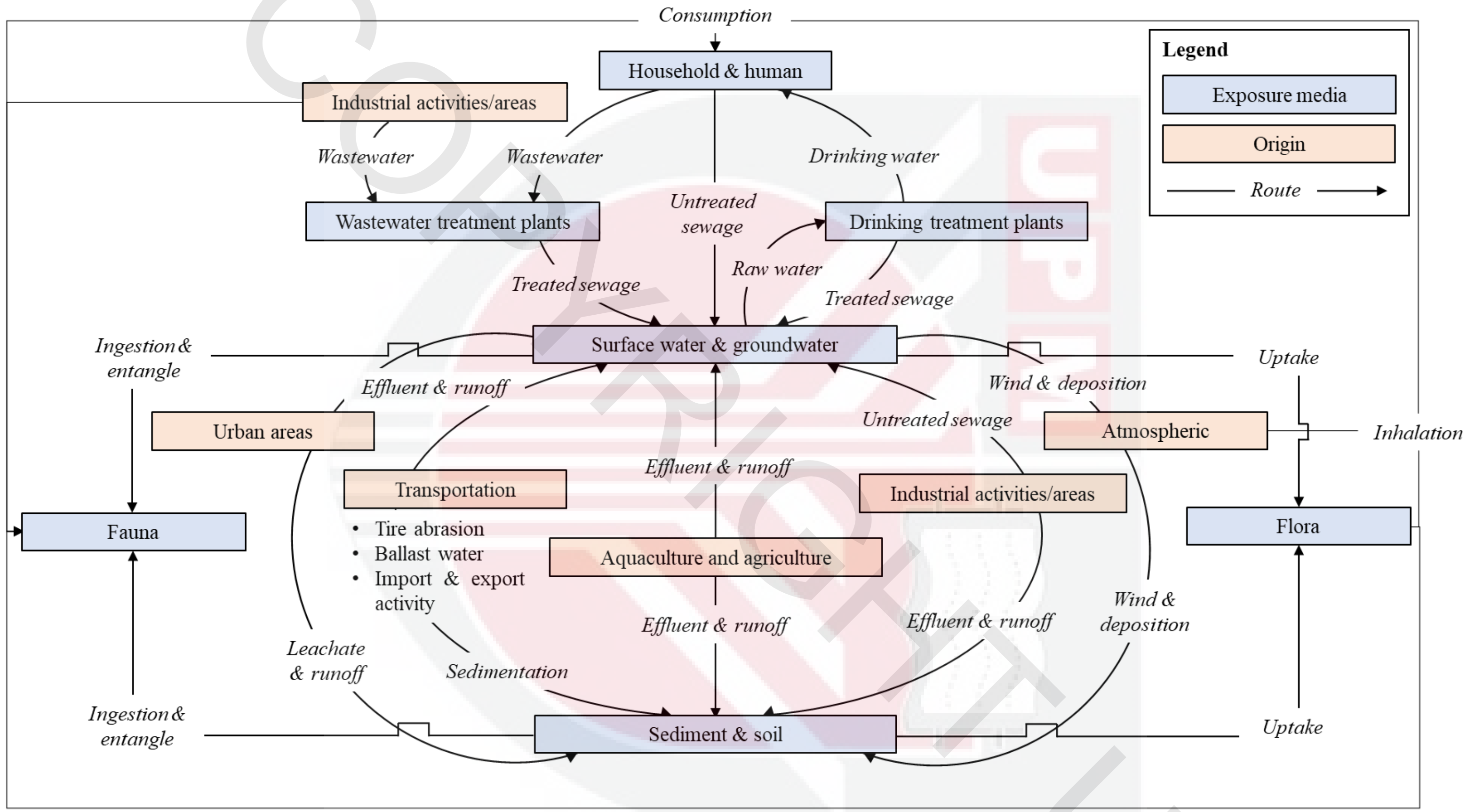

Figure 2.1: Conceptual map of the origin, exposure media, route, and fate of microplastics and EDCs in the environment, exposed and introduced from various sources, and eventually making their way up to the food chain

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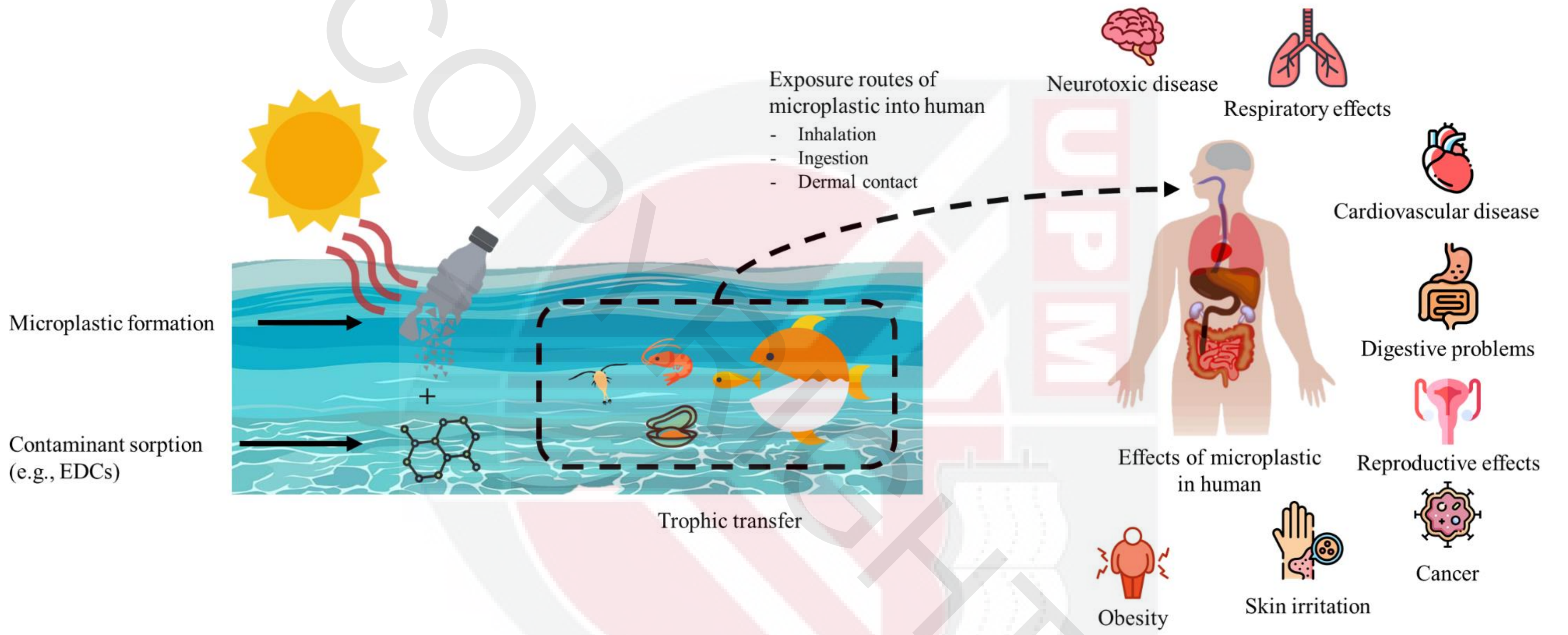

Figure 2.2: Potential associated risks and combined effects of microplastics and EDCs in the aquatic environment

In contrast, Pittura et al. (2018) indicated that adsorbed benzo[a]pyrene from microplastics slightly induced marked ecotoxicological effects at the molecular level in the Mediterranean mussel (*Mytilus galloprovincialis*) after 28 days of exposure. Similar species have also been reported by Avio et al. (2015), where the adsorption of pyrene to various tissues, including digestive tissues, hemolymph, and gills, caused adverse effects at both the molecular and cellular levels, such as immunological responses, peroxisomal proliferation, reduced antioxidant defenses, neurotoxicity, genotoxicity, and altered gene expression. Microplastics could also be a vector for inorganic contaminants, such as heavy metals. For instance, Lee et al. (2019b) observed inhibition in the production of reactive oxygen species (ROS) from the combined effects of nanoplastics and heavy metals in zebrafish. This is concerning as EDCs may interfere with cellular signalling pathways at the molecular level, causing changes in cellular functions such as hormone control, metabolism, and gene expression (Herrero et al., 2015; Zhang et al., 2022). Microplastics may harm cells and tissues by causing mechanical stress or releasing harmful compounds, such as plasticizers and EDCs.

Microplastics and EDCs may also affect individual and organ levels of aquatic organisms. Exposure to microplastics and EDCs has been reported to cause a wide range of adverse effects on individual physiological processes, including reproductive and growth rates, neurotoxicity, immune system dysfunction, and organ damage. Aquatic organisms may be exposed to these contaminants through ingestion, inhalation, and dermal exposure, thus increasing toxicity and the potential for chronic health effects. Because microplastics and EDCs are persistent in the environment, these contaminants not only affect specific exposed individual organisms but also

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could bioaccumulate and biomagnify to various trophic levels. Batel et al. (2016) demonstrated the trophic transfer of benzo[a]pyrene between shrimp nauplii (*Artemia* sp.) and zebrafish. This biomagnification was facilitated by microplastics, highlighting the potential of these harmful chemicals to accumulate and concentrate as these particles are passed on to organisms higher up in the food chain.

Furthermore, the accumulation of microplastics and EDCs has been reported to increase mortality and inhibit the growth rate of organisms. At the individual and organ levels, the combined effect of microplastics and EDCs may have considerable effects on organism health and survival, as well as wider ecosystem-level effects. The use of chemical additives in plastics has raised significant concerns regarding the potential harm to aquatic organisms through both direct and indirect ingestion of microplastics. Such exposure may result in severe negative impacts on the reproductive, respiratory, and neurotoxicological systems of aquatic life, as well as induce stress responses. Moreover, it is possible that these chemicals could also pose health risks to humans. Therefore, it is critical to continue exploring the effects of these compounds and develop measures to limit their environmental and human health implications. Overall, microplastics and EDCs could have a combined effect at the molecular, cellular, individual, and organ levels when these contaminants enter the aquatic environment and interact with aquatic organisms (**Figure 2.3**).

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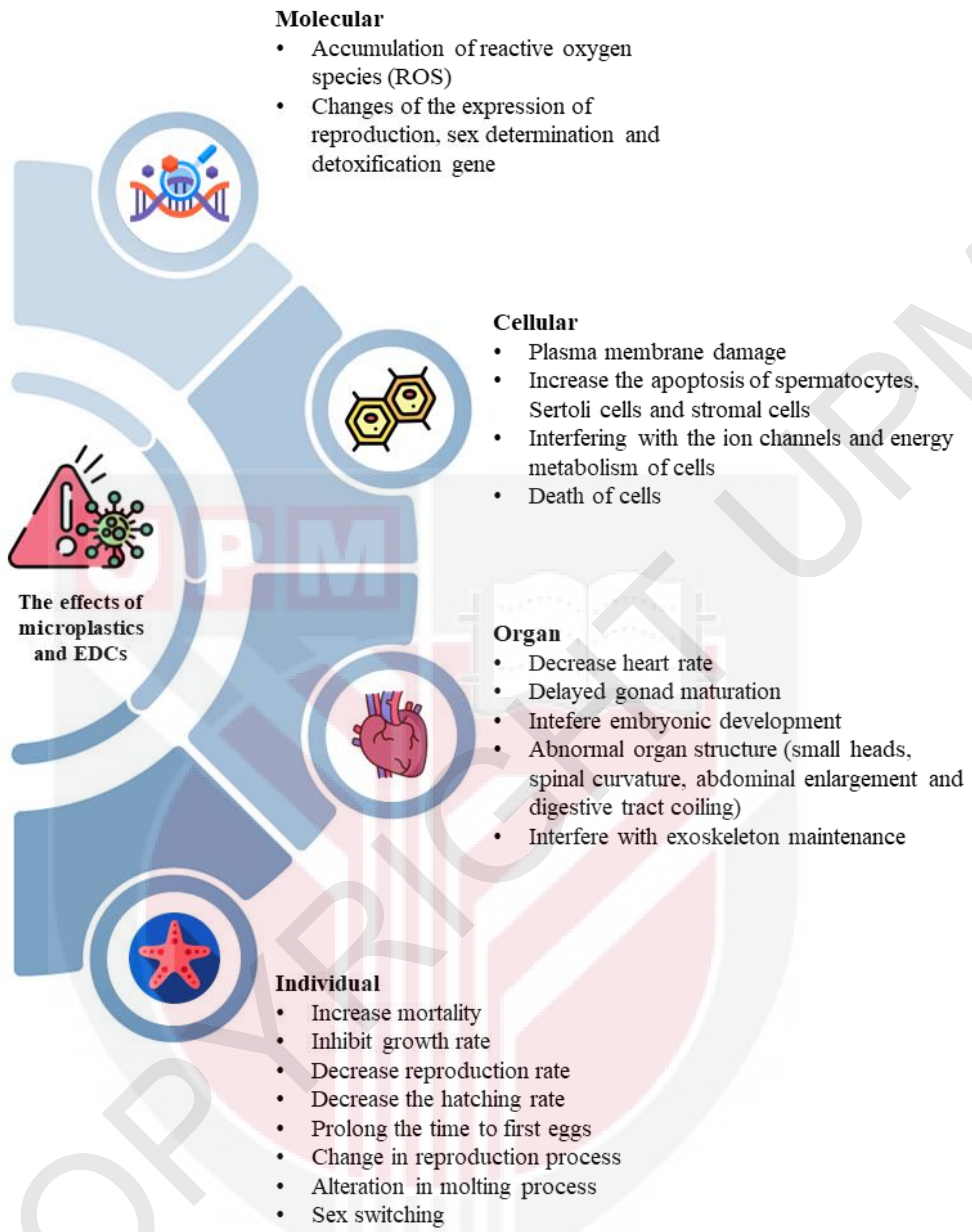

Figure 2.3: Potential associated risk and effects of co-occurring microplastics and EDCs on aquatic organisms (molecular, cellular, individual, and organ). Data retrieved from Hong et al. (2020), Liu et al. (2022), Martins et al. (2020), Wang et al. (2019b), and Zhang et al. (2020)

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Common plastics such as PP, PE, PET, PS, PA, PMMA, and PVC have been used in a wide array of applications, including household goods, personal care products, electronics, medical equipment, fishing gear, and agriculture (Cole et al., 2011; PlasticsEurope, 2019; Ouyang et al., 2020). When exposed to the environment, these micro-sized plastic particles have the potential to undergo transformation into nanoplastics. This transition highlights the emergence of nanoplastics as significant pollutants, extending the risk beyond microplastics and/or EDCs alone and posing threats to the environment and ecosystems. Although concerns, sources, and risks associated with nanoplastics have been widely discussed and debated in scientific, governmental, and public communities, there are limited reports on their occurrence in marine environments. Adequate investigation of the ecological effects of nanoplastics on marine organisms is hindered by the lack of standardized methods for detecting and quantifying nanoplastics in complex environmental matrices (Ter Halle et al., 2017; Alimi et al., 2018). Ter Halle et al. (2017) has provided the first evidence of the occurrence of nanoplastics in the North Atlantic subtropical gyre, including common synthetic polymers such as PE, PS, PVC and PET in the size range of 1–999 nm.

Furthermore, the occurrence of these common plastics, particularly spherical PS and PMMA nanoplastics, has been examined in ecotoxicological studies on marine organisms (Gonçalves and Bebianno, 2021). Although specific environmental nanoplastic concentrations have not been reported, ecotoxicological studies have shed light on the potential implications for aquatic organisms. **Figure 2.4** illustrates the possible ingestion, bioaccumulation, and biomagnification of nanoplastics by marine organisms, such as phytoplankton, zooplankton, crustaceans, mollusks, and fish

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species, in aquatic environments. Exposure to nanoplastics has been associated with impairments and inhibitions in the growth, development, reproduction, behavior, and mortality of various aquatic organisms across trophic levels (Ferreira et al., 2019; Barría et al., 2020; Gonçalves and Bebianno, 2021).

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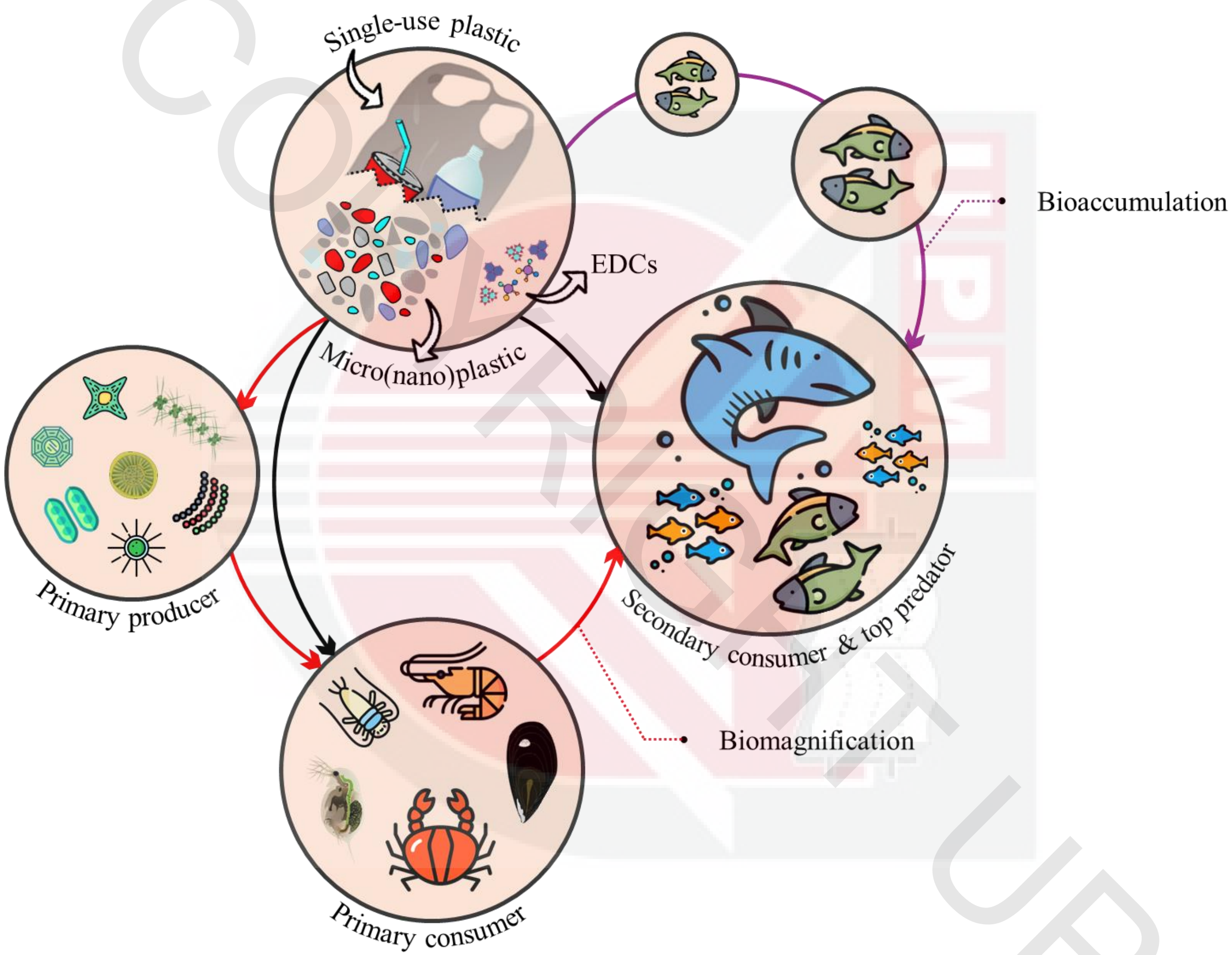

Figure 2.4: Trophic transfer and pathway of microplastics and nanoplastics from primary producer to secondary consumer and top predator

CHAPTER 3

MICROPLASTICS IN SURFACE WATER AND SEDIMENT OF THE SELANGOR RIVER BASIN

3.1 Introduction

The widespread presence and effects of microplastics have piqued the attention of scientists and the public in understanding the origins and fate of microplastics in aquatic environments. While the majority of microplastics originate from sewage effluents and river runoff, they have also been directly introduced from estuaries and the open ocean (Tang et al., 2018; Robin et al., 2020). In particular, the sources of microplastic contamination in marine ecosystems can be primarily attributed to land-based activities, including the discharge of sewage treatment waste, industrial operations, agricultural practices, and commercial activities, which collectively account for approximately 80% of the overall contamination (Andrady, 2017; McCormick et al., 2014). Previous studies have indicated that the occurrence of microplastics can be attributed to specific anthropogenic wastes on land, such as cosmetic products (Lei et al., 2017; Praveena et al., 2018; Zainuddin et al., 2022), washing clothes (De Falco et al., 2018; Liu et al., 2019a), and recreational activities (Waite et al., 2018; Egessa et al., 2020).

This chapter is based on the papers:

a) Zaki, M.R.M., Sukatis, F.F., Roslan, M.Q.J., Isa, N.M., Yusoff, F.M., & Aris, A.Z. (2023). Microplastic pollution in freshwater sediments: Abundance and distribution in Selangor River Basin, Malaysia. Water, Air, & Soil Pollution, 234(7), 1–13. [Q2, IF: 2.9].

b) Zaki, M.R.M., Zainuddin, A.H., Saifuddin, M.F.U., Razak, M.R., Isa, N.M., Ash'aari, Z.H., & Aris, A.Z. Microplastic contamination and ecological risk assessment in tropical riverine surface water of Selangor River Basin, Malaysia [ACS ES&T Water – Accepted, Q1, IF: 5.3, Top 15%].

c) Zaki, M.R.M., Saifuddin, M.F.U., Ash'aari, Z.H., & Aris, A.Z. Microplastics and endocrine disrupting compounds in urbanized tropical riverine water: A comparative analysis, pollution load and land-use effect [Preparing for journal submission].

d) Zaki, M.R.M., Razak, M.R., & Aris, A.Z. Microplastics and plasticisers in the freshwater environments: A perspective on the distribution and associated risk [Science of the Total Environment – Under review, Q1, IF: 9.8, Top 10%].

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The occurrence and abundance of microplastics, as well as their transportation and potential sources from rivers to coastal areas, have not been comprehensively explored, especially in the surface water and sediment of river basins. Although sediment is the final sink for microplastics, marine sediment has received considerable attention compared to riverine sediment. Analyzing riverine surface water and sediment could provide insights into the transportation pathways of microplastics to the marine environment. Notably, Malaysia is one of the developing countries with poor plastic waste management practices and awareness, which may contribute to microplastic pollution in the future. Additionally, there is a notable research gap regarding the abundance and distribution of microplastic pollution in tropical river basins. Despite the growing global concern over microplastic pollution, studies specifically addressing these aspects in tropical river systems remain limited, posing significant challenges (Anuar et al., 2023).

Freshwater ecosystems, particularly rivers, act as critical conduits for the transport of MPs from terrestrial to marine environments. Rivers carry microplastics from urban runoff, untreated wastewater, and industrial discharge downstream (Woodward et al., 2021; Zhou et al., 2023). The hydrodynamics of river systems, including flow velocity, turbulence, and sedimentation, influence the movement and retention of microplastics (Kumar et al., 2021; Zaki and Aris, 2022). Investigating the spatial distribution and resuspension processes in riverine environments is crucial for understanding their fate and ecological risks. Because river basins provide essential freshwater resources, assessing microplastic dynamics is necessary for developing monitoring frameworks and mitigation strategies.

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Moreover, microplastics pose an emerging risk to the environment and humans. Microplastic ingestion and incidents have been extensively reported in a vast array of aquatic organisms in marine ecosystems (Li et al., 2016; Abreo et al., 2019). Microplastics have been detected ubiquitously in a variety of economic seafood (Ogunola et al., 2022; Pan et al., 2022), airborne (Amato-Lourenço et al., 2022; Torres-Agullo et al., 2022), and drinking water (Pivokonsky et al., 2018; Mintenig et al., 2019). This widespread presence suggests that humans are at risk of exposure to MPs via the consumption of contaminated seafood, inhalation of airborne particles, and drinking water. These exposure pathways have potential implications for human health. Therefore, understanding the sources, transport mechanisms, and fate of microplastics in river basins is essential for effective mitigation strategies.

3.2 Methodology

3.2.1 Chemicals and materials

The chemicals and reagents employed in this study served their purpose in facilitated the preparation and extraction of microplastics from both surface water and sediment. Specifically for the extraction of microplastics, ferrous sulphate ($FeSO_4$) was obtained from Fisher Scientific (Leicestershire, UK). Hydrogen peroxide (H_2O_2), sodium chloride (NaCl, 1.2 g/cm^3) solution, and zinc chloride ($ZnCl_2$, 1.5 g/cm^3) were purchased from Merck (Darmstadt, Germany). For microplastics filtration, this study bought 0.45 µm cellulose nitrate filter paper from Whatman (Buckinghamshire, UK). Recovery analysis was performed using homopolymer polypropylene granules (US Pharmacopeia, USA), polyethylene granules (Sigma-Aldrich, USA), low-density polyethylene (LDPE, European Reference Materials) granules, polyethylene

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terephthalate granules (Sigma-Aldrich, USA), poly(vinyl chloride) granules (Sigma-Aldrich, USA), and polystyrene granules (Goodfellow Cambridge Ltd., UK).

3.2.2 Study area

This study aimed to comprehensively investigate the abundance and spatial distribution of microplastics in the Selangor River Basin, located in Selangor, Malaysia. The research encompasses various sample types, including surface water and sediment, to provide a more comprehensive understanding of the presence of microplastics and their potential association with EDCs. In the Selangor River Basin, surface water and sediment samples were obtained for both microplastic and EDCs analysis. This dual sampling approach enabled us to analyze the distribution of microplastics in both water and sediment, providing insights into their transport and deposition patterns of microplastics and EDCs in the aquatic environment.

Selangor River Basin is in the northern part of Kuala Lumpur in Malaysia and is approximately 110 km long with a catchment area of 2200 km^2. The basin is one of the major rivers in Selangor, which flows from Selangor Dam, which is considered a pristine and less populated area, to an urbanized area in Kuala Selangor, and then flows into the Straits of Malacca. The population in Selangor has increased from 5.79 million in 2013 to 7 million in 2021 (Department of Statistics Malaysia, 2022), which may indicate that a substantial number of emerging pollutants will be introduced to the environment. In addition, the main river of the Selangor River Basin received urban runoff from major tributaries, including the Rawang River, Kerling River, and Tinggi River. The Selangor River Basin encompasses multiple water treatment plants and

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agricultural, industrial, and ecotourism areas, all of which could add to the human-made waste that flows downstream (**Table 3.1**).

The basin is mainly covered by natural forests (57%) and agricultural land (23%) (Othman et al., 2018). The basin was chosen because of the presence of various anthropogenic activities that could influence the contamination of riverine sediments. Previous studies have detected various contaminants in the Selangor River Basin, including pesticides and plasticizers (Santhi and Mustafa, 2013), polychlorinated biphenyls (PCBs) (Sakai et al., 2016), heavy metals (Sakai et al., 2017a), and linear alkylbenzene sulfonate (Sakai et al., 2017b). Santhi and Mustafa (2013) detected high concentrations of plasticizers such as BPA and PAEs in urbanized areas downstream of the main river of the Selangor River Basin. The main source of plasticizers in the environment is mainly originated from the municipal and industrial sewage systems (Wee et al., 2019). Plasticizers are plastic additives that potentially leach out of plastics and/or microplastics and thus pollute riverine ecosystems (Cole et al., 2011; Wu et al., 2018).

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Table 3.1: Geographic information of sampling points with coordinates and site descriptions along the Selangor River Basin

Sampling point	Region	Coordinate	Site description
SP1	Upstream	3.538425, 101.669071	Residential area, receives water flow from Selangor River dam
SP2	Upstream	3.506960, 101.633820	Residential area, agriculture, after drinking water treatment plant intake
SP3	Upstream	3.483870, 101.615650	Receives input from Batang Kali River
SP4	Upstream	3.458015, 101.586849	Receives input from agriculture and poultry farm
SP5	Upstream	3.484200, 101.538600	Peat area, receives input from agriculture
SP6	Upstream	3.432092, 101.497437	Peat area, receives input from agriculture
SP7	Tributaries	3.539329, 101.569774	Kerling River, peat area
SP8	Tributaries	3.469606, 101.642671	Batang Kali River, residential area
SP9	Tributaries	3.493908, 101.517232	Tinggi River, peat area, receives input from agriculture and Tinggi River dam
SP10	Tributaries	3.399100, 101.671850	Liam River, receives input from industry (plastic and rubber manufacturing)
SP11	Tributaries	3.366932, 101.591335	Serendah River, residential area, industrial area and receives input from mining activities nearby
SP12	Tributaries	3.239600, 101.538490	Rawang River, residential area, industrial area, receives input from domestic waste
SP13	Tributaries	3.249731, 101.550869	Rawang River, residential area, industrial area, receives input from domestic waste
SP14	Tributaries	3.280709, 101.516467	Rawang River, residential area, receives input from domestic waste
SP15	Tributaries	3.318980, 101.514698	Rawang River, residential area, industrial area, receives input from domestic waste
SP16	Tributaries	3.300568, 101.599205	Rawang River, residential area, near Rawang bypass highway, receives input from domestic waste
SP17	Tributaries	3.316780, 101.571081	Rawang River, residential area, receives input from domestic waste

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Table 3.1: Continued

SP18	Tributaries	3.334154, 101.560331	Rawang River, residential area, near highway, receives input from domestic waste
SP19	Tributaries	3.383385, 101.476915	Rawang River, peat area, reserved forest, receives input from agriculture
SP20	Downstream	3.402268, 101.441801	Peat area, receives input from agriculture, Rawang River and Guntong River
SP21	Downstream	3.406817, 101.430381	Peat area, receives input from university, residential and biogas power plant
SP22	Downstream	3.384498, 101.414488	Peat area, Selangor River Phase 2 raw water intake plant, receives input from palm oil plantation
SP23	Downstream	3.361707, 101.340697	Peat area, residential area, receives input from poultry farm and agriculture
SP24	Downstream	3.363797, 101.311090	Peat area, Rantau Panjang Water Treatment Plant, nearby jetty, receives input from fishery
SP25	Downstream	3.335072, 101.254770	Residential area, industrial area, recreational area, near river mouth of Selangor River and jetty, receives input from fishery and domestic waste

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3.2.3 Surface water and sediment sampling

To ascertain the abundance and spatial distribution of microplastics and EDCs, surface water and sediment were collected from 25 sampling points along the Selangor River Basin in May 2022 (**Figure 3.1**). The Selangor River Basin was divided into three major sampling regions: upstream (SP1–SP6), tributary (SP7–SP19), and downstream (SP20–SP25). The upstream of the Selangor River is in the northern part of Selangor and plays a crucial role in providing raw and drinking water to support domestic and agricultural activities throughout the Selangor River Basin. The lower streams were highly influenced by industrial activity and urban runoff from the upper streams and tributaries of the Selangor River Basin. Moreover, the tributary areas of the Selangor River Basin (e.g., Rawang, Serendah, Batang Kali, and Kerling) are of significant importance owing to the extensive industrialization and urbanization present in these regions. Tributaries serve as crucial focal points for investigating the occurrence of emerging contaminants, such as microplastics and EDCs, in the main river, as previously reported by Wong et al. (2020) and Zhao et al. (2020a). For instance, Sakai et al. (2016) reported that a high concentration of PCBs was detected in Rawang, which was attributed to industrial estates and plastic manufacturing factories. Thus, the combination of these sampling points and the prevalence of various anthropogenic activities are likely to have played a role in the occurrence of microplastics and EDCs in the Selangor River Basin.

Approximately 5 L and 0.5 L of surface water samples were collected for microplastic and EDCs analysis, respectively, using a purging pump. Surface water samples were stored in amber glass bottles. For sediment analysis of microplastics and EDCs, a total of 500 g of sediment was collected using an Ekman grab sampler. Both the surface

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water and sediment samples were promptly stored in an icebox at 4 °C. For in situ measurements, a YSI Pro Plus multiprobe meter (YSI Inc., USA) and WTW Multi 3630 IDS (Xylem Analytics GmbH, Germany) were utilized to measure various parameters, including pH, temperature, dissolved oxygen, conductivity, salinity, oxidation reduction potential (ORP), and total dissolved solids (TDS) (**Table 3.2**). Upon arrival at the laboratory, the surface water sample for EDCs analysis was filtered immediately using a 0.2 µm glass microfiber filter. Subsequently, the filtered sample was quenched with excess ascorbic acid and preserved at 4 °C. **Figure 3.2** depicts the comprehensive methodologies employed in the identification and quantification of microplastics in the surface water and sediment of the Selangor River Basin.

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Table 3.2: Physicochemical parameters of surface water in Selangor River Basin

Sampling point	pH	Temperature (°C)	Conductivity (mS/cm)	DO (mg/L)	Salinity (ppt)	ORP/redox (mV)	TDS (mg/L)
SP1	6.65	26.64	0.025	6.51	0.01	212.37	16.00
SP2	6.65	26.77	0.033	5.06	0.01	9.43	20.67
SP3	7.03	26.57	0.076	4.14	0.03	5.47	48.00
SP4	7.26	26.89	0.031	4.94	0.01	-16.50	19.67
SP5	7.02	28.76	0.040	7.59	0.02	4.97	24.67
SP6	7.09	26.58	0.073	5.60	0.03	-1.70	46.00
SP7	7.40	25.37	0.022	6.46	0.01	-27.53	14.33
SP8	6.69	26.62	0.034	6.00	0.01	21.27	21.67
SP9	6.65	29.10	0.025	7.58	0.02	14.53	26.33
SP10	6.26	26.01	0.021	5.95	0.01	49.23	13.00
SP11	6.58	26.99	0.059	4.90	0.02	22.73	37.00
SP12	6.67	27.67	0.156	4.78	0.07	16.90	96.33
SP13	6.82	28.46	0.180	4.85	0.08	7.60	109.33
SP14	6.66	27.79	0.180	5.04	0.08	7.43	110.67
SP15	6.73	28.25	0.175	4.88	0.05	7.70	107.00
SP16	6.36	24.54	0.062	6.32	0.03	-30.57	41.00
SP17	7.27	24.68	0.053	5.82	0.02	-18.27	34.00
SP18	7.50	25.02	0.058	5.88	0.03	-30.33	37.67
SP19	6.78	29.06	0.152	4.65	0.07	12.54	93.00
SP20	6.67	27.06	0.048	4.72	0.02	21.76	30.00
SP21	7.31	27.13	0.048	4.50	0.02	23.67	29.67
SP22	7.09	26.60	0.046	5.21	0.02	30.97	29.00
SP23	6.67	28.18	0.356	4.30	0.16	15.30	218.33
SP24	6.42	28.25	0.337	4.36	0.15	17.67	206.00
SP25	6.41	27.90	0.575	5.15	0.26	33.13	354.00

DO: Dissolved oxygen; ORP/redox: Oxidation-reduction potential; TDS: Total dissolved solids

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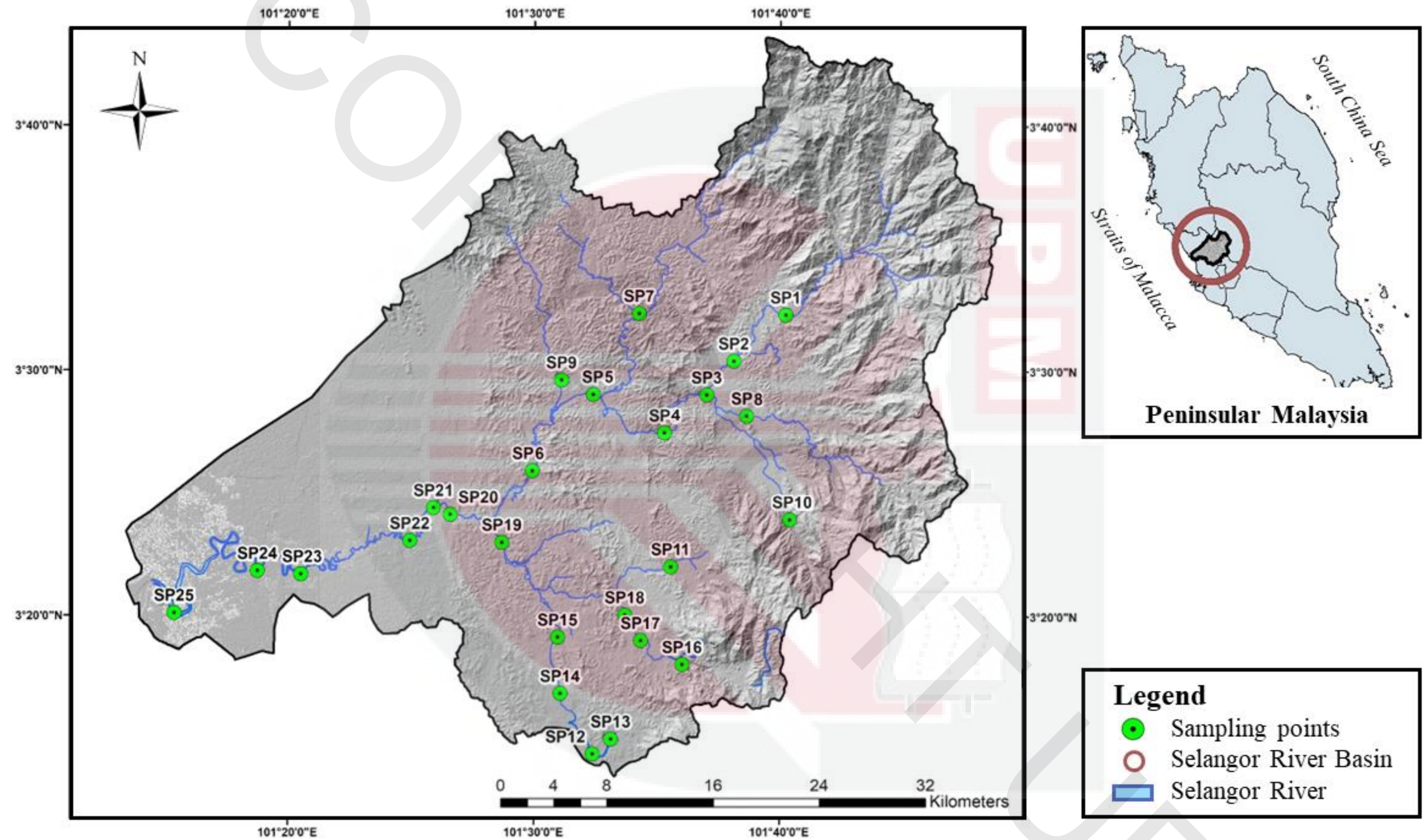

Figure 3.1: Sampling points along the Selangor River Basin, Malaysia. The sampling points comprised upstream (SP1–SP6), tributaries (SP7–SP19) and downstream (SP20–SP25)

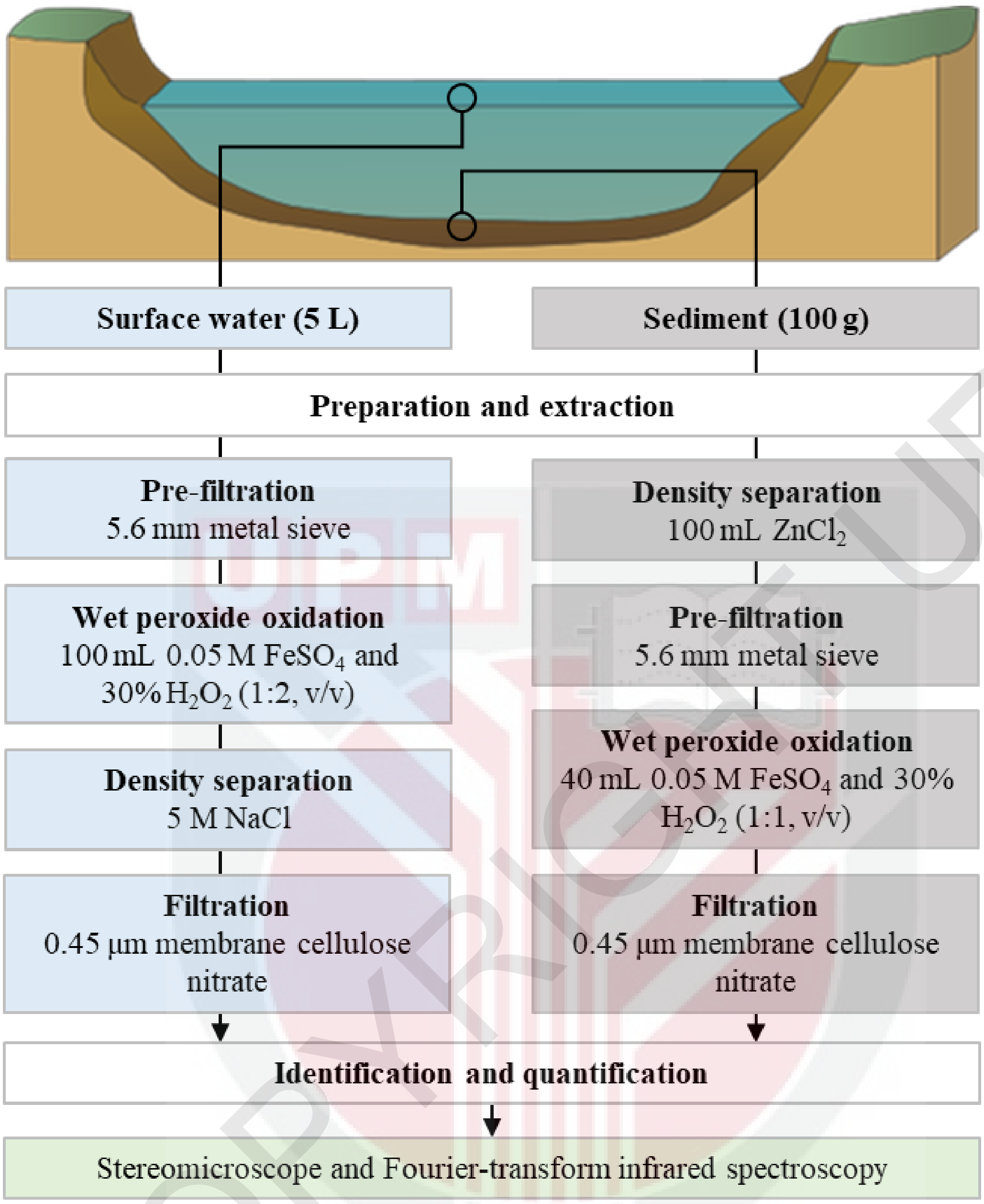

Figure 3.2: Flowchart illustrating the methodological overview of a study on microplastics in surface water and sediment of Selangor River Basin

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3.2.4 Preparation and extraction of microplastics

3.2.4.1 Surface water

Microplastics were extracted from the surface water using a modified method adopted from Masura et al. (2015) and Zaki et al. (2021a). As these methods were originally developed for analyzing microplastics in marine environments, this study incorporated minor optimizations to adapt the procedure to freshwater samples. The samples were extracted by wet peroxide oxidation, density separation, and filtration. Briefly, surface water was pre-filtered through a 5.6 mm metal sieve to remove large particles and residues. Subsequently, the sample was treated with 100 mL of a mixture of 0.05 M $FeSO_4$ and 30% H_2O_2 (1:2, v/v). For the rapid digestion of organic matter, the sample was stirred and heated to 75 °C for 5 min. As soon as gas bubbles were formed, the sample was swiftly withdrawn from the hot plate to slow down the boiling process. The cooling process was extended to room temperature for >12 h to avoid physical changes in the microplastic particles (Zaki et al., 2021a).

Prior to density separation, the samples were treated with a 5 M NaCl solution. Through density separation, microplastic particles (i.e., PE, PP, and PS) with densities lower than or equivalent to the NaCl solution (~1.2 g/cm^3) can be effectively recovered. NaCl solution is preferred for microplastic density separation owing to its low cost, non-toxicity, ease of use, and effectiveness in recovering common microplastics. Alternative solutions such as sodium iodide (NaI; ~1.8 g/cm^3), calcium chloride ($CaCl_2$; ~1.4 g/cm^3) and $ZnCl_2$ (1.5–1.8 g/cm^3) are more hazardous and can degrade certain polymers (Masura et al., 2015; Duong et al., 2022). Prior to filtration, the supernatant was retrieved after 8 h and filtered using a 0.45 µm membrane cellulose

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nitrate filter. The membrane filter was then transferred to a glass Petri dish and covered with aluminum foil to eliminate airborne and cross-contamination.

3.2.4.2 Sediment

Microplastics were extracted from the sediment samples using a modified method developed by the National Oceanic and Atmospheric Administration (NOAA) laboratory with minor modifications (Kabir et al., 2022; Masura et al., 2015). The wet sediment sample was pre-filtered through a 5.6 mm stainless steel sieve to discard larger debris and potential macro(meso)plastic that were irrelevant to the microplastic size in this study, which was less than 5 mm (Eriksen et al., 2014). The sample was oven-dried at 40 °C for 48 h and homogenized using stainless steel before density separation. Approximately 100 g of sediment (dry weight) was added to the beaker and treated to prepare 100 mL of the $ZnCl_2$ solution. Then, the mixture was homogenously agitated for 20 min and allowed to settle overnight. The supernatant was pre-filtered through a 5.6 mm sieve to discard larger residues. The target particle residues in the range of 0.05 to 5.6 mm were then washed multiple times with deionized water to ensure that all particles were recovered and transferred to a glass beaker.

Prior to wet peroxide oxidation, the beaker was oven-dried at 50 °C until dry. Wet peroxide oxidation was performed on a sediment sample in a beaker by treating it with 20 mL of 0.05 M $FeSO_4$ and 20 mL of 30% H_2O_2 solution. The mixture underwent repetitive heating on hotplates at 60 °C while being homogenously stirred for rapid digestion until there was no visible natural organic material. This temperature is widely used in microplastic digestion methods to ensure efficient organic matter removal

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while minimizing the risk of microplastic degradation (Pfeiffer and Fischer, 2020; Zhong et al., 2022; Şimşek and Sanin, 2024). An additional 10 mL of H_2O_2 was added to further digest any visible natural organic material. The residual microplastic was filtered using 0.45 µm filter paper. Subsequently, the filter paper was placed in a glass petri dish and wrapped with aluminum foil to avoid airborne contamination and cross-contamination prior to the visual observation of microplastics.

3.2.5 Visual and identification of microplastics

The microplastic sample underwent a comprehensive examination of its physical and chemical composition to determine both the physical characteristics and types of polymers present. Using microscopy and spectroscopy, microplastics were observed based on their size, shape, color, and polymer composition. This microscopy-based identification of microplastic types is crucial for understanding their sources and pathways, as different types of plastic particles may have diverse origins, such as microbeads from personal care products or microfibers from textiles. Simultaneously, spectroscopic techniques (e.g., Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopy) have provided valuable information on the chemical composition of microplastics. These data are vital for identifying the specific polymer type and additives present in microplastics, which can help trace the origin and potential sources of the particles.

The visual inspection of microplastics was adapted based on established procedures (Hidalgo-Ruz et al., 2012; Simon-Sánchez et al., 2019). A ZEISS stereomicroscope (Axioskop 2, Germany) was used to observe the microplastic characteristics, including size, shape, and color. The image of a physical characteristic was captured using a

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digital camera (Zeiss AxioCam ERc 5s, Germany) and analyzed using ZEN software (Zeiss, Germany). Subsequently, microplastic particles were verified, and the polymer composition of microplastics was identified using Fourier-transform infrared spectroscopy attached with attenuated total reflectance (FTIR-ATR; Thermo Fisher Nicolet 6700, USA). Infrared measurements were taken within a range of 400–4000 cm^{-1} and 4 cm^{-1} resolution over 16 background scans throughout the analysis. The obtained spectra were compared with a reference spectra library from Thermo Fisher Scientific (Hummel Polymer Sample Library and HR Spectra Polymers and Plasticizers). Only a degree of similarity of 60% or above was verified as microplastics (Frias et al., 2016).

3.2.6 Data analysis

The microplastic concentrations in the surface water were quantified as the mean (particles/L) ±SD, whereas sediment measurements were expressed as the mean (particles/kg) ± SD, facilitating direct comparison with a previous study. All statistical data were analyzed using IBM SPSS Statistics Software (IBM, USA) version 25. For surface water, the normality of the data was assessed using the Shapiro-Wilk test, while the homogeneity of variance was evaluated using Levene's tests. Furthermore, one-way analysis of variance (ANOVA) with Tukey's test was used to examine the statistical differences in microplastic concentrations among the three sampled regions (i.e., upstream, tributaries, and downstream). Pearson correlation coefficients were used to evaluate the correlation between the abundance of microplastics and their characteristics or physicochemical parameters. Meanwhile, for sediment, the correlation between microplastic characteristics (size, shape, color, and polymer

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composition) and microplastic abundance in sediment was evaluated using Pearson's correlation. Statistical significance was considered at a significance level of $p < 0.05$.

3.2.7 Quality assurance and quality control

Given the comprehensive scope of this study, which involved the analysis of multiple environmental samples, such as surface water and sediment, collected from riverine environments, it is imperative to implement specific measures for recovery, quality assurance, and quality control. To ensure the utmost accuracy and reliability of the data while minimizing the potential for contamination, a comprehensive set of quality assurance and quality control (QAQC) protocols were diligently implemented throughout the entire analytical procedure.

Since there are no standardized techniques that provide optimum efficiency while being cost-effective and less time-consuming for microplastic analysis, the surface water samples collected in this study were of small volume for reliability. Previous studies have often utilized large volumes of water samples through bulk sampling methods (Zhao et al., 2014; Wang et al., 2018a; Yan et al., 2019) or plankton tows (Yonkos et al., 2014; Di and Wang, 2018). However, a large volume of water sample is not sufficient to guarantee that samples are representative of microplastic occurrence and abundance in the environment. In addition, large-volume samples are costly and time-consuming to collect, and have a high potential for the loss of smaller microplastics (Pico et al., 2019). McEachern et al. (2019) conducted a comparative analysis of two distinct sampling techniques: discrete samples (1 L) and plankton tow samples (roughly 10,000 L). The results revealed that despite the smaller volume of

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the sample, the discrete samples exhibited a higher microplastic concentration than the plankton tow samples.

It was crucial to consider the cost and time efficiency of this study while ascertaining the occurrence and abundance of microplastics in riverine surface water samples. Thus, the methodology established by McEachern et al. (2019) was adopted, utilizing 5 L volumes for both the control and environmental surface water samples, along with appropriate replicates. Recovery analysis was performed to evaluate the efficiency of the microplastic extraction method using a standard reference material as the internal standard. For riverine surface water and sediment samples, a mixture of 0.5 g homopolymer PP granules, PE granules, LDPE, PET, PVC granules, and PS granules were spiked into clean riverine water and sediment to represent the riverine and environmental conditions. Optimum percentage recoveries (95–105%) were acquired for the optimization of microplastic extraction and analysis in riverine samples (surface water and sediment).

As a prior quality control, two replicate samples were collected at each sampling point, and procedural blank samples (n = 3) were included in the extraction method for both the control and environmental samples. The replicates enhance the precision and reproducibility of measurements by accounting for environmental variability, while procedural blanks serve as quality controls to detect potential contamination and ensure the accuracy of the analytical process. All preparation and extraction processes were carried out in a fume hood to avoid airborne and cross-contamination. All solutions and solvents, such as ultrapure water (UPW, Milli-Q), methanol (MeOH), $ZnCl_2$, $FeSO_4$, and H_2O_2, were filtered through 0.45 µm glass membrane filters prior

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to sample preparation and extraction. Furthermore, all glassware materials used in this study, including the vernier caliper and steel scalpel, were pre-cleaned and thoroughly rinsed with Decon 90, UPW, and MeOH prior to drying for 24 h at 65 °C. This precautionary step is crucial because particles coexisting in solutions or solvents may be mistaken for microplastics in the samples.

3.3 Results and discussion

3.3.1 Abundance and distribution of microplastics

The riverine samples encompassed two types: surface water and sediment, which were collected from 25 sampling points across the river basin. These sampling points were strategically chosen to cover areas affected by various anthropogenic activities, including residential, industrial, and agricultural. Through this comprehensive approach, this study aimed to assess the occurrence and distribution of microplastics in the riverine environment and gain insights into their potential contribution to pollution in estuaries and marine ecosystems downstream.

3.3.1.1 Surface water

Microplastics were detected in all surface water samples collected from 25 sampling points in the Selangor River Basin. The mean abundance of microplastics was 1.44 ± 0.62 particles/L and ranging from 0.7 to 3.4 particles/L (**Figure 3.3**). The distribution of microplastics varied across all sampling points, with coefficients of variation (CV) up to 42.69%, indicating a notable degree of fluctuation in microplastics detected in the Selangor River Basin. The average microplastic abundance in the upstream, tributaries, and downstream along the Selangor River Basin was 1.35 ± 0.61

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particles/L, 1.29 ± 0.33 particles/L, and 1.87 ± 0.95 particles/L, respectively. Although the average microplastic concentration in the tributaries is slightly lower than in the upstream, the overall trend indicates an increase as the river flows downstream, suggesting higher microplastic accumulation in urban areas. In addition, this variation may be attributed to localized factors, such as point-source pollution, dilution effects, or differences in land use along the tributaries. However, no statistically significant difference ($F(2,22) = 2.04$, $p > 0.05 = 0.155$) was observed in the abundance of microplastics among the upstream, tributary, and downstream locations. This suggests that the abundance of microplastics in the surface water was evenly distributed across the river basin.

The average microplastic abundance in the Selangor River Basin was relatively higher than that reported for the Hudson River (0.98 fibers/L; Miller et al., 2017), Pearl River (0.57 ± 0.71 particles/L; Fan et al., 2019), Orange-Vaal River (0.21 ± 0.27 particles/L; Weideman et al., 2019), Dafeng River (0.0006 ± 0.0005 particles/L; Liu et al., 2021b), and Ganges River (0.038 ± 0.004 particles/L; Napper et al., 2021), as summarized in **Table 3.3**. Notably, larger river basins with major urban areas (e.g., the Hudson River and Pearl River) exhibited lower microplastic abundances than the Selangor River Basin, despite having relatively smaller catchment areas. This implies that larger catchment areas may dilute the abundance of microplastics, whereas urban areas may contribute to higher microplastic pollution due to increased anthropogenic activity and waste production (McCormick et al., 2014; Barrows et al., 2018).

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Consequently, the microplastics levels observed in this study were considerably lower in magnitude compared to those reported for the Ciwalengke River (5.85 particles/L; Alam et al., 2019), Manas River (21–49 particles/L; Wang et al., 2020a), and Langat River (4.39 particles/L; Chen et al., 2021). Ding et al. (2019) also observed concentrations ranging from 3.67 to 10.70 particles/L in the Wei River, further highlighting the variability across different river systems. Moreover, the present study was comparable to concentration reported in studies within Suzhou River (1.80–2.40 particles/L; Luo et al., 2019), Orange-Vaal River (1.70 ± 5.10 particles/L; Weideman et al., 2020) and Tapi-Phumduang River (1.77 ± 0.28 particles/L; Chinfak et al., 2021). These variations underscore the complex interplay of factors influencing microplastic pollution levels, including geography, land use, hydrodynamic conditions, and anthropogenic activities within river catchments (Chen et al., 2020; Kumar et al., 2021; Talbot and Chang, 2022).

The concentration of microplastics downstream (i.e., SP25; 3.40 ± 0.28 particles/L) is anticipated to be elevated, as microplastics are susceptible to transportation and remobilization by factors such as surface runoff, high river flow, and heavy rainfall. Previous studies have consistently demonstrated that downstream regions tend to experience a higher influx of microplastic particles, primarily driven by the increased transport and remobilization resulting from surface runoff, high river flow, and heavy rainfall (Horton and Dixon, 2018; Meng et al., 2020; Wong et al., 2020; Liu et al., 2021a; Lu et al., 2023). As a result, microplastics are likely to accumulate in higher concentrations in such areas compared to locations further upstream, where these factors might not be as pronounced. Zhao et al. (2020a) also indicated that urban runoff

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has a major impact on microplastic accumulation (54–3379 particles/m^3) in the Qiantang River.

Furthermore, the high concentrations of microplastics downstream of the Selangor River Basin could be attributed to the high population density associated with intensive anthropogenic activities, particularly in areas with significant urbanization and industrial influence. Microplastic concentrations in the Langat River also showed increasing trends (more than 3-fold) downstream, considering that the Langat River is the longest urbanized river in Selangor compared to the Selangor River Basin (Chen et al., 2021). While the Langat River has been reported to release roughly 5 billion microplastic particles/day into the ocean, largely due to its extensive urbanized stretch and hydrodynamics (Chen et al., 2021), the Selangor River Basin, although less urbanized, is also subject to considerable microplastic input. This is because of the presence of densely populated residential areas, industrial zones, and aquaculture activities along its course. Given that the Selangor River serves as a crucial water source for Selangor and Kuala Lumpur, continuous wastewater discharge, stormwater runoff, and mismanaged plastic waste may have contributed to the observed microplastic accumulation. Hydrodynamic conditions, including river flow velocity and sediment transport, likely influence the retention and transport of microplastics within the basin. Thus, while the Langat River may exhibit a higher overall microplastic flux owing to its greater urban extent, the Selangor River's downstream microplastic concentrations may also be significant, necessitating further investigation into site-specific sources and hydrological influences.

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Numerous previous studies indicate a trend of low microplastic concentrations in upstream regions (Fahrenfeld et al., 2019; Feng et al., 2021; Xu et al., 2021), which aligns with the findings of this study, where upstream concentrations (1.35 ± 0.61 particles/L) in the Selangor River Basin were lower than those observed downstream regions (1.87 ± 0.95 particles/L). Notably, the lowest microplastic concentration was found at site SP22 (0.70 ± 0.14 particles/L), located downstream of three municipal water intakes (Sungai Selangor Phases 1, 2, and 3), suggesting a potential influence of water treatment processes on microplastic distribution. This water intake removes a substantial volume of river water (Kusin et al., 2016b); thus, microplastics can be retained or discarded in water treatment plants from being transported into the ocean. Jung et al. (2022) reported that Korean drinking water treatment plants have shown more than 90% reduction of microplastics concentration in the treated drinking water (0.02–0.11 particles/L) from raw water (0.5–7.0 particles/L).

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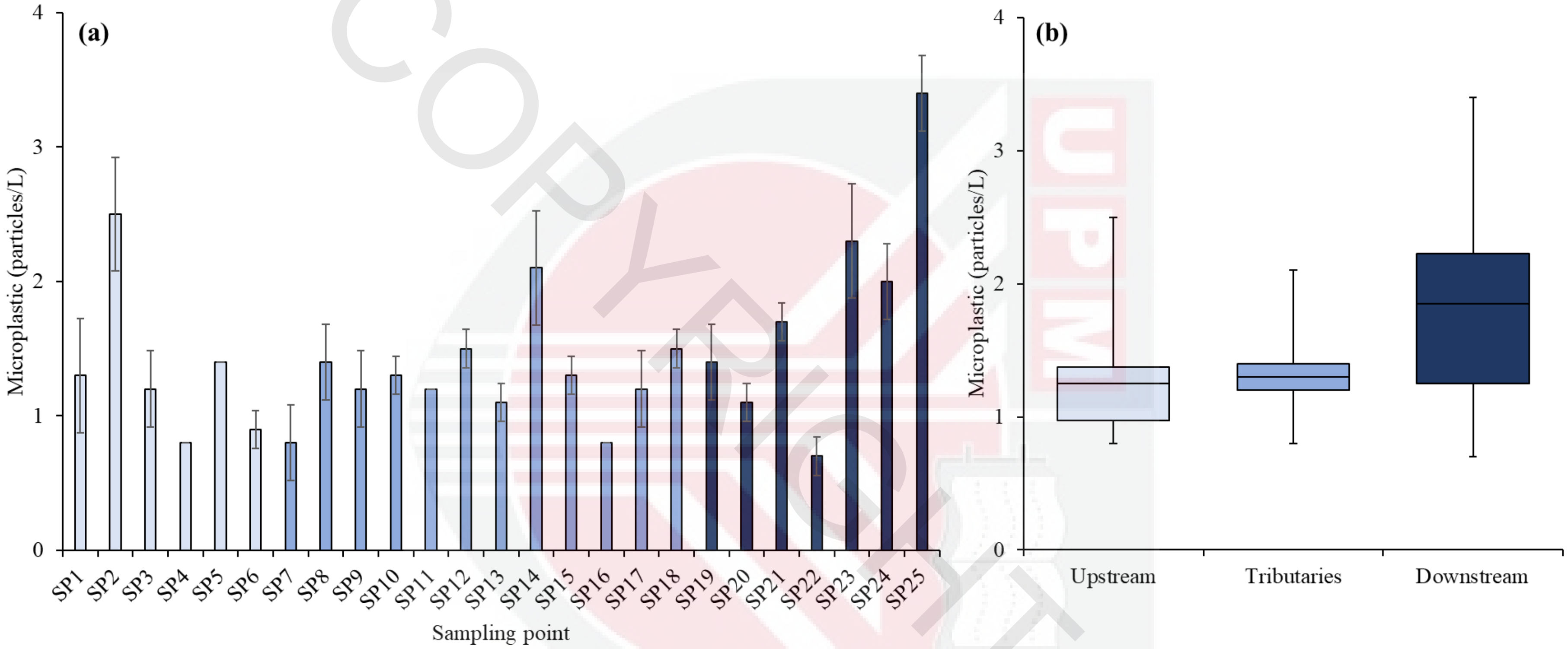

Figure 3.3: Abundance and distribution of microplastics (mean ± standard deviation) in surface water across (a) 25 sampling points and (b) upstream, tributaries, and downstream of the Selangor River Basin

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Table 3.3: Comparison of microplastic abundance in the surface water of the Selangor River Basin to other riverine surface waters

Location	Catchment area (km^2)	Microplastic (particles/L)		Risk Quotient	References
		Average	Range		
Hudson River, USA	21,565	0.98[a]		0.04	Miller et al. (2017)
Gallatin River, USA		1.20	0–67.50	0.05	Barrows et al. (2018)
Ciwalengke River, Indonesia		5.85 ± 3.28		0.23	Alam et al. (2019)
Wei river, China			3.67–10.70		Ding et al. (2019)
Pearl River, China		0.57 ± 0.71		0.02	Fan et al. (2019)
Suzhou River, China			1.80–2.40		Luo et al. (2019)
Orange-Vaal River, South Africa		0.21 ± 0.27		0.01	Weideman et al. (2019)
Yulin River, China	3,861	0.013[b]	0.007–0.017[b]	5.16E–04	Mao et al. (2020)
		0.195[c]	0.02–0.70[c]	0.01	
Manas River, China	21,000		21 ± 3–49 ± 3		Wang et al. (2020a)
Orange-Vaal River, South Africa		1.70 ± 5.10		0.07	Weideman et al. (2020)
Langat River, Malaysia	2,663	4.39		0.17	Chen et al. (2021)
Tapi-Phumduang River, Thailand	6,125	1.77 ± 0.28	0.68–2.81	0.07	Chinfak et al. (2021)
Chishui River, China	1,788		1.77–14.33		Li et al. (2021a)
Dafeng River, China		0.0006 ± 0.0005		2.38E–05	Liu et al. (2021b)
Ganges River, India		0.038 ± 0.004		1.51E–03	Napper et al. (2021)
Yangtze River, China		1.27 ± 0.83	0.02–2.58	0.05	Yuan et al. (2022)
Selangor River, Malaysia	2,200	1.44 ± 0.62	0.70–3.40	0.06	This study

[a] Fibers/L; [b] Mainstream; [c] Tributaries

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3.3.1.2 Sediment

Microplastics were identified across 25 sampling points in the sediment of the Selangor River Basin. The abundance of microplastics ranged from 10 to 55 particles/kg, with an average of 28.40 ± 10.18 particles/kg (**Figure 3.4**). A high abundance of microplastics was recorded at SP25, which was in the downstream area. Meanwhile, low abundance was found at SP22, situated downstream of phases 2 and 3 of raw water intake, aligning with the observed trends in microplastic distribution within surface water samples. Microplastic abundances decrease gradually in the midstream areas (i.e., SP6) due to the lack of anthropogenic activities as compared to upstream areas (i.e., SP1, SP2, SP3, SP4, SP5, SP7, and SP8) of the Selangor River Basin. Anthropogenic wastes from tourism, industry, agriculture, and extensive residential areas have been subjected to microplastic pollution. These wastes can be transported to downstream areas by river flow and accumulate in riverine sediment.

Besides, downstream areas tended to exhibit higher concentrations of microplastics due to the presence of various human activities, including industrial, tourism, and fishing (Ferreira et al., 2018; Huang et al., 2021a). The high abundance of microplastics downstream of the Selangor River Basin was consistent with the Rhine River (Mani et al., 2019), Atoyac River basin (Shruti et al., 2019), and St. Lawrence River (Crew et al., 2020). In addition, slower river flow velocity in downstream areas could be another factor influencing the prevalence of microplastics in the sediment (Lin et al., 2021; Xu et al., 2022a). Despite inputs from terrestrial sources, microplastics may also originate from marine activities, influencing microplastic abundance in the riverine environment. Marine-based sources, such as discarded fishing gear, shipping activities, aquaculture waste, and plastic debris from coastal

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regions, can introduce microplastics into nearby water bodies (Zaki et al., 2021a; Zainuddin et al., 2022). Additionally, tidal influences and estuarine mixing can transport microplastics from marine environments back into riverine systems (Kumar et al., 2021; Malli et al., 2022). In estuaries and coastal rivers, seawater intrusion and tidal currents can carry marine-derived microplastics upstream, adding to the overall microplastic load in the river. This interaction between marine and freshwater sources complicates microplastic pollution dynamics, demonstrating that riverine microplastic abundance is not solely dependent on terrestrial inputs but is also influenced by marine-derived contamination.

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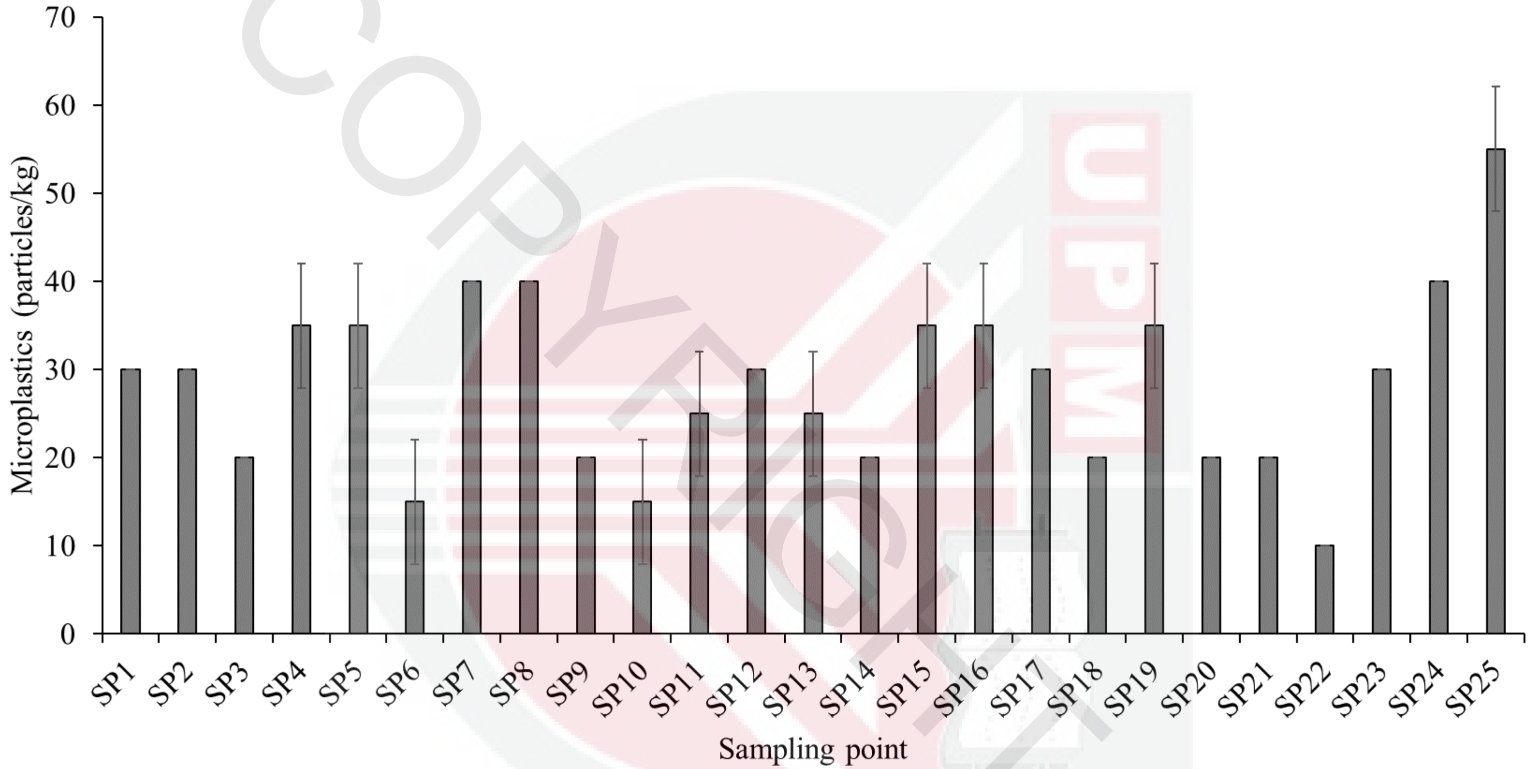

Figure 3.4: Microplastic concentration and distribution across all sampling points in sediment along the Selangor River Basin. The error bars indicate the standard deviations

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Although both sewage treatment facilities and water treatment plants can serve as pathways and sources for microplastic pollution, these facilities may also help diminish the prevalence of microplastics. Sewage treatment plants primarily receive wastewater from domestic and industrial sources, with varying degrees of microplastic removal depending on the treatment efficiency. In contrast, water treatment plants are designed to provide potable water, may intentionally introduce microplastics during the treatment process, and have the capability to reduce their abundance. For instance, Wang et al. (2020b) demonstrated that the use of coagulation combined with sedimentation (40.5–54.5%) and granular activated carbon filtration (56.8–60.9%) considerably decreased the abundance of microplastics in an advanced drinking water treatment plant. Thus, this demonstrates the impact of water treatment plants on reducing microplastic abundance. Overall, the average microplastic abundance in the sediments of the Selangor River Basin is much lower than those reported previously, i.e., 32,947 ± 15,342 particles/L in the Wen-Rui Tang River (Wang et al., 2018b) and 159.2 ± 138.4 particles/L in the Danube River Basin (Pojar et al., 2021) (**Figure 3.5**).

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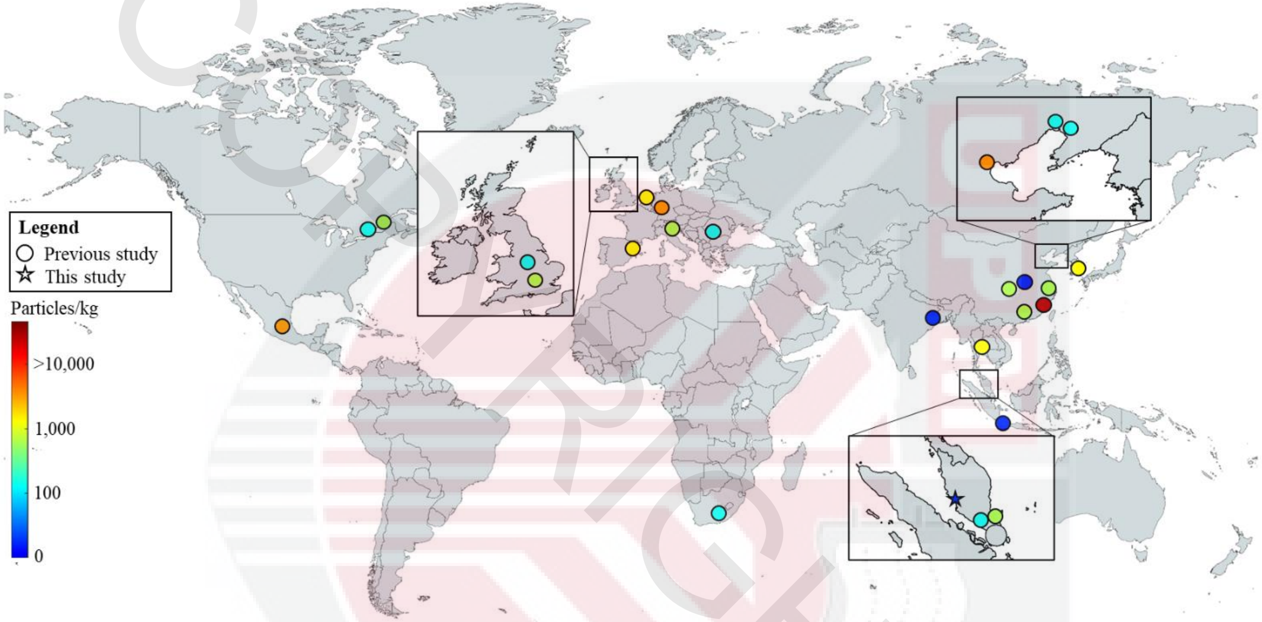

Figure 3.5: Comparison of the average abundance of microplastics in the sediments of the Selangor River Basin with in that other regions of riverine sediment. Data retrieved from Klein et al. (2015), Horton et al. (2017), Leslie et al. (2017), Vermaire et al. (2017), Nel et al. (2018), Peng et al. (2018), Sarijan et al. (2018), Tibbetts et al. (2018), Wang et al. (2018a), Alam et al. (2019), Eo et al. (2019), Fan et al. (2019), Piehl et al. (2019), Shruti et al. (2019), Simon-Sánchez et al. (2019), Crew et al. (2020), Li et al. (2020), Rao et al. (2020), Ta et al. (2020), Xu et al. (2020), Liu et al. (2021c), Pojar et al. (2021) and Singh et al. (2021)

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3.3.2 Microplastic characteristics

3.3.2.1 Surface water

The microplastics were visually identified based on their physical characteristics (size, shape, and color) and captured using a stereomicroscope with an attached camera (**Figure 3.6**). The average size of the microplastics was 627.70 ± 520.51 µm which ranged from 20.21 to 2798.74 µm. Microplastics ranging from 100 to 1000 µm exhibited the highest abundance, comprising 22.7%, 19.7%, and 26.3% for size fractions of 100–300 µm, 300–600 µm, and 600–1000 µm, respectively (**Figure 3.7a**). In addition, microplastic abundance was positively correlated ($p < 0.01$) with 100–300 µm ($r = 0.565$), 300–600 µm ($r = 0.554$), and 600–1000 µm ($r = 0.718$), suggesting the ubiquity of microplastic sizes within 100–1000 µm compared to other sizes. Meanwhile, microplastic sizes of 1000–5000 µm (19.1%) and <100 µm (12.2%) were the least observed, indicating that the microplastic concentration decreased along the <100 µm and >1000 µm directions, respectively. This result was comparable to surface water from the Gallatin River watershed (Barrows et al., 2018), Taihu Lake (Su et al., 2016), Tapi-Phumduang River (Chinfak et al., 2021), and Klang River estuary (Zaki et al., 2021a). Microplastics have also been extensively used in ecotoxicological studies, suggesting that they have detrimental effects on aquatic species. Hence, the presence of microplastics in aquatic ecosystems is a matter of significant concern because of the potential threats posed to aquatic organisms.

Approximately four different morphological shapes of microplastics, including fibers, fragments, beads, and films, were identified. Microplastic fibers were the dominant microplastic shape, accounting for 64.3% (232 particles), followed by fragments (81

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particles, 22.4%), beads (40 particles, 11.1%), and films (8 particles, 2.2%) (**Figure 3.7b**). Microfibers are common microplastics in aquatic environments and are ubiquitous owing to their extensive use in the production of fishing gear and textiles (Andrady, 2011; Wang et al., 2019a). This could potentially explain the association between the presence of microfibers and residential areas, proximity to sewage treatment plants (SP2 and SP24), and the vicinity of the jetty (located downstream of the Selangor River Basin). Most microplastic fibers in the environment are commonly reported to originate from fishing gear and textiles (Hidalgo-Ruz et al., 2012; Pan et al., 2020; Zaki et al., 2021a).

Moreover, disposable face masks in the environment are a potential source of the predominance of fibers. Since the spread of coronavirus disease 2019 (COVID-19) worldwide, the mass production of personal protective equipment, such as face masks, has rapidly increased. Generally, face masks are composed of microfibers that contain common polymeric materials, such as PE, PP, PS, and PC (Aragaw, 2020). Initially, the use of a face mask was intended to minimize the spread of COVID-19. However, tons of fibers suspected to have originated from face masks have been found in various environmental compartments worldwide (De-la-Torre et al., 2021; Mghili et al., 2022; Wang et al., 2022a). This indicates that the extensive use of fiber in broad applications contributes to microplastic pollution, potentially amplifying the abundance and risks of microfibers to aquatic ecosystems and human health (Aragaw, 2020; Li et al., 2021b; Shen et al., 2021). Therefore, this study proposes that the intense usage of disposable face masks during the pandemic could contribute as another factor to microplastic pollution.

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Microplastics exhibit various colors, including transparent, green, red, blue, or black. Among these, transparent was the most commonly observed color, accounting for 51.8%), followed by black (28%), red (9.1%), green (7.5%), and blue (3.6%) (**Figure 3.7c**). The high proportions of transparent microplastics in the surface water of the Selangor River Basin are comparable to those found in Dong Lake (Wang et al., 2017b), which indicates that fishery and fishing activities may contribute to their abundance (Wang et al., 2017b; Zaki et al., 2021a). This reflects the low occurrence of transparent microplastic particles in the water and sediment samples of the Vistula River because of the lack of fishing activities (Sekudewicz et al., 2021). Therefore, the ubiquity of transparent microplastics is due to fishing activities as well as anthropogenic discharge from residential and industrial areas.

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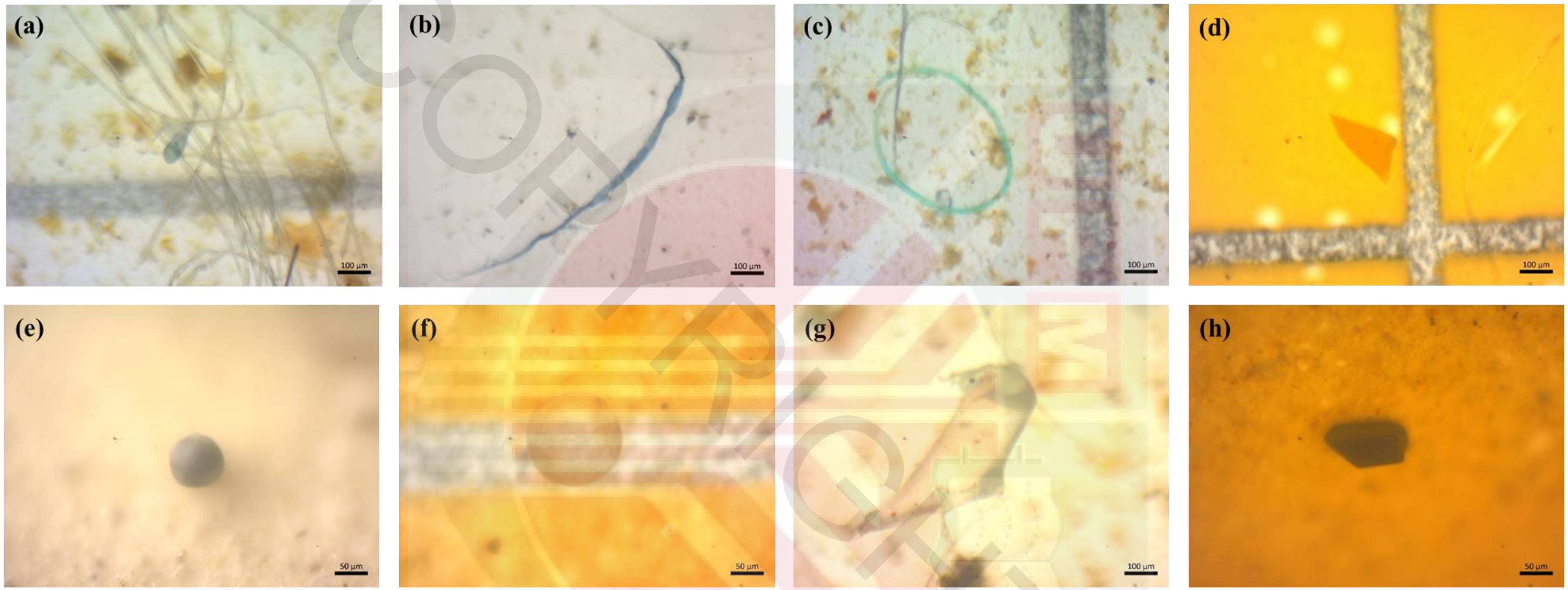

Figure 3.6: Microscopic images captured by stereomicroscopy show suspected microplastics from the surface of water, consisting of (a–c) fibers, (d) film, (e and f) beads, and (g and h) fragments

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Approximately 132 particles were randomly selected for polymer composition analysis by FTIR spectroscopy (**Figure 3.7d**). Prior to the analysis, the selected particles were rinsed with UPW to remove any surface contaminants and then dried in a clean, controlled environment. Each particle was placed on the ATR crystal for direct spectral acquisition within a wavenumber range of 4000–400 cm^{-1}, with 16 background scans at a resolution of 4 cm^{-1}. The main polymer composition of the microplastics was PE (38.6%), followed by PP (26.5%), PET (15.9%), and ethylene/vinyl acetate (EVA, 6.1%). Meanwhile, 17 particles were unidentified, accounting for 12.9% of the total. PE, PP, and PET are common synthetic polymers with high economic value owing to their light weight, durability, and cost-effectiveness. Therefore, these materials have been extensively used in consumer products, particularly in single-use plastics, plastic bottles, and containers. The distribution of microplastics in the environment is commonly influenced by anthropogenic activities because of the broad range of consumer products and their industrial applications. However, low-density microplastics, such as PE and PP, tend to float in surface water and are easily transported by river runoff.

This indicates that their density in surface water may also have influenced the distribution of microplastics along the Selangor River Basin. The high abundance of PE and PP in the present study is consistent with previous studies, including those of the Manas River Basin (Wang et al., 2020a), Renhuai River Basin of the Chishui River (Li et al., 2021a), and Yangtze River Basin (Yuan et al., 2022a). High-density microplastics, specifically PET with a density of 1.38 g/cm^3, were also detected, indicating that environmental factors such as UV radiation, rapid water flow, wave action, turbulence, and biofouling (i.e., attached biomass) may influence the

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prevalence and distribution of microplastics (Wang et al., 2016a; Andrady, 2017; Zaki et al., 2021a).

3.3.2.2 Sediment

A stereomicroscope (Axioskop 2, Zeiss, Germany) and camera (AxioCam ERc 5 s, Zeiss, Germany) were used to visually distinguish microplastics in the sediment of the Selangor River Basin based on their physical features, such as size, shape, and color (**Figure 3.8**). Microplastics varied in size from 50.58–3617.47 µm, with an average of 352.46 ± 466.55 µm. Microplastics are classified into five categories (<100 µm, 100–299 µm, 300–599 µm, 600–999 µm, and 1000–5000 µm). The most abundant size of microplastics was found between 100–299 µm with 45% proportions ($p < 0.05$), followed by <100 µm (25%), 300–599 µm (12%), 1000–5000 µm (9%) and 600–999 µm (8%) (**Figure 3.9a** and **Figure 3.10a**). Microplastics ranging in size from 100 to 299 µm were detected at all sampling points, except for SP10, which is considered to be less urbanized.

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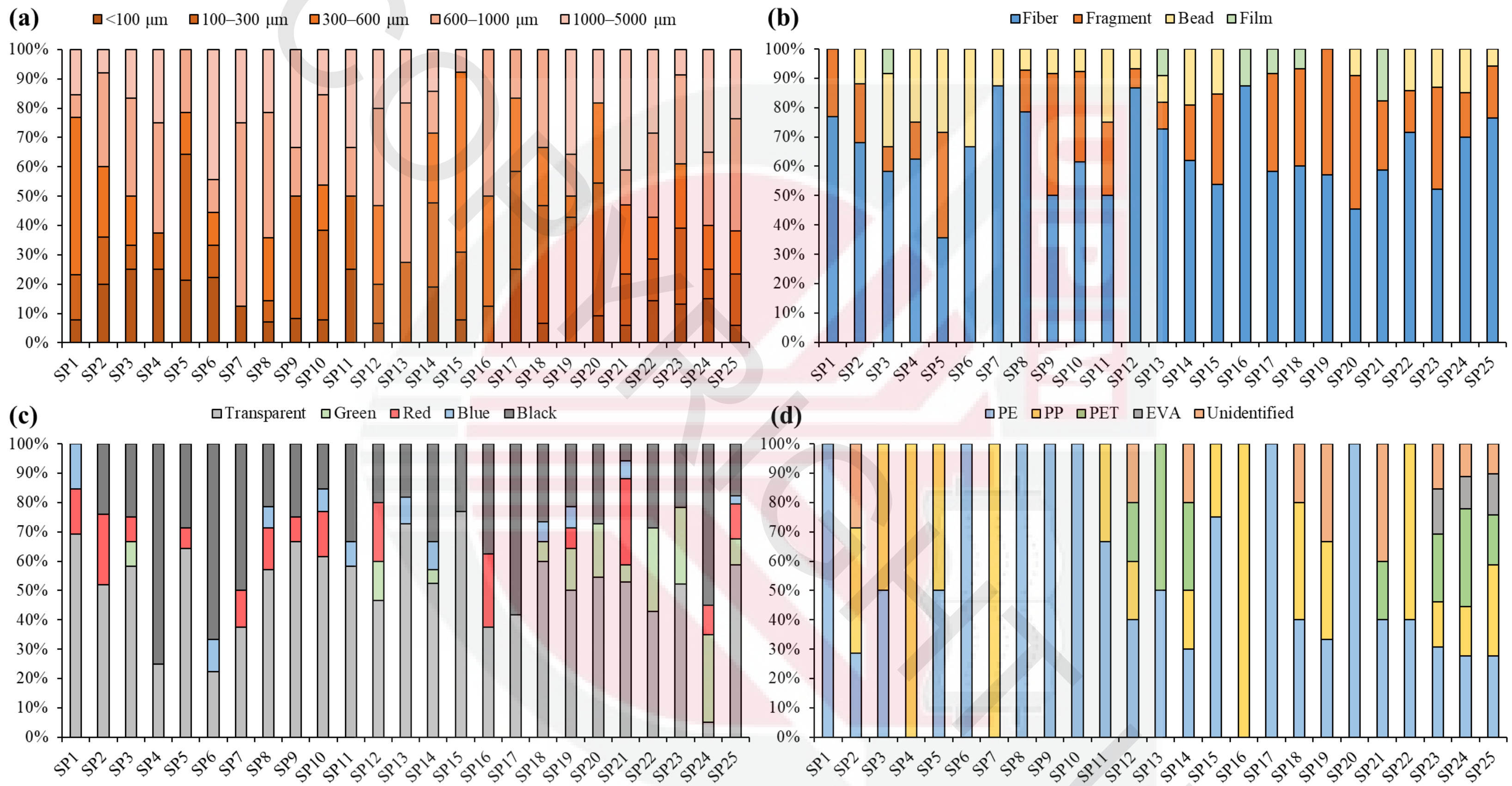

Figure 3.7: Microplastic characteristics in surface water of the Selangor River Basin, categorized by (a) size, (b) shape, (c) color, and (d) polymer composition

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Approximately, a total of 16 sampling points in the Selangor River Basin exhibited a high proportion of small microplastics (<300 μm), ranging from 67 to 100%. Moreover, SP24, which was located after the Rantau Panjang water treatment plant, indicated the lowest proportion (13% of small microplastics). Similar results were observed in the Ciwalengke River (Alam et al., 2019), Haihe River (Liu et al., 2021c), and Nakdong River (Eo et al., 2019). Even though sewage treatment plants are supposedly designed to reduce microplastics, it is notable that sewage systems are also the major sources of microplastics in the environment. Previous research has observed the abundance of microplastics in sewage effluents and waste discharge (Kalčíková et al., 2017; Mak et al., 2020), which pollutes the riverine environment.

Spherical in shape (beads) dominated and made up almost half proportions of microplastics with 43%, followed by fragment (32%), fiber (23%) and film (2%) (**Figure 3.9b** and **Figure 3.10b**). The bead shape was predominantly observed in the downstream areas (SP20–SP25), with a detection range of 25% to 100%. The predominance of beads in the present study was comparable to the urban rivers in Shanghai (Peng et al., 2018), Winnipeg (Anderson et al., 2017), and Antuã (Rodrigues et al., 2018). Even though beads had the highest proportions, there was no significant correlation between microplastic abundance in the sediment of the Selangor River Basin. Instead, the films were found to be correlated with microplastic abundance ($p < 0.01$).

The presence of films in the environment is strongly linked to their usage in various sectors, including plastic packaging (e.g., food packaging, wrapping materials, and disposable plastic products), agricultural activities (e.g., plastic mulch), and plastic

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manufacturing (Horodytska et al., 2018; Shen et al., 2020). Similarly, the predominance of beads is associated with their widespread use in personal care products (e.g., exfoliating scrubs) and industrial plastic processes (Fu and Wang, 2019; Galafassi et al., 2019). These types of microplastics can be transported via urban and stormwater runoff, eventually accumulating in rivers and sediments. Thus, the abundance and distribution of microplastics, such as beads, fragments, fibers, and films, are associated with an increase in domestic, industrial, and agricultural waste discharge from upstream to downstream in the Selangor River Basin.

In terms of color, microplastics were divided into transparent, green, red, blue, and black. Black and transparent were the most common and abundant microplastic colors in all sediment samples, accounting for 49% and 39%, respectively. The black color of microplastics was observed across all sampling points, with the majority observed more prominently in the upstream areas where residential and industrial activities were more intense. Deng et al. (2020) and Liu et al. (2021c) also distinguished the predominant color of black microplastics in riverine sediments in the textile industrial area in Shaoxing City and the Haihe River, respectively. Meanwhile, colored microplastics, such as blue (6%), others (3%), and red (2%), were detected in low proportions across all sampling points (**Figure 3.9c** and **Figure 3.10c**). Colorful microplastics are attributed to two primary sources: shedding of microfibers from clothes and degradation of packaging materials (Miller et al., 2017; Wang et al., 2017a). These processes contribute to the prevalence of vibrant microplastic particles in the environment. Transparent microplastics were detected at all sampling points, except for two sampling points (SP3 and SP6), which were located far from residential and urban areas. It is also possible that transparent microplastics did not sink into the

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sediment but were instead transported by river runoff (Kim et al., 2015; Wright and Kelly, 2017).

FTIR coupled with ATR was used to identify the polymer composition of the microplastic particles. In this study, 77.05% of the randomly selected particles in the sediment samples were verified as microplastics. A total of three polymer compositions were technically recognized as PP, PE, and polyester (**Figure 3.9d** and **Figure 3.10d** and **Figure 3.11**). The dominant polymer composition was PP (50.82%), followed by polyester (22.95%) and PE (9.84%). The results indicated that the occurrence of PP, PE, and polyester may be closely associated with anthropogenic sources (Peng et al., 2018; Yan et al., 2019). PP, PE, and polyester are the most extensively used single-use packaging materials (plastic bottles, plastic bags, and containers), textiles (clothes, carpets, rope, and nets), and personal care products. These microplastics have been observed to float in the surface water of both marine and freshwater environments. This is because the density of microplastics allows them to float and drift in aquatic environments. Microplastics can also settle and sink in sediments.

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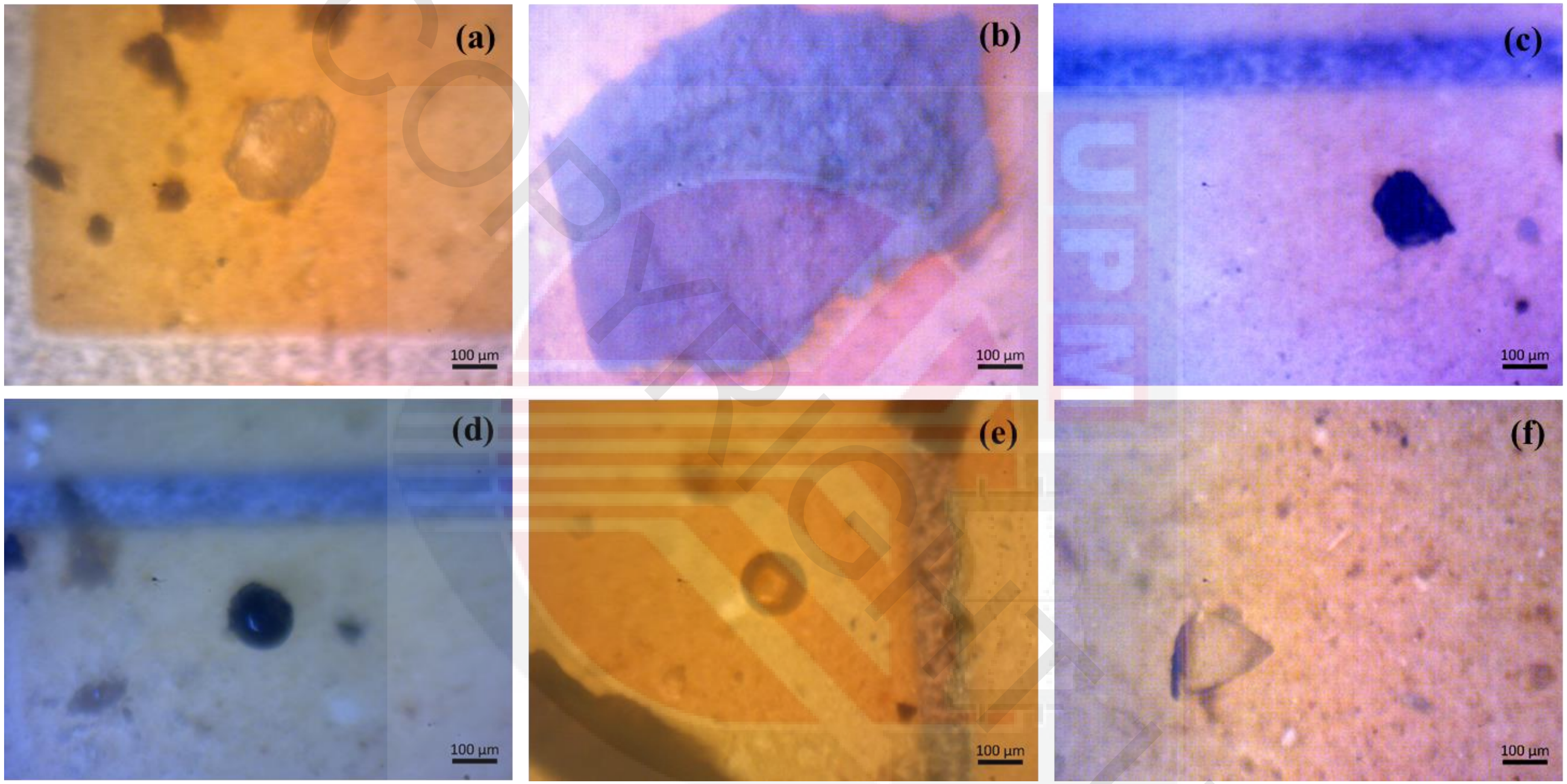

Figure 3.8: Microscopic images of common microplastics under a stereomicroscope found in the sediment of the Selangor River Basin consist of (a–c) fragments, (d and e) beads, and (f) film

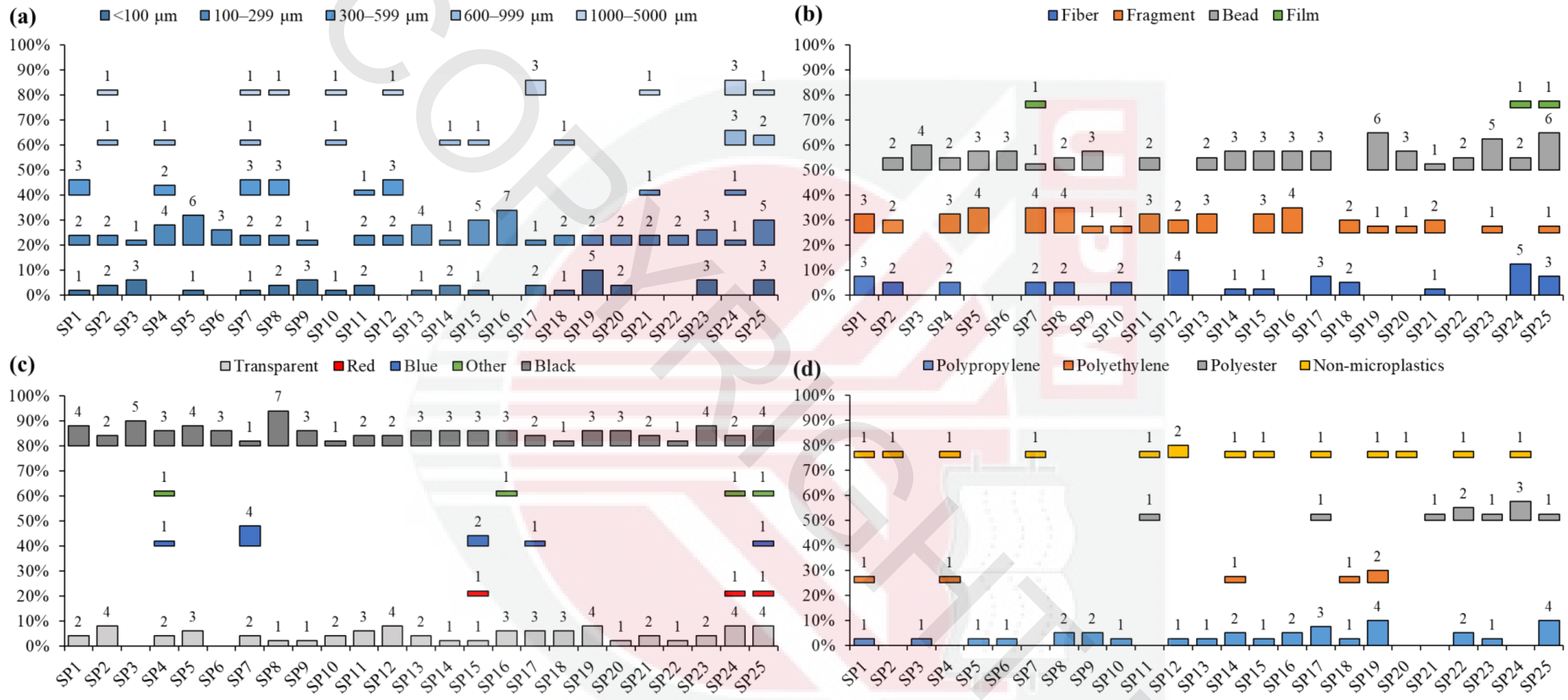

Figure 3.9: The distribution of (a) size, (b) shape, (c) color, and (d) polymer composition of microplastics in the Selangor River Basin

Microplastic characteristics (e.g., size, density, and buoyancy) and environmental factors (e.g., sedimentation, biofouling, and turbulence) may influence the ubiquity of microplastics in the sediment. Microplastics have a large surface-area-to-volume ratio compared to large plastics, which results in the adherence of microplastics to microbes and/or organic matter (Huang et al., 2021a). This phenomenon, known as biofouling, affects the density of microplastics and causes them to sink into the sediment. Microplastics have their own density, with low-density microplastics floating on the surface and high-density microplastics sinking in the sediment. This could be attributed to the ubiquity of microplastics in the sediments. For instance, Di and Wang (2018) detected the predominance of PS in the sediment of the Three Gorges Reservoir. Similarly, other types of high-density microplastics, such as PVC (1.16–1.58 g/cm^3), polyester (1.24–2.3 g/cm^3) and PA (1.02–1.05 g/cm^3) was found in the sediment (Dai et al., 2022; Egessa et al., 2020; Hope et al., 2021).

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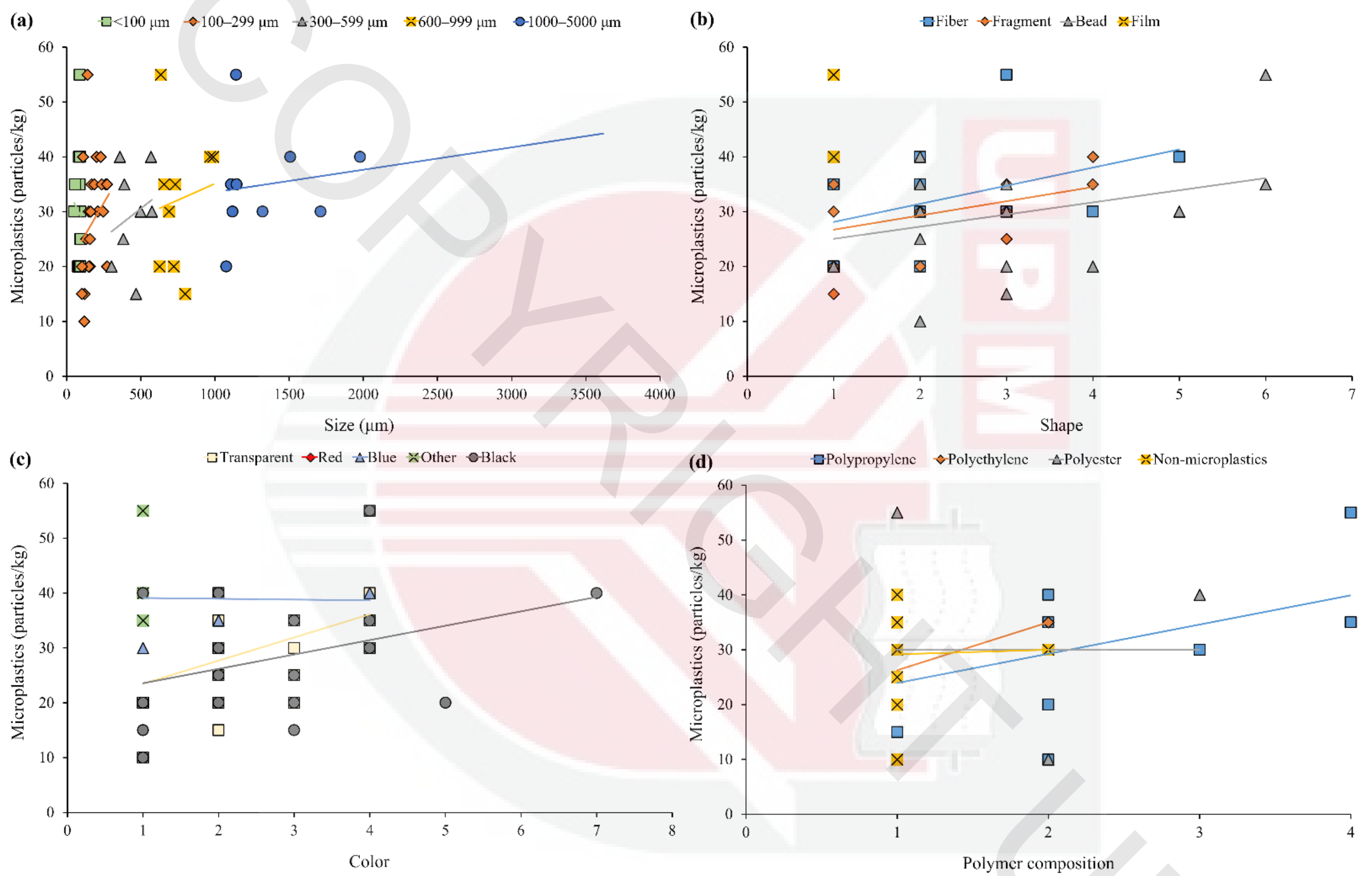

Figure 3.10: Relative microplastic abundance and distribution for (a) size, (b) shape, (c) color, and (d) polymer composition in the sediments of the Selangor River Basin

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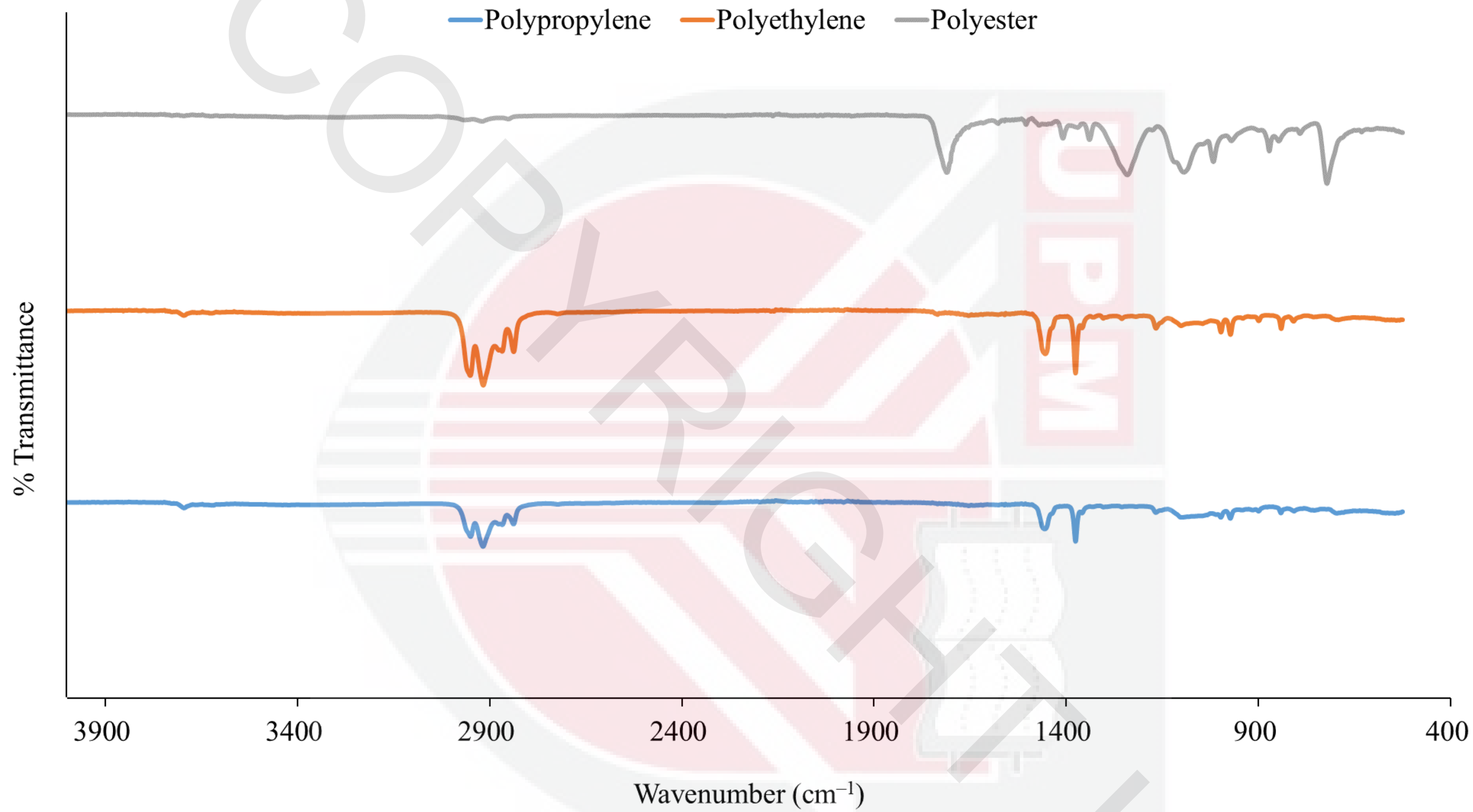

Figure 3.11: Fourier-transform infrared (FTIR) spectra coupled with attenuated total reflectance (ATR) spectra of typical selected microplastics

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3.3.3 Comparison of microplastics in the riverine environment

Microplastics were detected across 25 sampling points in the surface water and sediment samples. The independent sample *t*-test revealed a significant statistical difference in microplastic concentration between surface water and sediment of the Selangor River Basin ($t(48) = -13.22$, $p < 0.01 = 0.000$), with sediment exhibiting a notably higher microplastic concentration (**Figure 3.12**). The result of this study aligns with a previous study conducted in the urbanized section of the Vistula River in Poland, where microplastic concentrations were detected up to 2.55 particles/L in surface water and 580 particles/kg in sediment (Sekudewicz et al., 2021). Furthermore, several other studies have consistently demonstrated a higher prevalence of microplastics in sediment than in surface water (Ding et al., 2019; Wu et al., 2020; Huang et al., 2021a). The predominance of microplastics in sediment suggests their tendency to accumulate and persist owing to their unique characteristics and interactions in the environment. For instance, the majority of microplastics have relatively similar or higher density than water (up to 1.8 g/cm^3), which makes them settle and accumulate in sediment (Li et al., 2018b).

However, in environmental settings, the density of microplastics may vary depending on various chemical (e.g., adsorption and desorption), physical (e.g., degradation, weathering, and waves), and biological (e.g., biofilm) interactions (Cole et al., 2011; Tang et al., 2018). Additionally, their higher surface-area-to-volume ratio enables a greater sorption capacity for other organic pollutants and particles (i.e., biofilms), further promoting their deposition in sediment (Cole et al., 2011; Tourinho et al., 2019). However, it is important to note that the distribution and concentration of

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microplastics can vary depending on factors such as the specific environment, sources of pollution, hydrodynamic conditions, and regional factors.

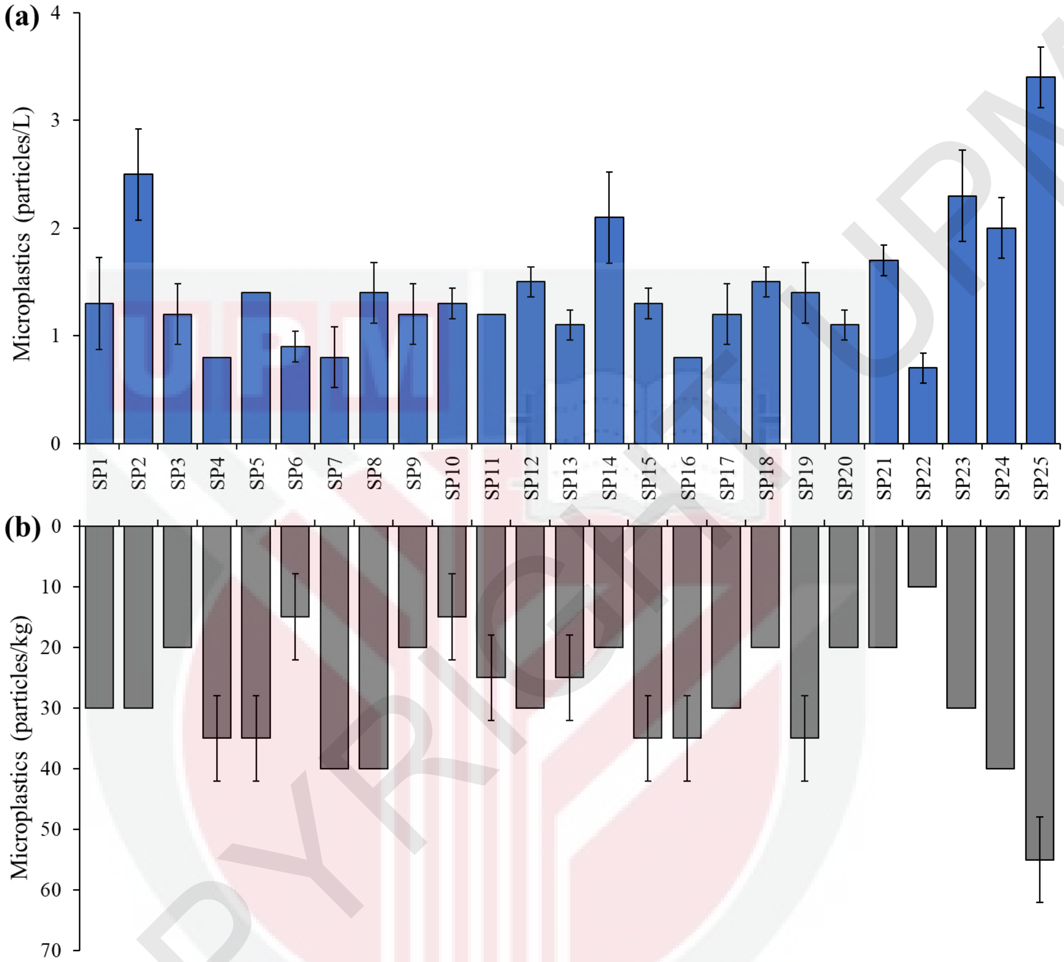

Figure 3.12: The mean concentration and distribution of microplastics in (a) surface water and (b) sediment of Selangor River Basin

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3.4 Conclusion

The measured levels of microplastics in the surface water of the Selangor River Basin range from 0.70 to 3.40 particles/L, with the downstream areas experiencing a significant burden of microplastic pollution. This higher concentration downstream is likely attributed to substantial anthropogenically driven waste discharge from both upstream sources and tributaries. Consequently, the Selangor River Basin appears to play a pivotal role as a potential source of microplastics entering the oceans. This study revealed that particles below 1000 µm in size, with fiber-like structures, transparent particles, and composed of PE, were prevalent in the surface water of the Selangor River Basin. In contrast, sediment analysis indicated the dominance of smaller microplastics (less than 300 µm), displaying bead-like shapes and black coloration, with concentrations ranging from 10 to 55 particles/kg. The primary polymer constituents in the sediment were identified as PP, followed by polyester and PE. The presence and distribution of microplastics in both surface water and sediment within the Selangor River Basin are profoundly influenced by extensive urbanization and various anthropogenic activities, including industrial, commercial, residential, and tourism-related sources in the basin. Remarkably, these specific microplastic characteristics exhibit a consistent predominance in various environmental compartments globally, highlighting their widespread occurrence and potential risks to ecosystems and human health. These findings underscore the imperative need for comprehensive, large-scale monitoring assessments across different river regions with varying land-use patterns and seasonal variations (**Table 3.4**). Such assessments are essential for a thorough understanding of the nature, extent, and fate of microplastics in the environment and for the effective mitigation of their adverse impacts on the environment, aquatic organisms, and human well-being.

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Table 3.4: Summary of the research matrix for the occurrence of microplastics in surface water and sediment

Objective	Activities	Findings/Outputs	Publications
1. To ascertain the concentration, spatial distribution, and compare the occurrence of microplastics in samples of surface water and sediment collected from the Selangor River Basin.	✓ Comprehensive examination of the occurrence and distribution of microplastics in surface water and sediment. ✓ Surface water (5 L) and sediment (100 g) sampling and extraction analysis. ✓ Physicochemical properties for descriptive analysis. ✓ Statistical analysis: - One-way analysis of variance (ANOVA) examined the statistical differences in microplastic concentrations among the three sampled regions (i.e., upstream, tributaries, and downstream). - Pearson correlation coefficients evaluated the correlation between the abundance of microplastics and their characteristics or physicochemical parameters.	✓ Preliminary study of microplastic concentration in surface water and sediment. ✓ Establishing the occurrence and spatial distribution of microplastics in both surface water and sediment. ✓ Determination of physical and chemical properties of microplastics in both surface water and sediment. ✓ Anticipated factors or sources influencing the occurrence of microplastics in the riverine environment.	✓ Zaki, M.R.M., Sukatis, F.F., Roslan, M.Q.J., Isa, N.M., Yusoff, F.M., & Aris, A.Z. (2023). Microplastic pollution in freshwater sediments: Abundance and distribution in Selangor River Basin, Malaysia. Water, Air, & Soil Pollution, 234(7), 1–13. [Q2, IF: 2.9]. ✓ Zaki, M.R.M., Zainuddin, A.H., Saifuddin, M.F.U., Razak, M.R., Isa, N.M., Ash'aari, Z.H., & Aris, A.Z. Microplastic contamination and ecological risk assessment in tropical riverine surface water of Selangor River Basin, Malaysia [ACS ES&T Water – Accepted, Q1, IF: 5.3, Top 15%]. ○ Zaki, M.R.M., Saifuddin, M.F.U., Ash'aari, Z.H., & Aris, A.Z. Microplastics and endocrine disrupting compounds in urbanized

Table 3.4: Continued

	- Pearson correlation analysis ascertained the correlation between microplastics in both matrices (e.g., surface water and sediment) of Selangor River Basin.		tropical riverine water: A comparative analysis, pollution load and land-use effect [Preparing for journal submission].

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CHAPTER 4

MULTICLASS ENDOCRINE DISRUPTING COMPOUNDS IN SURFACE WATER AND SEDIMENT ACROSS THE SELANGOR RIVER BASIN

4.1 Introduction

EDCs have garnered increasing attention as emerging contaminants since 1965 because of their ubiquitous presence in the environment and their potential to disrupt the hormonal systems of aquatic organisms and humans (Daughton, 2016). EDCs encompass a broad spectrum of emerging environmental contaminants, including plasticizers, pesticides, hormones, pharmaceuticals, and personal care products (Wee and Aris, 2017b). Plasticizers, such as phenolic compounds, PAEs, and polyhalogenated compounds, are commonly incorporated as additives in plastic production to enhance the durability and resilience of plastics. Furthermore, plasticizers have extensive applications in various industrial processes, serving as additives for the manufacturing of consumer goods such as fragrances, fire retardants, detergents, plastics, coatings, antiseptics, fabrics, and construction materials.

This chapter is based on the papers:

a) Zaki, M.R.M., Razak, M.R., Haron, D.E.M., Isa, N.M., Yusoff, F.M., & Aris, A.Z. Multiclass endocrine disrupting compounds in surface water from Selangor River Basin: Occurrence and ecological risk assessment [ACS ES&T Water – under review, Q1, IF: 5.3, Top 15%].
b) Zaki, M.R.M., Wee, S.Y., Haron, D.E.M., Isa, N.M., Yusoff, F.M., & Aris, A.Z. Occurrence and distribution of multiclass endocrine disrupting compounds in sediment of anthropogenically impacted Selangor River Basin, Malaysia [Preparing for journal submission].
c) Zaki, M.R.M., Saifuddin, M.F.U., Ash'aari, Z.H., & Aris, A.Z. Microplastics and endocrine disrupting compounds in urbanized tropical riverine water: A comparative analysis, pollution load and land-use effect [Preparing for journal submission].
d) Zaki, M.R.M., Razak, M.R., & Aris, A.Z. Microplastics and plasticisers in the freshwater environments: A perspective on the distribution and associated risk [Science of the Total Environment – Under review, Q1, IF: 9.8, Top 10%].

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BPA, bisphenol F (BPF), bisphenol S (BPS), NP, and OP are types of phenolic compounds widely used in making plastics, antioxidants, detergents, and additives. BPA is a synthetic building block that is commonly used in the production of PC plastics and epoxy resins. These materials are used extensively in various consumer products, packaging, and construction. For example, PC plastics are used to make things like plastic bottles, toys, household appliances, and medical devices. Epoxy resins are used as protective coatings for food containers, adhesives, and vehicle parts. Notably, both epoxy resins and PC plastics can withstand high temperatures of up to 145°C, which has led to the production of over 10 million tons of BPA annually by 2022 (Giulivo et al., 2016; Staples et al., 2018; Huelsmann et al., 2021). Furthermore, BPA exposure has been associated with the inhibition of genes related to fetal development because it accumulates in fatty tissues owing to its ability to dissolve in fats (lipophilic properties) (Valentino et al., 2016; Yilmaz et al., 2020).

Subsequently, numerous compounds sharing identical physicochemical properties and molecular structures with BPA have been employed in the manufacture of PC plastics and epoxy resins. These compounds are also known as BPA analogs or substitutes, which consist of 16 compounds, including BPS, BPF, and bisphenol AF (BPAF), that have been utilized for industrial purposes (Chen et al., 2016). Due to their extensive production and wide use in consumer products, these compounds have also been frequently detected in the environment (Yamazaki et al., 2015; Jin and Zhu, 2016), food products (Liao and Kannan, 2013; Wang et al., 2020e; Lim et al., 2022), and the atmosphere (Liao et al., 2012). Exposure to these compounds may result in toxic effects similar to those caused by BPA, affecting the health of both aquatic organisms and humans.

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Other than that, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are synthetic chemicals belonging to the PFAS family and have been widely used in various manufacturing processes. For instance, PFOA is commonly used in the production of Teflon, a popular non-stick coating for cookware, whereas PFOS is frequently found in various products, such as stain-resistant textiles, fire-fighting foams, and food packaging. Owing to previous evidence on the ubiquity and toxicity of PFOS and PFOA in the environment, many industries in the world have phased out the production of these compounds (Ahrens et al., 2009; Domingo and Nadal, 2019). These compounds have been frequently detected in various environmental compartments, including freshwater environments. For instance, Skutlarek et al. (2006) detected the abundance of PFOA in the Moehne River located in Germany, with levels measuring as high as 3640 ng/L. These compounds have been detected in drinking water (Mastrantonio et al., 2018; Domingo and Nadal, 2019; Crone et al., 2019).

To address concerns regarding the potential health risks associated with PFOS and PFOA exposure, the USEPA has established a drinking water health advisory level of 70 ng/L for these chemicals and their combined concentrations. Meanwhile, the European Union has implemented a stringent environmental quality standard for PFOS, setting the limit at an annual average of 0.65 ng/L in inland surface waters. These regulatory guidelines aim to mitigate the risks posed by these substances to human health and the environment. Exposure to these compounds has been associated with numerous adverse health effects, including hepatotoxicity, neurotoxicity, reproductive toxicity, immunotoxicity, thyroid disruption, cardiovascular toxicity,

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pulmonary toxicity, and renal toxicity in laboratory animals as well as in vitro human systems (Zeng et al., 2019; Ojo et al., 2021).

Therefore, the study of EDCs is of paramount importance as EDCs encompass a wide range of environmental contaminants, including plasticizers, pesticides, hormones, pharmaceuticals, and personal care products, and have gained significant attention due to their widespread presence and their potential to disrupt hormonal systems in aquatic organisms and humans. In light of these findings, ongoing research and monitoring of EDCs, BPA analogs, PFAS, and other emerging disrupting compounds are essential to fully understand their environmental impact and potential threats to human health, ultimately guiding effective regulatory measures to mitigate these risks.

4.2 Methodology

4.2.1 Chemicals and materials

A total of ten multiclass EDCs were analyzed, comprising plasticizers (BPA, BPS, and BPF), pharmaceuticals (propranolol), PFAS (PFOA and PFOS), and hormones (testosterone, progesterone, 17β-estradiol (E2), and 17α-ethynylestradiol (EE2)). The analytical external standards of the EDCs were supplied by Dr. Ehrenstorfer (Augsburg, Germany). Meanwhile, internal standards for bisphenol A-d8, 17α-ethynylestradiol-d4 and 17β-estradiol-d4 and progesterone-d9 were supplied by Toronto Research Chemicals (Toronto, Canada) and Cambridge Isotope Laboratories (Massachusetts, USA), respectively. Analytical grade chemical reagents, including MeOH, acetonitrile (ACN), hydrochloric acid (HCl) solution, methyl-tributyl ether (MTBE), acetone, and ammonium hydroxide (NH_4OH, 25% ammonia solution) were

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acquired from Fisher Scientific (New Jersey, USA). Ascorbic acid, 0.2 µm glass microfiber filter, tetrasodium ethylenediamine-tetraacetate dihydrate (Na_2EDTA), and UPW were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), Whatman (Buckinghamshire, UK), Merck (Darmstadt, Germany), and Millipore (Massachusetts, USA), respectively. Aluminum oxide (Al_2O_3) was supplied by Merck (Darmstadt, Germany), whereas the hydromatrix was purchased from Agilent Technologies (California, USA). Polytetrafluoroethylene (PTFE) syringe filters (0.2 µm, 4 mm) and solid phase extraction (SPE) cartridges Strata-X polymeric reversed phase (200 mg/6 mL, C18) were supplied by Phenomenex (California, USA).

4.2.2 Study area

As detailed in Chapter 3, Section 3.2.2, Page 54.

4.2.3 Surface water and sediment sampling

As detailed in Chapter 3, Section 3.2.3, Page 58.

4.2.4 Sample extraction and clean-up

4.2.4.1 Surface water

A comprehensive flowchart illustrating the sample preparation, extraction, and quantification of EDCs in surface water and sediment is depicted in **Figure 4.1**. The extraction of EDCs was performed using a validated multi-residue SPE-LC-MS/MS, which was adapted from existing studies (Ismail et al., 2019; Wee et al., 2019). The pH of the extracted surface water sample was adjusted to 2.0 ± 0.5 through the addition

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of a 1 M HCl solution. Additionally, the sample was spiked with internal standards at a concentration of 50 ng/mL to enhance the precision of the measurements. Subsequently, the samples were treated with Na_2EDTA at a concentration of 300 mg/L. Prior to extraction, the cartridge Strata-X 33u Polymeric Reversed Phase (200 mg/6 mL) was pre-conditioned with 10 mL of MeOH, followed by 5 mL of UPW and 5 mL of UPW (pH 2.0 ± 0.5). Consequently, the cartridge was loaded with the sample and then washed with 10 mL of UPW to remove excess Na_2EDTA. The analytes in the cartridge were then eluted using 10 mL of MeOH, subsequently supplemented with 5 mL of MeOH:acetone (1:1, v/v). Using a rotary evaporator, the eluent was reduced to 1–2 mL and subsequently concentrated to 1 mL using nitrogen blow. The eluent was reconstituted with ACN:UPW (3:7, v/v) in 1 mL of sample. Prior to instrumental analysis, the reconstituted sample was filtered using a 0.2 µm PTFE syringe filter.

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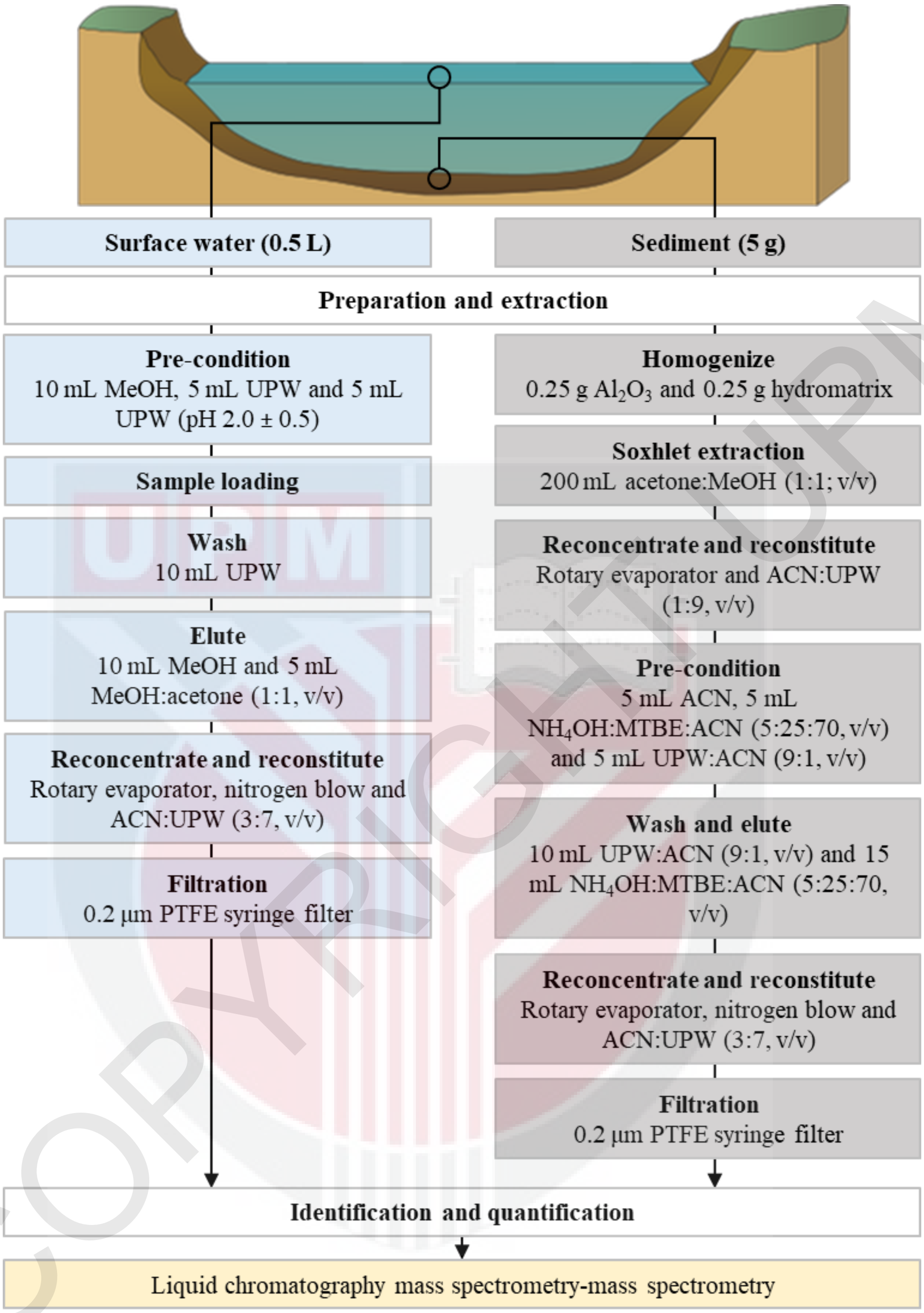

Figure 4.1: Flowchart illustrating the methodological overview of a study on EDCs in surface water and sediment of Selangor River Basin

4.2.4.2 Sediment

The EDCs extraction was adapted with slight modifications by using Soxhlet extraction and SPE based on Omar et al. (2017), Wee et al. (2019) and Ismail et al. (2020). A sediment sample of approximately 5 g (± 0.01) was weighed and homogenized with 0.25 g of Al_2O_3 and 0.25 g of hydromatrix. The mixture was transferred directly into a cellulose thimble after being finely ground using a mortar and pestle. The sample was spiked with internal standards (10 ng/g) and then extracted using Soxhlet extraction for 8 h using a 200 mL mixture of acetone:MeOH (50:50; v/v). The extraction was performed without the presence of light, and aluminum foil was used to wrap the glassware to avoid light penetration. The long exposure (8 h) of the sample to light during extraction might cause the degradation of targeted compounds (Omar et al., 2017). After extraction, a rotary evaporator was used to reduce the extracted sample to approximately 1–2 mL. Prior to SPE analysis, the extracted sample was further reconstituted with ACN:UPW (10:90, v/v) to 15–20 mL. The SPE cartridge was pre-conditioned with 5 mL of ACN, 5 mL of NH_4OH:MTBE:ACN (5:25:70, v/v) and 5 mL of UPW:ACN (90:10, v/v). The sample was transferred immediately without allowing the cartridge to dry and washed using 10 mL of UPW:ACN (90:10, v/v). The analyte was eluted with 15 mL of NH_4OH:MTBE:ACN (5:25:70, v/v) after the cartridge was allowed to dry for 5–10 min. Consequently, a rotary evaporator was used to pre-concentrate the extract to 1–2 mL and further concentrate under nitrogen blow. The final volume of the extracted sample (1 mL) was prepared by reconstituting the analytes with ACN:UPW (30:70, v/v). The reconstituted sample was filtered through a 0.2 μm PTFE syringe filter prior to instrumental analysis by liquid chromatography-mass spectrometry (LC-MS/MS).

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4.2.5 Instrumental analysis

For instrumental analysis of EDCs, the sample was quantified using chromatographic techniques with mass spectrometry. This combination allowed for the precise identification and quantification of EDCs with high sensitivity and specificity. Quantification of EDCs in the surface water and sediment samples was performed using an LC-MS-8050 Tandem Quadrupole Mass Spectrometer. The LC-MS-8050 utilized two mobile phases (A and B) to enhance the ionization efficiency of multiclass EDCs. The analytical method was adapted from the optimized method developed by Wee et al. (2019). Detailed information on the instrumental analysis, as well as the chromatographic conditions, can be found in **Table 4.1**.

Table 4.1: Analytical setup and chromatographic condition for instrumental analysis

Chromatographic condition		
Liquid chromatography system	Shimadzu, Japan	
Mass spectrometry	LC-MS-8050 Tandem Quadrupole Mass Spectrometer	
Column	Kinetex EVO C18 100A column (150 × 2.10 mm, 5 µm)	
Ionization mode	Electrospray ionization, positive and negative	
Method	Basic	
Mobile phase	0.01% NH_4OH in UPW (A) and 100% MeOH (B)	
Injection volume (µL)	10	
Column temperature (°C)	40	
Flow rate (mL/min)	0.35	
Ion spray voltage (kV)	4.5	
CID gas (kPa)	230	
Drying gas flow (L/min)	15	
Heat block temperature (°C)	400	
Nebulising gas flow (L/min)	3	
Gradient	Time (min)	% B
	0.00	30
	1.00	30
	3.00	90
	6.00	90
	6.01	30
	10.00	30

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4.2.6 Data analysis

The concentrations of EDCs in the surface water samples are reported as mean (ng/L) ± SD. An analysis of variance (one-way ANOVA) test was conducted to ascertain the statistical significance of differences in EDCs concentrations across the three regions of the sampling sites (upper stream, tributaries, and lower stream). Pearson correlation analysis was used to assess the correlations between EDC concentrations and physicochemical parameters. Meanwhile, for sediment, descriptive statistics were used to observe the spatial distribution and variation of EDCs in the sediment of the Selangor River Basin. Statistically significant differences in EDCs concentrations in the upper stream, tributaries, and lower stream along the Selangor River Basin were evaluated using one-way analysis of variance (ANOVA). All statistical data were analyzed using IBM SPSS Statistics Software (IBM, USA) version 25.

4.2.7 Quality assurance and quality control

Meanwhile, the recovery analysis for EDCs quantification in both surface water and sediment involved the use of a control sample collected from the upper stream of the Selangor River Basin. This control sample was spiked with isotope-labelled internal standards, following the developed method demonstrated by Wee et al. (2019), to achieve optimal recovery results. Internal standards comprising bisphenol A-d8, 17α-ethynylestradiol-d4, 17β-estradiol-d4, and progesterone-d9 were prepared for spiking in both the control and environmental samples. The parameters for the extraction of EDCs, such as the method recovery, relative standard deviation (RSD), method detection limit (MDL), matrix effect (ME), and linearity (r), were included to verify the accuracy of the analytical method for EDCs quantification (**Table 4.2**).

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For surface water, the method recovery ranged from 66.51–129.66% at spiking concentrations of 50 ng/mL, while MDL ranged from 0.26–27.04 ng/L. Prior to reliability and sensitivity of instrumentation performance, a five-point calibration curve (5–250 ng/mL) of the targeted compounds ranged from 0.9665–0.9984, which indicated high positive linearity of the targeted compounds. ME test was then conducted to ascertain the influence of the riverine water matrix on the electrospray ionization and MS response efficiencies. The ME of the riverine water samples in this study was <100%. ME <100% indicated that the signal was suppressed, whereas >100% suggested that the signal was amplified. Therefore, isotope-labelled internal standards have been used to suppress ionization for recovery correction of compounds (Wu et al., 2010; Omar et al., 2017).

Meanwhile for sediment, the method recovery for the targeted compounds varied from 78.39–138.40% at spiking concentrations of 10 ng/g, whereas MDL computed using a signal-to-noise ratio (S/N, 3:1) ranged from 0.002–1.60%. Moreover, the ME was analyzed to determine the effect of the riverine sediment matrix on the effectiveness of electrospray ionization. The percentage of ME was calculated and adopted from Omar et al. (2017), where all the targeted EDCs encountered an ionization suppression (ME <100%). Matuszewski et al. (2003) suggested that the ME with <100% indicated as the ionization suppression, meanwhile ME with >100% showed as the signal enhancement. Five-point calibration curves of the targeted EDCs, ranging from 5 to 250 ng/mL, suggested high positive linearity with correlation coefficients ranging from 0.9665 to 0.9984.

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Table 4.2: Quality assurance and quality control parameters for targeted compounds in surface water and sediment samples of Selangor River Basin

Environmental sample	EDC group	Compounds	QAQC parameter				
			Recovery (%)	RSD (%)	MDL (ng/L)	ME (%)	*r*
Surface water	Plasticizer	BPA	84.77	3.40	1.82	–81.94	0.9932
		BPF	77.31	7.93	24.86	–84.60	0.9922
		BPS	78.34	7.25	0.26	–76.89	0.9855
	Pharmaceutical	Propranolol	66.51	20.04	4.02	–92.27	0.9936
	PFAS	PFOS	77.44	9.46	27.04	–90.66	0.9901
		PFOA	105.00	3.45	10.26	–77.93	0.9968
	Hormone	Testosterone	83.43	4.58	24.72	–94.30	0.9965
		Progesterone	129.66	7.93	4.04	–56.08	0.9984
		EE2	75.65	11.45	4.7	–91.09	0.9908
		E2	73.83	13.69	1.58	–91.11	0.9665
Sediment	Plasticizer	BPA	86.23	10.46	0.10	–85.63	0.9932
		BPF	90.29	7.22	1.60	–85.44	0.9922
		BPS	81.44	14.47	0.002	–73.64	0.9855
	Pharmaceutical	Propranolol	92.45	5.55	0.01	–89.42	0.9936
	PFAS	PFOS	104.91	3.39	0.08	–83.43	0.9901
		PFOA	115.35	10.08	0.26	–81.58	0.9968

Table 4.2: Continued

Hormone	Testosterone	138.40	22.78	1.12	–71.46	0.9965
	Progesterone	130.67	18.80	0.10	–64.37	0.9984
	EE2	78.39	17.13	0.55	–95.32	0.9908
	E2	113.13	8.71	0.07	–80.13	0.9665

BPA: Bisphenol A; BPF: Bisphenol F; BPS: Bisphenol S; PFAS: Perfluoroalkyl substance; PFOS: Perfluorooctane sulfonate; PFOA: Perfluorooctanoic acid; EE2: 17α-ethynylestradiol; E2: 17β-estradiol; RSD: Relative standard deviation; MDL: Method detection limit; ME: Matrix effect; *r*: Linearity

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For quality control, the procedures are outlined in Chapter 3, Section 3.2.7, Page 67.

4.3 Results and discussion

4.3.1 Occurrence and spatial distribution of endocrine disrupting compounds

EDCs have been identified in diverse environmental compartments worldwide, underscoring the importance of assessing their prevalence and distribution. Their persistent presence raises concerns owing to the array of adverse effects posed on both ecological systems and human health. Notably, prior research has suggested a link between microplastics and the release of various plasticizers categorized as EDCs. Considering this association, the present study investigated the occurrence and spatial distribution of microplastics and multiclass EDCs within the Selangor River Basin. The primary aim of the present study was to unveil potential correlations, differentiate controlling mechanisms, and precisely identify contributing factors that influence the occurrence and contamination of these hazardous substances within riverine environments, potentially causing disruptions to river ecosystems.

4.3.1.1 Surface water

Approximately seven EDCs were detected, including three plasticizers (BPA, BPF, and BPS), two PFAS (PFOS and PFOA), and two hormones (EE2 and E2). One pharmaceutical (propranolol) and two hormones (testosterone and progesterone) were below the MDL. Amongst the identified EDCs, only BPA and E2 were detected at all sampling points (100% detection) with concentrations ranging from 25.43 to 358.05 ng/L and 6.64 to 50.18 ng/L, respectively. Other compounds such as EE2 (<4.7–30.74

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ng/L), BPS (<0.26–14.71 ng/L), and BPF (<24.86–1098.40 ng/L) were present with relatively high percentages of detection at 84%, 56%, and 44%, respectively. The least compounds detected in the surface water were two PFAS compounds, namely, PFOS (20%) and PFOA (4%). The majority of the EDCs found in this investigation had a CV of more than 30%. This indicated that EDC concentrations varied greatly across different sampling points (**Figure 4.2**, **Figure 4.3** and **Table 4.3**).

The presence of all three plasticizers (i.e., BPA, BPF, and BPS) in the surface water suggests that the river was heavily impacted by plastic pollution. For instance, BPA is a commonly used plasticizer additive with widespread applications in the production of PC plastics, epoxy resins, and various household products such as bottles, food containers, toys, and water pipes. Similarly, bisphenol analogs (i.e., BPF and BPS) have been extensively used as BPA alternatives and substitutes (Yang et al., 2014; Jin and Zhu, 2016; Zainuddin et al., 2023). The vast application, usage, and high demand for BPA, BPF, and BPS may contribute to their ubiquity in the environment. Moreover, BPF and BPS have been widely detected in environmental compartments, such as sewage sludge (Lee et al., 2015b; Yu et al., 2015; Qian et al., 2021), drinking water (Zhang et al., 2019a), indoor dust (Liao et al., 2012), and biota (Rios-Fuster et al., 2022). In this study, BPF was detected at the highest concentration (1098.40 ng/L) compared to BPA (358.05 ng/L) and BPS (14.71 ng/L). The measured concentrations of BPF in the present study were comparable with the results reported by Yamazaki et al. (2015) for the Han River (121–1300 ng/L) and Pearl River (448–1110 ng/L), which were attributed to anthropogenic waste discharge from populated and urbanized areas.

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Meanwhile, BPA concentrations in the Selangor River Basin surface water were relatively low compared to those reported in other Malaysian waters by Nazifa et al. (2020) in the Klang River (up to 12000 ng/L) and Pahang River (up to 37000 ng/L). In contrast, Wee et al. (2019) observed considerably lower concentrations of BPA (1.18–8.24 ng/L) in the Langat River compared to the present study. Wee et al. (2019) highlighted that the restriction and regulation of BPA usage were prompted by the presence of BPA at low concentrations within the Langat River. However, even with the ban in place, there is no assurance that the occurrence of BPA will decrease because of its physicochemical properties, bioaccumulation, and persistence in the environment. Malaysian tap water has been demonstrated that contains alarming levels of BPA, reaching as high as 66.4 ng/L (Wee et al., 2020).

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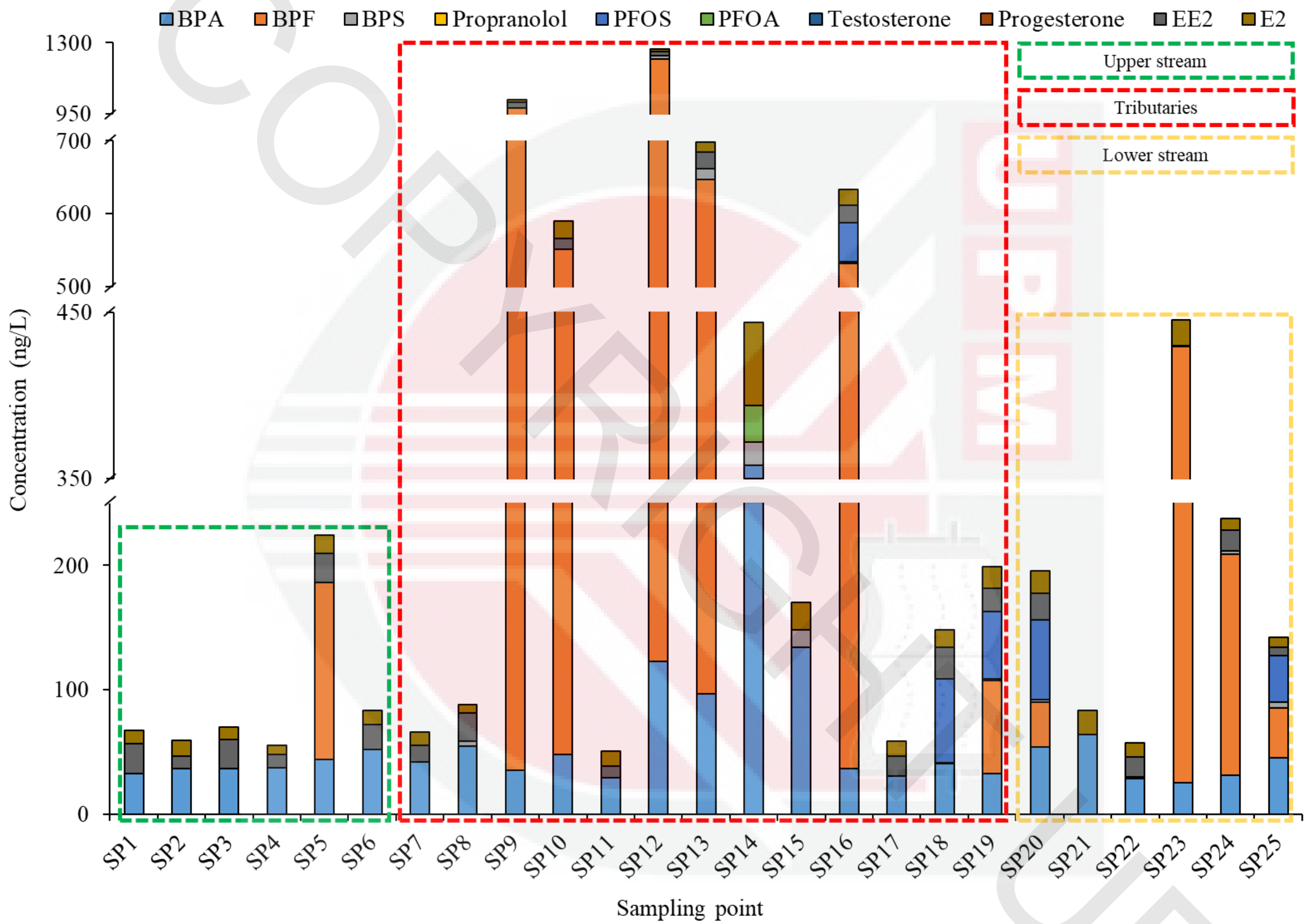

Figure 4.2: Spatial distribution of EDCs concentrations in surface water from the upper stream, tributaries, and lower stream of the Selangor River Basin

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Table 4.3: Occurrence and distribution of EDCs in the surface water of Selangor River Basin

Sampling point	Mean concentration of EDCs (ng/L)									
	BPA	BPF	BPS	Propranolol	PFOS	PFOA	Testosterone	Progesterone	EE2	E2
SP1	32.51	<24.86	<0.26	<4.02	<27.04	<10.26	<24.72	<4.04	24.06	10.53
SP2	36.30	<24.86	<0.26	<4.02	<27.04	<10.26	<24.72	<4.04	10.11	13.06
SP3	36.54	<24.86	<0.26	<4.02	<27.04	<10.26	<24.72	<4.04	23.40	10.19
SP4	37.09	<24.86	<0.26	<4.02	<27.04	<10.26	<24.72	<4.04	10.57	7.74
SP5	44.03	142.00	<0.26	<4.02	<27.04	<10.26	<24.72	<4.04	23.92	14.53
SP6	51.66	<24.86	<0.26	<4.02	<27.04	<10.26	<24.72	<4.04	20.20	11.57
SP7	41.56	<24.86	<0.26	<4.02	<27.04	<10.26	<24.72	<4.04	13.59	10.78
SP8	54.57	<24.86	3.91	<4.02	<27.04	<10.26	<24.72	<4.04	23.12	6.64
SP9	35.50	942.36	<0.26	<4.02	<27.04	<10.26	<24.72	<4.04	30.74	9.22
SP10	47.72	503.78	<0.26	<4.02	<27.04	<10.26	<24.72	<4.04	14.71	23.93
SP11	29.40	<24.86	<0.26	<4.02	<27.04	<10.26	<24.72	<4.04	8.87	12.27
SP12	122.64	1098.40	14.71	<4.02	<27.04	<10.26	<24.72	<4.04	22.97	9.98
SP13	96.38	551.07	14.25	<4.02	<27.04	<10.26	<24.72	<4.04	22.44	14.03
SP14	358.05	<24.86	13.97	<4.02	<27.04	21.68	<24.72	<4.04	<4.7	50.18
SP15	134.16	<24.86	13.73	<4.02	<27.04	<10.26	<24.72	<4.04	<4.7	22.45
SP16	36.57	495.87	1.93	<4.02	53.63	<10.26	<24.72	<4.04	23.63	22.17
SP17	30.35	<24.86	0.50	<4.02	<27.04	<10.26	<24.72	<4.04	15.40	12.61
SP18	40.78	<24.86	0.38	<4.02	67.60	<10.26	<24.72	<4.04	25.31	13.98
SP19	32.72	74.92	0.79	<4.02	54.73	<10.26	<24.72	<4.04	18.77	17.08
SP20	53.74	36.26	1.91	<4.02	64.00	<10.26	<24.72	<4.04	21.91	17.54

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Table 4.3: Continued

SP21	63.89	<24.86	<0.26	<4.02	<27.04	<10.26	<24.72	<4.04	<4.7	19.65
SP22	28.32	<24.86	1.61	<4.02	<27.04	<10.26	<24.72	<4.04	15.93	11.74
SP23	25.43	404.11	0.30	<4.02	<27.04	<10.26	<24.72	<4.04	<4.7	15.61
SP24	30.97	177.73	3.23	<4.02	<27.04	<10.26	<24.72	<4.04	16.76	8.83
SP25	45.02	40.17	4.90	<4.02	37.14	<10.26	<24.72	<4.04	7.18	7.56
Range	25.43–358.05	<24.86–1098.40	<0.26–14.71	<4.02	<27.04–67.60	<10.26–21.68	<24.72	<4.04	<4.7–30.74	6.64–50.18
Mean ± SD	61.84 ± 67.74	406.06 ± 361.63	5.44 ± 5.88	NA	55.42 ± 11.83	21.68	NA	NA	18.74 ± 6.28	14.95 ± 8.74
CV (%)	109.55	89.06	108.18	NA	21.35	NA	NA	NA	33.51	58.45
MDL (ng/L)	1.82	24.86	0.26	4.02	27.04	10.26	24.72	4.04	4.7	1.58
Detection (%)	100	44	56	0	20	4	0	0	84	100

SD: standard deviation; CV: Coefficients of variation; MDL: Method detection limit; BPA: Bisphenol A; BPF: Bisphenol F; BPS: Bisphenol S; PFOS: Perfluorooctane sulfonate; PFOA: Perfluorooctanoic acid; EE2:17α-ethynylestradiol; E2:17β-estradiol; NA: Not available; <: Denotes value below the MDL

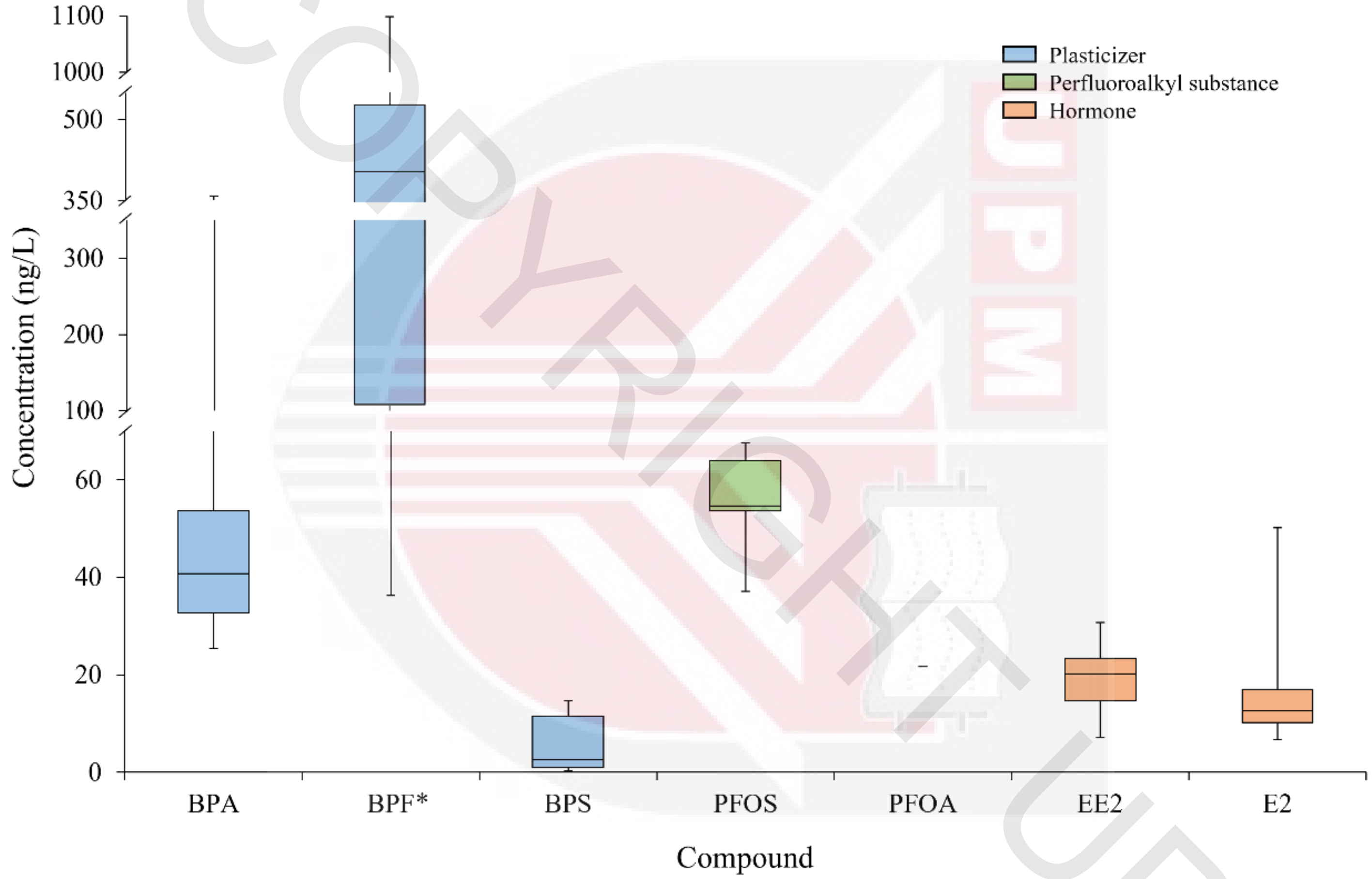

Figure 4.3: Concentration of EDCs in the surface water of the Selangor River Basin. * Significant at $p < 0.05$

The prevalence of hormones (i.e., E2 and EE2) indicates the introduction of various anthropogenic discharges from industrial and domestic activities in the Selangor River Basin. In this study, the concentration of E2 (6.64–50.18 ng/L) was relatively higher compared to EE2 (<4.7–30.74 ng/L). The concentrations of E2 and EE2 observed in this study were relatively higher than those reported in the Bahe River (Wang et al., 2018c), Langat River (Wee et al., 2019), Klang River (Nazifa et al., 2020), and Mille Ile River (Goeury et al., 2022). However, the findings of the present study were comparable to those documented by Gorga et al. (2015) in the Iberian River with E2 and EE2 ranging up to 2.2 ng/L and 7.8 ng/L, respectively. Similarly, Manickum and John (2014) observed that E2 (119 ng/L) had a higher concentration than EE2 (30 ng/L) in an activated sewage system in Pietermaritzburg, South Africa. The aquatic ecosystem is heavily impacted by populated areas that are primarily introduced through sewage systems.

The widespread presence of E2 in the environment can be attributed to its natural secretion and exposure as well as the extensive usage and consumption of oral contraceptive pills (Zhang et al., 2011). E2 is a naturally occurring estrogen, whereas EE2 is a synthetic estrogen. Both compounds are commonly used in the formulation of synthetic estrogen medications and in the creation of combined oral contraceptive pharmaceuticals (Aris et al., 2014; Cunha et al., 2016). These hormones are released into the aquatic environment mainly through sewage treatment effluents and urban runoff, which carry waste from (i) livestock activities, (ii) industrial effluents, (iii) agriculture, (iv) hospital effluents, and (v) residential areas (Aris et al., 2014; Pessoa et al., 2014). This includes waste from human excretion (i.e., from the adrenal cortex,

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placenta, testis, and ovary) and diagnostic laboratory analysis (e.g., urine samples) (Avberšek et al., 2011).

Considering that this study was performed after the COVID-19 pandemic, it is possible that this caused an increase in EDCs in the environment. For instance, the pandemic has increased the demand for plastics (face masks, gloves, and other single-use plastics). In fact, 8.4 million metric tons of plastic waste were produced by the pandemic alone, with more than 25,000 metric tons ending up in the ocean (Peng et al., 2021). Notably, these plastics can leach out their plastic additives after exposure to the environment (Hermabessiere et al., 2017); thus, there is also the potential to intensify the concentration of BPS in the environment. Hence, the prevailing presence of BPA and other EDCs in the Selangor River Basin may be associated with factors, such as the recent COVID-19 pandemic and various anthropogenic waste discharges.

Three regions, the upper stream (SP1–SP6), tributaries (SP7–SP19), and lower stream (SP20–SP25) of the Selangor River Basin, were evaluated to distinguish the occurrence, spatial distribution, and potential sources of EDCs corresponding to different anthropogenic and land-use activities. The tributaries of the Selangor River Basin had relatively high EDC concentrations ranging from 0.38–1098.40 ng/L, followed by the lower stream (0.30–404.11 ng/L) and upper stream (7.74–142.00 ng/L). This suggests that the tributaries were heavily impacted by various anthropogenic activities in industrial and residential areas, particularly in Rawang. However, an ANOVA test found that there was no statistically significant difference between all detected EDCs in the upper stream, tributaries, and lower stream of the Selangor River Basin ($p > 0.05$).

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The variation and distribution of EDCs could be due to the presence and influence of various anthropogenic wastes from industrial, residential, and agricultural activities along the sampling point of the Selangor River Basin. Pearson's correlation revealed no significant correlations between EDC concentrations ($p > 0.05$) and physicochemical parameters, except for dissolved oxygen ($r = 0.507$, $p < 0.01$). This also indicates that the physicochemical parameters in the Selangor River Basin did not significantly influence the concentration, which is comparable to other EDCs (i.e., pesticides) from the Linggi River (Zainuddin et al., 2020). The elevated concentrations of BPF and other EDCs (e.g., BPA, BPS, PFOS, PFOA, EE2, and E2) within the Rawang area (SP11–SP18) appear to be related to extensive industrial effluents and domestic discharge. Othman et al. (2018) and Sakai et al. (2017b) also indicated that industrial activities in the Rawang sub-basin contributed to heavy metal and linear alkylbenzene sulfonate (LAS) pollution, respectively. This suggests that various anthropogenic activities in the Rawang sub-basin contributed to the occurrence of EDCs in the tributaries of the Selangor River Basin.

Among all the EDCs detected, BPF was detected at the highest concentration (1098.40 ng/L) at SP12, followed by BPA (358.05 ng/L) at SP14 and PFOS (67.60 ng/L) at SP18, which were introduced from urban discharge (e.g., industrial and domestic wastes). Moreover, BPF was identified at a significantly higher concentration than the other EDCs ($p < 0.05$). Similarly, PFOS (<27.04–67.60 ng/L) and PFOA (<10.26–21.68 ng/L) were frequently detected only in tributary areas, particularly in the Rawang sub-basin area and flowed through the mainstream and lower stream of the Selangor River Basin (up to 64 ng/L at SP20). This indicates that the introduction of PFOA and PFOS into the surface water of the Selangor River Basin is associated with

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intense terrestrial anthropogenic activities. The Rawang sub-basin is the most populated area, with more than 1000 people/km^2, and is heavily impacted by various anthropogenic wastes from industrial, commercial, and residential activities.

The maximum concentration of PFOS (67.60 ng/L) in the Selangor River Basin is much higher than in Chinese rivers, including the Hai River Basin (8.89 ng/L; Li et al., 2020b), Yangtze River (39.60 ng/L; Yu et al., 2022) and Shandong Province (4.71 ng/L; Feng et al., 2023). Nevertheless, the PFOS levels observed in the present study remained below the health advisory level of 70 ng/L for drinking water set by the USEPA. Nevertheless, the occurrence of PFOS must be considered because of its persistence, bioaccumulation, long-distance migration, and high resistance to biodegradation in the environment (Liang et al., 2021). The exposure of aquatic ecosystems and humans to PFOS has been associated with various adverse effects, including endocrine system disruption (Galloway et al., 2015; Wee and Aris, 2017b).

In contrast, the maximum concentration of PFOA in this study was 21.68 ng/L which is relatively lower than most of the previous studies in Chinese rivers, including the Hai River (57.5 ng/L; Wang et al., 2016b), Liao River Basin (61.6 ng/L; Chen et al., 2015) and Yangtze River (94.2 ng/L; Yu et al., 2022). The prevalence of China as the largest global producer and consumer of PFOA can be attributed to several factors, including insufficient regulations and mitigation measures. These factors have facilitated the widespread use of PFOA in various industries. In recent years, substantial population growth in China has heightened the demand for products containing PFOA (Meng et al., 2017). Feng et al. (2023) detected extremely high concentrations of PFOA ranging from 932.09–650418.64 ng/L, which were attributed

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to the largest manufacturing facilities of a fluorochemical industrial park in Huantai County, Shandong Province, China.

PFOS and PFOA are widely used in the production of water repellents, coatings, surfactants, and impregnation agents in textiles, leather, food packaging, cosmetics, and paper products. These organic compounds are ubiquitous in the environment because of their broad usage in consumer products and have been identified in various environmental compartments. Moreover, these compounds have been associated with various endocrine disruption consequences (e.g., neurotoxicity and carcinogenicity) owing to their environmental persistence, high resistance, and bio-accumulative nature (Wee and Aris, 2017b).

4.3.1.2 Sediment

The occurrence of multiclass EDCs in riverine sediments has received scant attention. The present study investigated the occurrence of ten EDCs which comprised of plasticizers (BPA, BPF, and BPS), pharmaceuticals (propranolol), PFAS (PFOS and PFOA), and hormones (testosterone, progesterone, EE2, and E2) in the sediment of the Selangor River Basin. As depicted in **Table 4.4** and **Figure 4.4**, all targeted compounds were detected, except for testosterone, which was below the MDL. Among all the EDCs, BPA was observed to have the highest concentration in the sediment ($p < 0.05$), varied between 3.74 ng/g and 117.20 ng/g (mean concentration of 35.00 ± 37.29 ng/g) with 100% detection at all sampling points. In fact, the abundance of BPA in the sediment of the Selangor River Basin was comparable to that reported by Salgueiro-González et al. (2015) in the Minho River, Spain-Portugal border (<2.9–130 ng/g).

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The ubiquity of BPA in riverine sediment is mainly influenced by the presence of anthropogenic activities from agricultural, industrial, residential, and fishing. For instance, Gorga et al. (2015) indicated that heavy anthropogenic activities in Iberian rivers, Spain, are the major contributors to BPA contamination, up to 430 ng/g in the sediment. Similarly, Huang et al. (2020) also observed high concentrations of BPA in the Dongjiang River and Zhujiang River, ranged from 26.6–1860 ng/g and 118–689 ng/g, respectively. In contrast, the concentration in this study was considerable high to those reported in Japanese rivers (<1.2–22.0 ng/g; Kitada et al., 2008), Klang River estuary, Malaysia (8.50–16.84 ng/g; Omar et al., 2018) and Yellow River, China (3.2–65.1 ng/g; Zhao et al., 2020b). Despite this, the occurrence of BPA in the environment could not be taken lightly due to its adverse effects and toxicity to aquatic organisms even at trace concentrations (Wee and Aris, 2017b).

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Table 4.4: Concentration and distribution of EDCs in sediment samples of Selangor River Basin

Sampling point	Mean concentration of EDCs (ng/g dry weight)									
	BPA	BPF	BPS	Propranolol	PFOS	PFOA	Testosterone	Progesterone	EE2	E2
SP1	23.99	1.70	<0.002	0.21	0.19	0.91	<1.12	<0.10	<0.55	0.50
SP2	23.70	<1.60	0.073	0.05	<0.08	0.70	<1.12	<0.10	<0.55	1.53
SP3	22.81	<1.60	0.178	0.11	<0.08	0.79	<1.12	0.12	1.84	1.06
SP4	6.27	<1.60	<0.002	0.03	0.13	2.38	<1.12	<0.10	3.18	3.73
SP5	4.36	<1.60	<0.002	0.02	0.31	1.75	<1.12	<0.10	5.05	2.68
SP6	3.74	<1.60	<0.002	0.02	0.17	2.54	<1.12	0.14	4.72	1.62
SP7	4.36	<1.60	<0.002	0.01	<0.08	1.37	<1.12	<0.10	3.26	2.13
SP8	4.11	<1.60	<0.002	0.04	0.14	1.97	<1.12	<0.10	4.85	2.07
SP9	5.50	<1.60	<0.002	<0.01	0.17	2.95	<1.12	<0.10	4.20	2.63
SP10	5.34	<1.60	0.126	<0.01	1.19	4.20	<1.12	<0.10	8.90	3.51
SP11	10.36	2.26	0.131	<0.01	0.29	1.97	<1.12	<0.10	5.50	2.82
SP12	33.75	1.77	0.378	0.03	0.57	2.71	<1.12	0.16	9.08	2.04
SP13	3.97	<1.60	<0.002	0.02	0.12	1.70	<1.12	<0.10	3.20	2.50
SP14	4.20	<1.60	<0.002	<0.01	0.20	1.47	<1.12	<0.10	3.12	1.89
SP15	8.55	<1.60	<0.002	<0.01	0.31	0.70	<1.12	<0.10	3.93	3.48
SP16	38.67	5.51	<0.002	<0.01	<0.08	0.58	<1.12	<0.10	3.66	2.47
SP17	55.62	4.34	0.115	<0.01	0.39	0.71	<1.12	<0.10	5.78	2.90
SP18	15.72	3.87	0.179	0.03	0.33	0.92	<1.12	<0.10	3.98	1.90
SP19	30.08	<1.60	<0.002	<0.01	<0.08	0.64	<1.12	<0.10	4.28	1.82
SP20	117.20	6.59	<0.002	<0.01	0.20	1.05	<1.12	0.20	6.11	2.07
SP21	90.20	5.40	<0.002	<0.01	0.27	1.11	<1.12	<0.10	4.22	3.89
SP22	99.20	5.32	0.114	<0.01	<0.08	1.34	<1.12	0.18	10.35	4.51
SP23	100.01	9.06	0.439	<0.01	0.28	1.16	<1.12	0.17	11.68	4.08

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Table 4.4: Continued

SP24	84.72	3.30	0.354	0.02	0.86	0.80	<1.12	<0.10	18.96	2.60
SP25	78.69	5.22	<0.002	<0.01	0.25	<0.26	<1.12	<0.10	20.89	2.26
Range	3.74–117.20	<1.60–9.06	<0.002–0.439	<0.01–0.21	<0.08–1.19	<0.26–4.20	<1.12	<0.10–0.20	<0.55–20.89	0.50–4.51
Mean ± SD	35.00 ± 37.29	4.53 ± 2.14	0.209 ± 0.131	0.05 ± 0.06	0.34 ± 0.27	1.52 ± 0.91	NA	0.16 ± 0.03	6.55 ± 4.90	2.51 ± 0.96
CV (%)	106.52	47.25	62.615	118.94	80.41	59.84	NA	17.46	74.76	38.19
MDL (ng/g)	0.10	1.60	0.002	0.01	0.08	0.26	1.12	0.10	0.55	0.07
Detection (%)	100	48	40	48	76	96	NA	24	92	100
Distribution (%)	73.58	4.57	0.18	0.05	0.54	3.06	NA	0.08	12.67	5.27

SD: Standard deviation; CV: Coefficients of variation; MDL: Method detection limit; BPA: Bisphenol A; BPF: Bisphenol F; BPS: Bisphenol S; PFOS: Perfluorooctane sulfonate; PFOA: Perfluorooctanoic acid; EE2: 17α-ethynylestradiol; E2: 17β-estradiol; NA: Not available; <: Denotes value below the MDL

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Moreover, BPF and BPS, which are commonly known as BPA analogs, were observed in this study ranging from <1.60–9.06 ng/g and <0.002–0.439 ng/g with 48% and 40% detection, respectively. The concentration was relatively low compared to those reported in the sediment of Zhujiang River for BPF (up to 341 ng/g) and BPS (up to 5.60 ng/g) (Huang et al., 2020). In fact, the occurrence of BPF and BPS in riverine sediment remains scarcely reported despite being extensively used as BPA alternatives and susceptible to bioaccumulation and biomagnification in the aquatic environment. These analogs have been extensively produced and commonly used as plastic adhesives, dental sealants, epoxy glues, coatings, and varnishes (Yang et al., 2014; Rochester and Bolden, 2015; Chen et al., 2016; Wu et al., 2018; Usman et al., 2019).

The production of BPA analogs was originally to reduce the usage of BPA itself, which has been banned in certain countries in France, China, Canada, and Malaysia due to their toxicity towards organisms (Fan et al., 2012; Eladak et al., 2015; Zhao et al., 2020b; Gu et al., 2022). In addition, these analogs have shown toxicity levels that are comparable to or even greater than those of BPA owing to the similarities in their molecular structures and physicochemical characteristics (Eladak et al., 2015; Xue et al. 2015). Consequently, these compounds have been widely detected in the environment, posing potential environmental risks to aquatic organisms and human health.

Among the monitored hormones, all compounds were detected, except for testosterone, which was detected below the MDL. EE2 was the highest concentration observed, ranging from <0.546–20.890 ng/g, followed by E2 (0.50–4.51 ng/g) and progesterone (<0.10–0.20 ng/g). These concentrations were comparable with those

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reported in the sediment from the Pearl River system in South China, where EE2 was observed at a maximum concentration of 21.2 ng/g (Shijing River) and E2 up to 4.12 ng/g in Zhujiang River (Zhao et al., 2011). Another study in the Langat River found that EE2 and E2 were extremely low compared to the present study, where the concentration observed ranged from undetectable to 0.000329 ng/g and 0.0053–0.0281 ng/g, respectively (Praveena et al., 2016). This may explain the ubiquity of hormones in the sediment, given that the Selangor River Basin is influenced by various anthropogenic wastes from domestic, industrial, and agricultural activities.

Steroid hormones (e.g., EE2 and E2) are commonly utilized in a broad range of industrial and consumer applications. For instance, EE2 has been extensively used in human medications for birth control (contraceptive pills), menopause syndrome treatment, osteoporosis, and livestock production and growth (Aris et al., 2014). Consequently, these compounds may be introduced to the aquatic environment through direct discharge of untreated domestic and industrial wastewater. In the environment, these hormones are persistent, bio-accumulative, non-biodegradable, and have a high sorption capacity to sediment owing to their physicochemical (e.g., hydrophobic and low volatility) properties. This indicates that the sediment could act as a sink for these emerging compounds and pose an emerging risk to aquatic organisms and human health.

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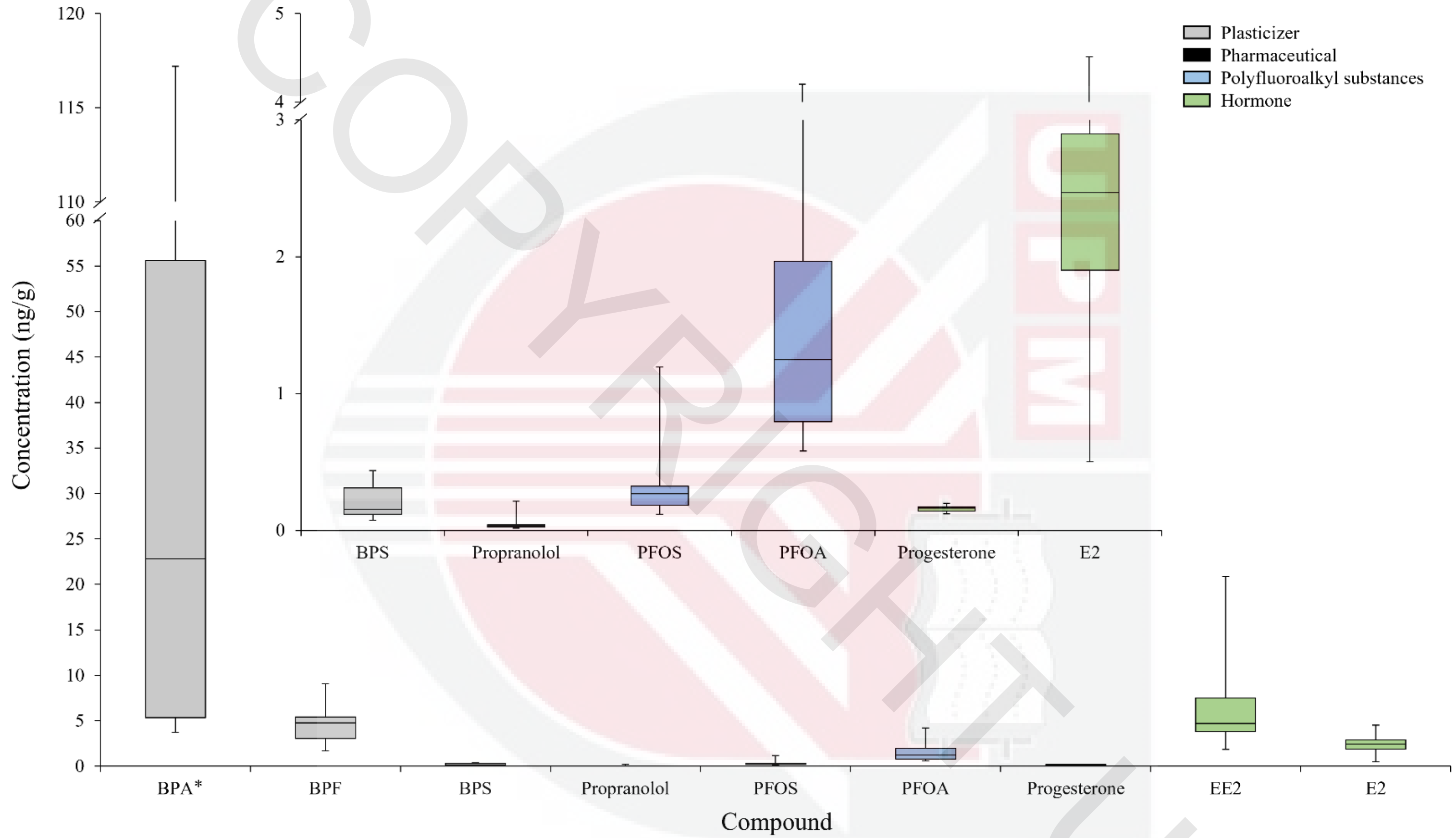

Figure 4.4: Concentration and abundance of EDCs in the sediment of the Selangor River Basin. * Significant at $p < 0.05$

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The ubiquity of EDCs in the environment is highly influenced by the presence of anthropogenic activity. The present study analyzed the spatial distribution of EDCs in the sediment of the Selangor River Basin by dividing it into three major regions (i.e., upper stream, tributaries, and lower stream) based on anthropogenic activities. The upper stream (SP1–SP6) of the Selangor River Basin is mainly covered by forested and agricultural land (Santhi and Mustafa, 2013). Meanwhile, tributaries (SP7–SP19) and lower streams (SP20–SP25) are heavily populated and occupied by plastic factories, industrial estates, and residential areas (Sakai et al., 2016). Therefore, these regions could serve as significant occurrences and sources of EDCs in the Selangor River Basin and the Straits of Malacca.

As depicted in **Figure 4.5**, the highest concentration of EDCs was observed at SP20 (BPA, 117.20 ng/g), whereas the lowest concentration was observed at SP7 (propranolol, 0.01 ng/g). SP20 was located in the lower stream of the Selangor River Basin, while SP7 was located in tributaries (Kerling River). As anticipated, the lower stream tends to have a higher abundance of EDCs because SP20 is surrounded by the plastic manufacturing industry and receives various anthropogenic inputs from the Rawang and Guntong Rivers. Previous research on PCBs pollution in the surface water of the Selangor River Basin discovered that the concentration of PCBs was highest in Rawang, which is known to be the most urbanized and populated area in the Selangor River Basin (Sakai et al., 2016). CV showed a relatively high percentage, varying from 38% to 119%, except for progesterone. High CV implies high variation in EDCs concentration and spatial distribution across sampling points of the Selangor River Basin. Thus, this result suggests that the occurrence of EDCs may have originated from

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industrial and domestic wastes from urbanized areas in Rawang and may have been transported and accumulated in the lower stream of the Selangor River Basin.

Despite the influence of anthropogenic wastes, the occurrence of EDCs could also be influenced by sediment adsorption. Owing to their highly hydrophobic nature (octanol–water partition coefficient, log K_{ow}) and high sorption to sediment or soil (soil sorption coefficient, K_{oc}) in the environment (Aris et al., 2014; Wee and Aris, 2017b), EDCs may accumulate in the sediment for long periods. Based on Wee and Aris (2017b), K_{oc} of EE2 (4.8×10^4) is relatively high compared to E2 (3.0×10^4), which indicates the predominance of EE2 than E2 in the sediment of Selangor River Basin. In addition, EE2 is typically resistant to biodegradation compared to E2 owing to its non-polar characteristic (Aris et al., 2014). Similarly, the sediment or soil adsorption of BPA was relatively higher than most of the EDC compounds, which corresponded to the highest concentration in the sediment of the Selangor River Basin. Thus, the ubiquity and retention of EDCs in the sediment may vary depending on the anthropogenic waste input as well as their physicochemical parameters in the environment.

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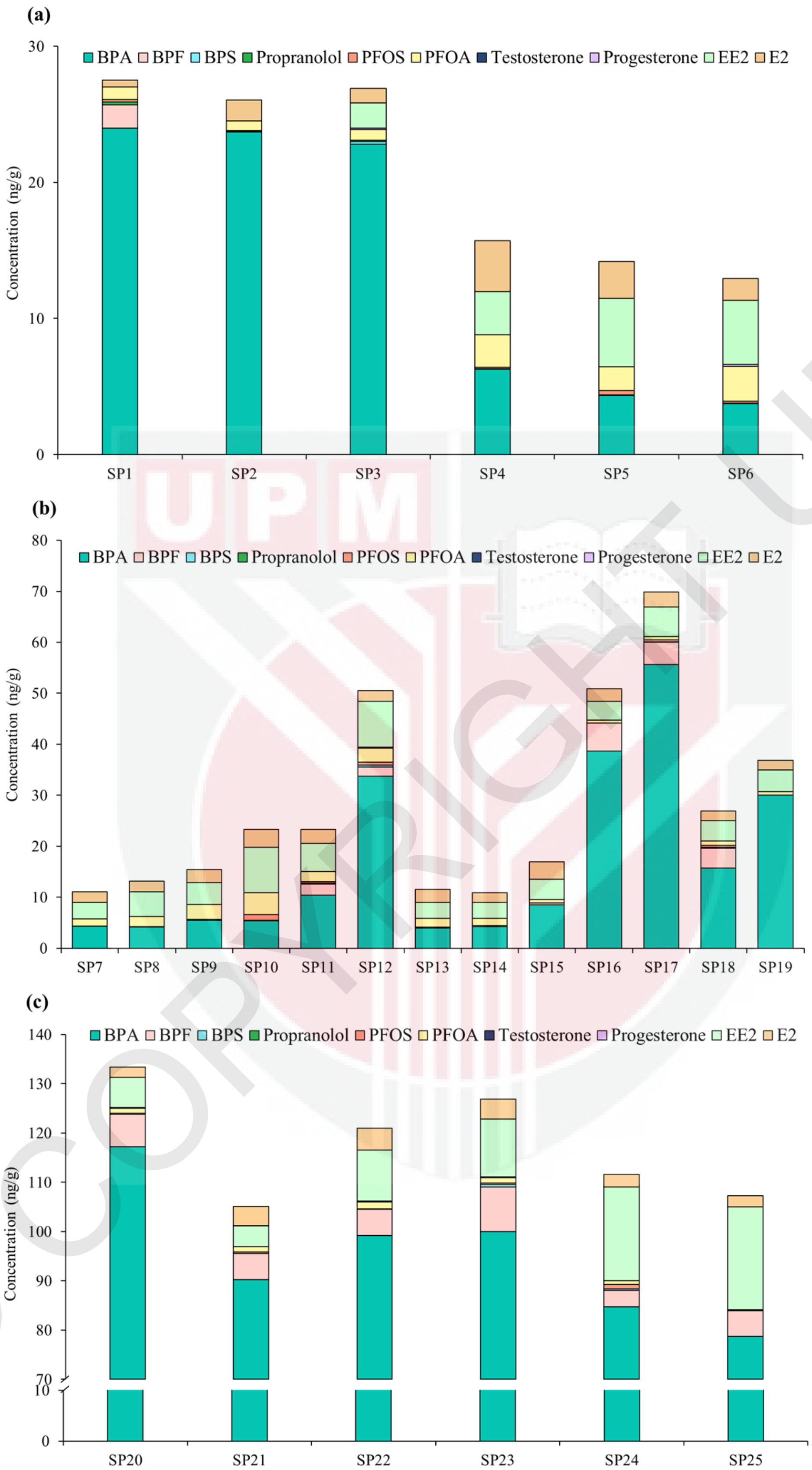

Figure 4.5: The concentration and distribution of EDCs detected at (a) upper stream, (b) tributaries and (c) lower stream of Selangor River Basin

4.3.2 Comparison of endocrine disrupting compounds in the riverine environment

Meanwhile, for EDCs, targeted compounds such as BPA, BPF, BPS, PFOS, PFOA, EE2, and E2 were detected in surface water and sediments across 25 sampling points in the Selangor River Basin. Other compounds (e.g., propranolol and progesterone) were only detected in the sediment. Only testosterone was detected below the MDL for both surface water and sediment samples. The mean concentrations of BPA, BPF, BPS, PFOS, EE2, and E2 were relatively higher in surface water than in sediment (**Figure 4.6**). Notably, organic compounds such as BPA and PFAS are moderately soluble in water, which illustrates their abundance in surface water (Careghini et al., 2015; Cortés-Arriagada, 2021; Lewis et al., 2022). Propranolol, PFOA, and progesterone were detected at relatively high levels and/or only in the sediment. Plasticizers such as BPF ($t(48) = 2.84$, $p < 0.01 = 0.007$) and BPS ($t(48) = 2.88$, $p < 0.01 = 0.006$) indicated that there was a statistically significant difference between the concentrations in surface water and sediment, except for BPA ($t(48) = 1.74$, $p > 0.05 = 0.089$).

Meanwhile, for hormones, EE2 and E2 were found to have statistically significant differences, with $t(48) = 4.69$, $p < 0.01 = 0.000$ and $t(48) = 7.08$, $p < 0.01 = 0.000$, respectively. The concentration of EDCs in both the surface water and sediment of the Selangor River Basin indicates the influence of anthropogenic discharges originating from urbanized areas. These areas, characterized by diverse anthropogenic activities, such as industrial, commercial, and residential activities, have emerged as the primary sources of EDC contamination. The occurrence of EDCs in the Selangor River Basin also indicated the inefficiency of sewage treatment plants in removing emerging

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organic contaminants, while the Selangor River Basin provides prominent water resources to the residents.

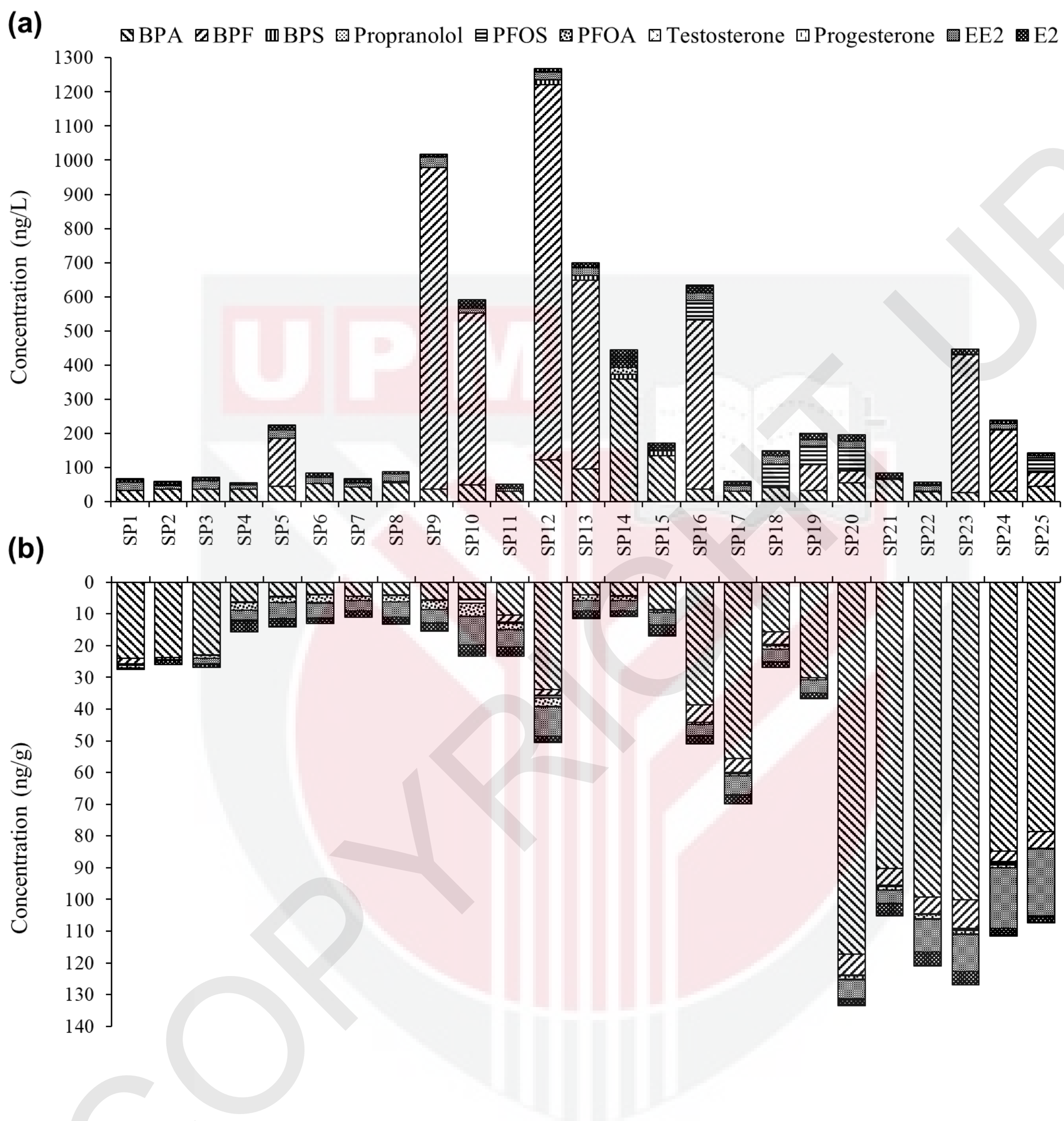

Figure 4.6: The mean concentration and distribution of multiclass EDCs (e.g., plasticizers, pharmaceutical, PFAS and hormones) in (a) surface water and (b) sediment of Selangor River Basin

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4.4 Conclusion

This study examined the occurrence and spatial distribution of ten multiclass EDCs, including BPA, BPF, BPS, propranolol, PFOS, PFOA, testosterone, progesterone, EE2, and E2, in both surface water and sediment samples collected from 25 sampling points across the Selangor River Basin in Selangor, Malaysia (**Table 4.5**). In surface water, plasticizers such as BPA, BPF, and BPS, as well as hormones such as EE2 and E2, were consistently observed and distributed across the Selangor River Basin. Propranolol, progesterone, and testosterone were detected at levels below the MDL. Conversely, in the sediment samples, all EDCs were detected, except for testosterone, which was below the MDL. Among the identified EDCs, BPA had the highest detection rate, occurring in all samples and ranging from 3.74 ng/g to 117.20 ng/g. Additionally, EE2 and BPF were notable for their relatively high concentrations and widespread presence throughout the samples, indicating the potential environmental significance of these compounds. The highest concentrations of EDCs were observed downstream in the Selangor River Basin, suggesting input from both specific and diffuse pollution sources originating from the upstream and tributaries. This contamination signifies contributions from industrial, commercial, and residential waste sources in the area. Therefore, our study established a foundational dataset that can address knowledge gaps regarding the occurrence of EDCs in surface water and sediment, facilitating future comparisons. Furthermore, it is imperative to consider spatial and temporal distributions on a larger scale to understand the pathways and extent of EDC contamination in the environment. Given the potential adverse effects of EDC contamination on aquatic organisms and human health, addressing the accumulation and persistence of EDCs in the environment is imperative.

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Table 4.5: Summary of the research matrix for the occurrence and distribution of EDCs in surface water and sediment

Objective	Activities	Findings/Outputs	Publications
2. To elucidate and compare the occurrence and spatial distribution of EDCs in surface water and sediment samples collected from the Selangor River Basin.	✓ Comprehensive examination of the occurrence and distribution of EDCs in surface water and sediment. ✓ Surface water (500 mL) and sediment (5 g) sampling. ✓ Extraction and quantification 10 compounds of EDCs. ✓ Physicochemical properties for descriptive analysis. ✓ Statistical analysis: - One-way analysis of variance (ANOVA) ascertained the statistical significance of differences in EDCs concentration across the three regions of the sampling sites (upper stream, tributaries, and lower stream). - Pearson correlation coefficients assessed the correlations between EDC concentrations and physicochemical parameters.	✓ Preliminary study of EDCs content in surface water and sediment. ✓ Establishing the occurrence and spatial distribution of EDCs (10 compounds) in both surface water and sediment. ✓ Anticipated factors or sources influencing the occurrence of EDCs (10 compounds) in the riverine environment.	o Zaki, M.R.M., Razak, M.R., Haron, D.E.M., Isa, N.M., Yusoff, F.M., & Aris, A.Z. Multiclass endocrine disrupting compounds in surface water from Selangor River Basin: Occurrence and ecological risk assessment [ACS ES&T Water – under review, Q1, IF: 5.3, Top 15%]. o Zaki, M.R.M., Wee, S.Y., Haron, D.E.M., Isa, N.M., Yusoff, F.M., & Aris, A.Z. Occurrence and distribution of multiclass endocrine disrupting compounds in sediment of anthropogenically impacted Selangor River Basin, Malaysia [Preparing for journal submission]. o Zaki, M.R.M., Saifuddin, M.F.U., Ash'aari, Z.H., & Aris, A.Z. Microplastics and endocrine disrupting compounds in urbanized

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Table 4.5: Continued

	- Pearson correlation analysis ascertained the correlation between EDCs in both matrices (e.g., surface water and sediment) of Selangor River Basin.	tropical riverine water: A comparative analysis, pollution load and land-use effect [Preparing for journal submission].

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CHAPTER 5

POTENTIAL ECOLOGICAL RISKS ASSOCIATED WITH MICROPLASTICS AND ENDOCRINE DISRUPTING COMPOUNDS

5.1 Introduction

Microplastics, tiny plastic particles measuring less than 5 mm in diameter, have emerged as ubiquitous pollutants in aquatic environments worldwide. Their sources are diverse and include the fragmentation of larger plastic items, abrasion of synthetic textiles, and shedding of microbeads from personal care products. Microplastics can be found in various forms, including microfibers, microbeads, and fragmented plastic debris. Once introduced into rivers, these pollutants can persist for extended periods, posing threats to aquatic life. Concurrently, EDCs have gained recognition as a class of environmental contaminants capable of interfering with the endocrine systems of organisms, including humans. EDCs comprise a wide array of synthetic and natural chemicals, such as plasticizers, pesticides, hormones, pharmaceuticals, and personal care products. Their occurrence in river ecosystems can originate from various sources, including industrial discharge, agricultural runoff, and domestic wastewater effluents.

This chapter is based on the papers:

a) Zaki, M.R.M., Zainuddin, A.H., Saifuddin, M.F.U., Razak, M.R., Isa, N.M., Ash'aari, Z.H., & Aris, A.Z. Microplastic contamination and ecological risk assessment in tropical riverine surface water of Selangor River Basin, Malaysia [ACS ES&T Water – Accepted, Q1, IF: 5.3, Top 15%].

b) Zaki, M.R.M., Razak, M.R., Haron, D.E.M., Isa, N.M., Yusoff, F.M., & Aris, A.Z. Multiclass endocrine disrupting compounds in surface water from Selangor River Basin: Occurrence and ecological risk assessment [ACS ES&T Water – under review, Q1, IF: 5.3, Top 15%].

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Previous toxicity studies have highlighted the toxicological effects of microplastics and EDCs on aquatic organisms within river ecosystems, emphasizing the need for further investigation of their ecological impacts (Wang et al., 2020f; Sabuz Vidal et al., 2021; Wang et al., 2021; Rakib et al., 2023). Microplastic exposure has been linked to various adverse effects on freshwater aquatic organisms, including growth inhibition, intestinal inflammation, and oxidative stress. For instance, Yu et al. (2018) reported these effects in the Chinese mitten crab (*Eriocheir sinensis*), Redondo-Hasselerharm et al. (2018) observed similar impacts in the amphipod crustacean (*Gammarus pulex*), Qiao et al. (2019b) documented these responses in zebrafish. Meanwhile, previous studies have demonstrated species-specific adverse effects of EDCs exposure in freshwater species. Pascoe et al. (2002) reported that exposure to high concentrations resulted in increased mortality and structural and physiological impairments, and inhibited regeneration of the cnidarian (*Hydra vulgaris*), with significant effects observed at concentrations above 58 µg/L (EE2) and 42 µg/L (BPA). The exposure of spotted snakehead (*Channa punctata*) to 1.81 mg/L and 3.81 mg/L of BPA induced oxidative stress, as measured by elevated malondialdehyde levels and decreased antioxidant enzyme activity, ultimately leading to cellular damage (Sharma and Chadha, 2021). Zebrafish, the most commonly used model organism and widely distributed in South Asia, have also demonstrated that bisphenol compounds exert toxic effects in zebrafish embryos through oxidative stress, endocrine disruption, developmental toxicity, and immune dysregulation (Xu et al., 2013; Moreman et al., 2017).

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Although microplastics and EDCs represent separate environmental concerns, their co-occurrence in river ecosystems presents a complex and potentially amplified threat to aquatic life. Studies have suggested that microplastics can serve as carriers for EDCs, facilitating their transport and bioaccumulation in organisms. Moreover, the combination of these two contaminants may lead to synergistic or additive effects, with implications for ecological disruption in river habitats. Given the growing recognition of the interconnectedness between microplastics and EDCs in river environments and the pressing nature of these issues, this study aimed to build upon previous research and further investigate the potential ecological risks associated with their co-occurrence.

5.2 Methodology

5.2.1 Study area

As detailed in Chapter 3, Section 3.2.2, Page 54.

5.2.2 Surface water sampling

5.2.2.1 Microplastics

As detailed in Chapter 3, Section 3.2.3, Page 58.

5.2.2.2 Endocrine disrupting compounds

As detailed in Chapter 4, Section 4.2.3, Page 105.

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5.2.3 Preparation and extraction

5.2.3.1 Microplastics

As detailed in Chapter 3, Section 3.2.4.1, Page 63.

5.2.3.2 Endocrine disrupting compounds

As detailed in Chapter 4, Section 4.2.4.1, Page 105.

5.2.4 Ecological risk assessment

As microplastics and EDCs gain recognition as emerging contaminants that are capable of inflicting harm and various detrimental impacts on freshwater organisms and human health, it is essential to undertake a comprehensive evaluation of the potential risks posed by these pollutants in the freshwater environment. Therefore, this study aimed to assess the potential ecological risk posed by microplastic and EDCs pollution within the Selangor River Basin by employing the risk quotient (RQ) approach, as defined in Eq. (1).

$$\text{Risk quotient, } RQ = MEC/PNEC \quad (1)$$

where MEC is the measured environmental concentration (mean) of microplastics and targeted EDCs. PNEC is the predicted no-effect concentration, which is calculated using the species sensitivity distribution (SSD) approach (European Commission, 2006; Razak et al., 2021), as shown in Eq. (2).

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Predicted no effect concentration, PNEC = HC_5/AF (2)

where HC_5 is the hazardous concentration for 5% of the species, which was acquired from the SSD curve using the SSD Generator (USEPA, 2020a). To generate the SSD curve, pertinent toxicity data, including lethal concentration of 50% (LC_{50}) and effective concentration of 50% (EC_{50}) values, were acquired from the USEPA ECOTOX database (USEPA, 2022b). Freshwater organisms with four trophic levels, algae, crustaceans, mollusks, and fish, were selected from the ECOTOX database to represent possible risks in the freshwater environment. The assessment factor (AF = 5) employed in this study was determined by considering multispecies data and information from previous ecotoxicological studies that specifically focused on non-native species (European Commission Joint Research Centre, 2003). Prior to the RQ value, the risk was classified into four categories: negligible risk (<0.01); low risk (0.01–0.09), medium risk (0.1–0.99) and high risk (≥ 1).

5.3 Results and discussion

5.3.1 Ecological risks of microplastics

The ubiquity of microplastics may be associated with ecological risks. Therefore, the RQ and SSD approaches in this study were performed using no observed effect concentration (NOEC) from ecotoxicology studies to establish an ecological risk assessment of microplastics (**Figure 5.1**). Given the tropical setting of the Selangor River Basin, including species relevant to such environments enhances ecological validity. Tropical freshwater ecosystems differ from temperate systems in terms of species composition and exposure dynamics, thereby influencing microplastic toxicity.

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Thus, integrating data from tropical taxa provides a more accurate risk estimation for the Selangor River Basin. The risk assessment involved the utilization of approximately 17 chronic toxicity data points on microplastics, encompassing species from five taxonomic classes, including crustaceans, algae, mollusks, cnidarians, and fish (**Table 5.1**). The HC_5 for 5% of the freshwater species was 125.87 particles/L (95% confidence interval: 26.46–598.70 particles/L) with a PNEC value of 25.17 particles/L. The RQ was estimated by dividing the mean concentration of microplastics in the present study (1.44 particles/L) by the PNEC (25.17 particles/L). The RQ value was 0.06, which suggests that a low ecological risk was anticipated in the Selangor River Basin.

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Table 5.1: Chronic toxicity data for microplastics in freshwater species

Freshwater species		Microplastic		Exposure duration (days)	NOEC (particles/L)	Endpoint	References
		Shape	Size (µm)				
Algae	*Dolichospermum flos-aquae*	Fragment	<200	21	1.20E+06	Growth	Yokota et al. (2017)
	Dunaliella salina	Bead	203.84	6	3.00E+04	Chlorophyll content	Chae et al. (2019)
	Dunaliella tertiolecta	Bead	0.5–6		1.05E+05	Growth	Sjollema et al. (2016)
	Microcystis arruginosa	Fragment	<200	21	1.20E+06	Growth	Yokota et al. (2017)
Crustacean	*Ceriodaphnia dubia*	Fiber	100–400	8	2.40E+03	Reproduction	Ziajahromi et al. (2017)
	Daphnia magna	Fragment	1–10	21	5.00E+04	Reproduction	Jaikumar et al. (2019)
	Daphnia pulex	Fragment	1–10	21	5.00E+04	Reproduction	Jaikumar et al. (2019)
	Gammarus fossarum	Fragment	32–250	28	1.67E+05	Growth	Straub et al. (2017)
	Gammarus pulex	Fragment	10–150	48	4.00E+06	Mortality	Weber et al. (2018)
	Hyalella azteca	Bead	10–27	42	2.50E+06	Reproduction	Au et al. (2015)
Molluscs	*Corbicula fluminea*	Bead	1–5		1.80E+07	Feeding and ChE inhibition	Guilhermino et al. (2018)
	Dreissena polymorpha	Bead	1 and 10		5.00E+05	Neurological	Magni et al. (2018)
Cnidarian	*Hydra attenuata*	Fragment	<400		2.80E+03	Feeding and morphology	Murphy and Quinn (2018)
Fish	*Clarias gariepinus*	Fragment		4	700	Histological change	Karami et al. (2016)
	Danio rerio	Fiber	10–30	21	3.40E+02	Growth	Qiao et al. (2019a)

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Table 5.1: Continued

	Oreochromis niloticus	Bead	0.1	14	9.00E+08	Neurotoxicity and oxidative stress	Ding et al. (2018)
	Symphysodon aequifasciatus	Bead	70–88		403	Predatory performance, digestion and energy production	Wen et al. (2018)

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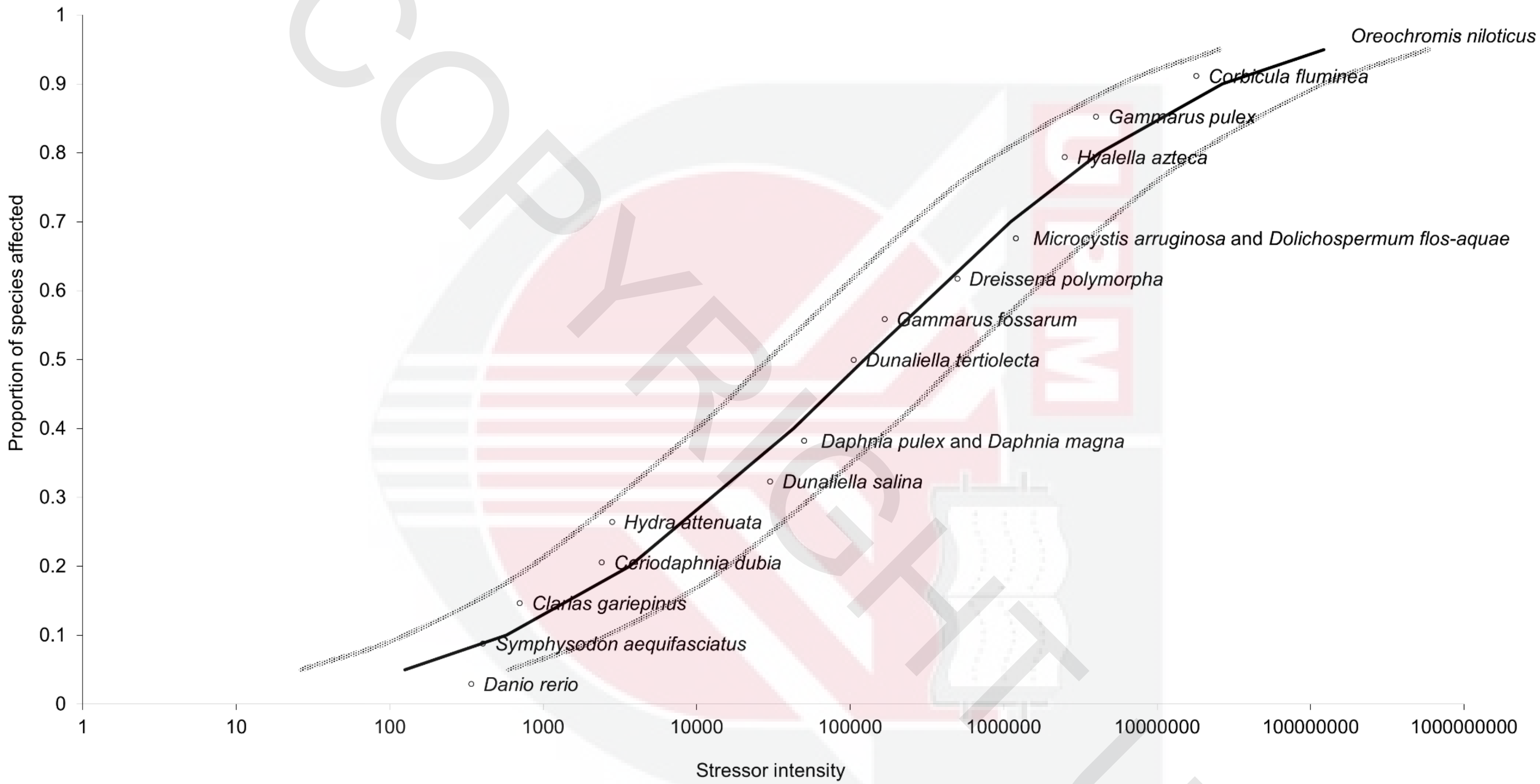

Figure 5.1: Species sensitivity distribution (SSD) curve of microplastics in freshwater ecosystems based on previous chronic toxicity data in freshwater species

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Prior to this comparison, the ecological risk posed by microplastics in the Selangor River Basin was compared to previous microplastic occurrences in other regions of the freshwater ecosystem. Negligible ecological risks from microplastics have been suggested for underdeveloped areas in the Yulin River (Mao et al., 2020) and remote areas in the Dafeng River (Liu et al., 2021b). Similarly, no possible ecological risk was found in a prominent river, such as the Ganges River, which has been providing water resources to 655 million people in India (Napper et al., 2021). This indicates that remote and rural areas with low anthropogenic activities are considerably safe from microplastic contamination by aquatic organisms, which may subsequently be safe for human health. However, it is important to include and analyze the ecological and human health risks in freshwater ecosystems, as humans are exposed to microplastics through the consumption of drinking water food.

The potential risk of microplastic contamination can be attributed to several factors associated with urbanization and industrialization. In previous studies, the Ciwalengke River has been highlighted for its notably high RQ value of 0.23, signifying a medium level of risk. Based on Alam et al. (2019), the Ciwalengke River is located in a highly populated area with a textile industry and poor sanitary facilities, thus increasing microplastic pollution and ecological risk. In contrast to the Selangor River Basin, medium risk (RQ = 0.17) has been recorded for the Langat River Basin, which is the largest river basin in Selangor, Malaysia. The Langat River Basin is heavily impacted by urbanization and industrialization from major cities, such as Kajang, Putrajaya, and Klang (Chen et al., 2021), compared to the Selangor River Basin. Thus, the results of the present study suggest that the current occurrence of microplastics in the Selangor River has few potential adverse effects on riverine ecosystems. Although the

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ecological risk of microplastics is relatively low, it cannot be ignored because bioaccumulation and biomagnification factors via trophic transfer may have various negative effects on ecological and human health. Further comprehensive and holistic monitoring and risk assessment that incorporates complex matrices (e.g., biota and sediment) are needed to understand the extent of microplastic risks in the environment.

5.3.2 Ecological risks of endocrine disrupting compounds

The prevalence of EDCs in aquatic ecosystems has become a global concern owing to their potential threat to both aquatic organisms and humans. A total of seven EDCs (BPA, BPF, BPS, PFOS, PFOA, EE2, and E2) were found to be present in the surface water of the Selangor River Basin. Given that propranolol, testosterone, and progesterone levels were below the MDL, these compounds were excluded from the risk assessment. An ecological risk assessment in the Selangor River Basin was conducted to determine the possible risks within freshwater organisms that could be associated with health risks for humans. Humans are exposed to various EDCs in contaminated food and drinking water (Liao and Kannan, 2013; Wee and Aris, 2017a).

All targeted EDCs were accessible for risk assessment using the ECOTOX database. However, propranolol, testosterone, and progesterone were excluded because of inadequate toxicity data and concentrations below the MDL. Whenever possible, toxicological data from previous studies were included in the risk assessment. The details of the risk assessment (e.g., RQ, MEC, PNEC, and HC_5) are provided in **Table 5.2** and **Figure 5.2**. Briefly, all plasticizers (e.g., BPA, BPF, and BPS) and PFAS (e.g., PFOS and PFOA) compounds showed negligible risks (RQ < 0.01). Hormones (e.g., EE2 and E2) compounds were estimated to have high risks (RQ ≥ 1), with RQ values

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of 82.11 and 29.43, respectively. This indicates that the risks of plasticizers and PFAS were relatively insignificant despite the high concentrations detected in the present study. Although plasticizers and PFAS were considered negligible risks, there was no guarantee that these emerging compounds would not threaten aquatic organisms and human health with long-term exposure. For instance, Akram et al. (2021) indicated that BPA affects the vital organs (e.g., liver, gills, brain, and kidneys) of freshwater bighead carp (*Aristichthys nobils*). Moreover, the growth and reproduction rates of two crustaceans, *D. magna* and *Moina macrocopa*, are negatively affected by long-term exposure to BPS (Park et al., 2019).

Among the monitored hormones, EE2 and E2 pose greater potential risks to aquatic organisms in the Selangor River Basin. The risks in this study were comparable to those from the Langat River (Wee et al., 2019), Yangtze River (Fan et al., 2021), and Pearl River Estuary (Chen et al., 2022), suggesting that rapid industrial activity and population growth contribute to hormone pollution in water bodies. EDCs are commonly found in sewage effluents and are released into the environment. Previous studies have demonstrated that effluent samples from wastewater treatment plants pose a high risk to aquatic organisms (Jiang et al., 2020; López-Velázquez et al., 2022). Although EE2 and E2 levels were relatively low in the present study when compared to plasticizers and PFAS, their concentrations were notably higher than those detected in most previous studies conducted in Malaysian waters (Praveena et al., 2016; Wee et al., 2019; Ismanto et al., 2022a; Nazifa et al., 2020). Moreover, synthetic estrogens are highly resistant to degradation in aquatic environments because of their physicochemical characteristics (e.g., poor water solubility, hydrophobicity, lipophilicity, and low volatility) (Langenbach, 2013; Aris et al., 2014). Therefore, the

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occurrence of EDCs in freshwater environments should not be neglected, despite the scarcity of their adverse effects and toxicity to humans.

This study provides critical insights into the ecological risks posed by emerging contaminants in the Selangor River Basin, emphasizing the need for ongoing monitoring and a broader risk assessment framework. While the current findings highlight their potential risks, future studies should incorporate cumulative risks to fully capture their long-term impact. The interactions between EDCs and other contaminants, their environmental persistence, and potential bioaccumulation may influence their overall toxicity. By integrating mixture toxicity assessments and real-world exposure scenarios, future research could build on these findings to develop more effective mitigation strategies and regulatory measures.

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Table 5.2: The concentration of EDCs in the surface water of Selangor River Basin and toxicological data retrieved from USEPA ECOTOX

EDC group	Compounds	MEC (ng/L)	PNEC (ng/L)	RQ	Risk characterization
Plasticizer	BPA	61.84	72880.27411	0.00085	Negligible risk
	BPF	406.06	68116.54493	0.00596	Negligible risk
	BPS	5.44	94955.56646	0.00006	Negligible risk
Pharmaceutical	Propranolol	<MDL	189144.30060	NA	NA
PFAS	PFOS	55.42	345046.44215	0.00016	Negligible risk
	PFOA	21.68	1349762.49991	0.00002	Negligible risk
Hormone	Testosterone	<MDL	NA*	NA	NA
	Progesterone	<MDL	NA*	NA	NA
	EE2	18.74	0.22823	82.11077	High risk
	E2	14.95	0.50806	29.42562	High risk

MEC: Mean measured environmental concentration; PNEC: Predicted no-effect concentration; RQ: Risk quotient; NA: Not available; MDL: Method detection limit; *: Denotes PNEC could not be generated due to insufficient data from USEPA ECOTOX and previous toxicological studies

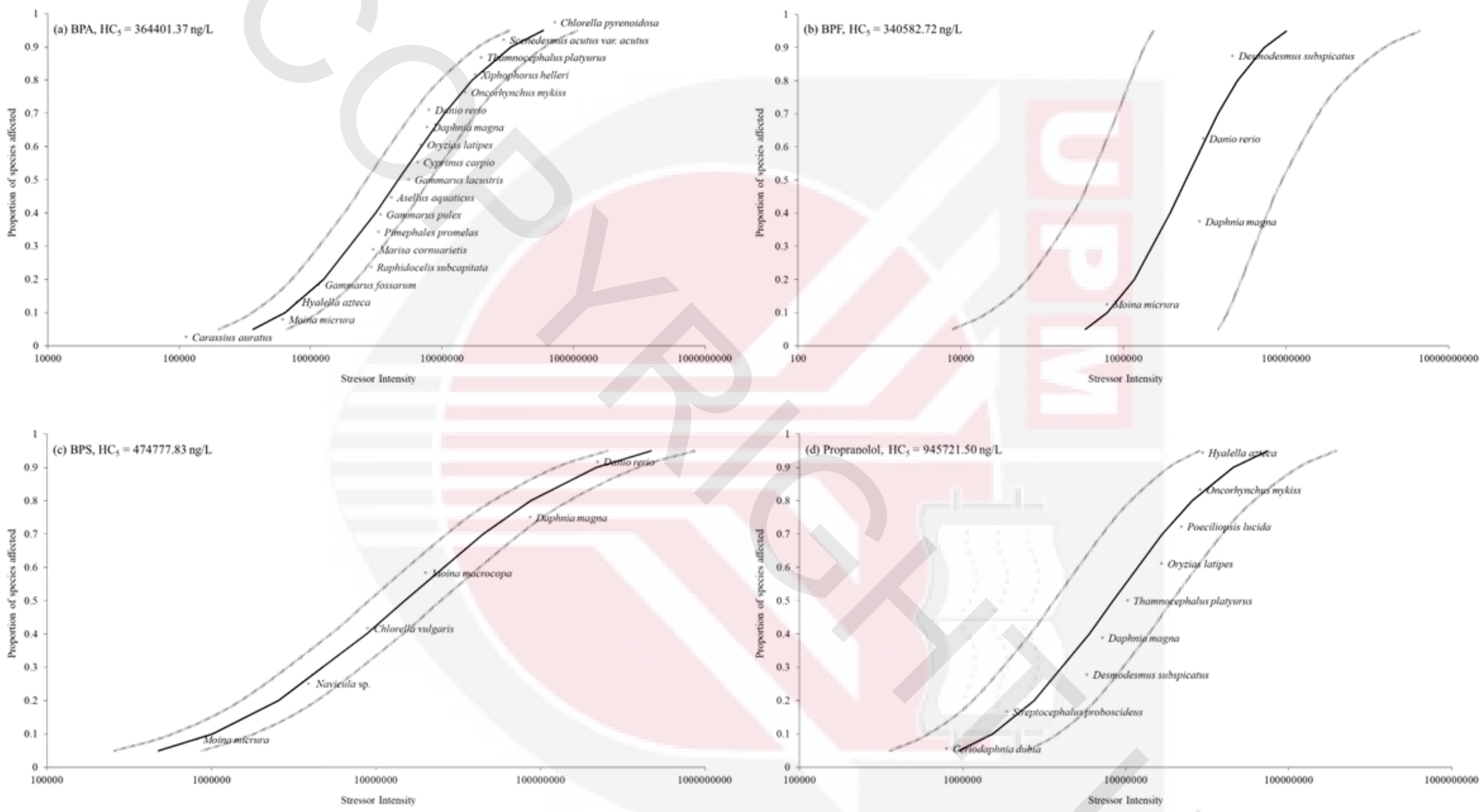

Figure 5.2: The species sensitivity distribution (SSD) curve for (a) BPA, (b) BPF, (c) BPS, (d) propranolol, (e) PFOS, (f) PFOA, (g) EE2 and (h) E2 based on toxicological data retrieved from USEPA ECOTOX database and previous toxicological studies

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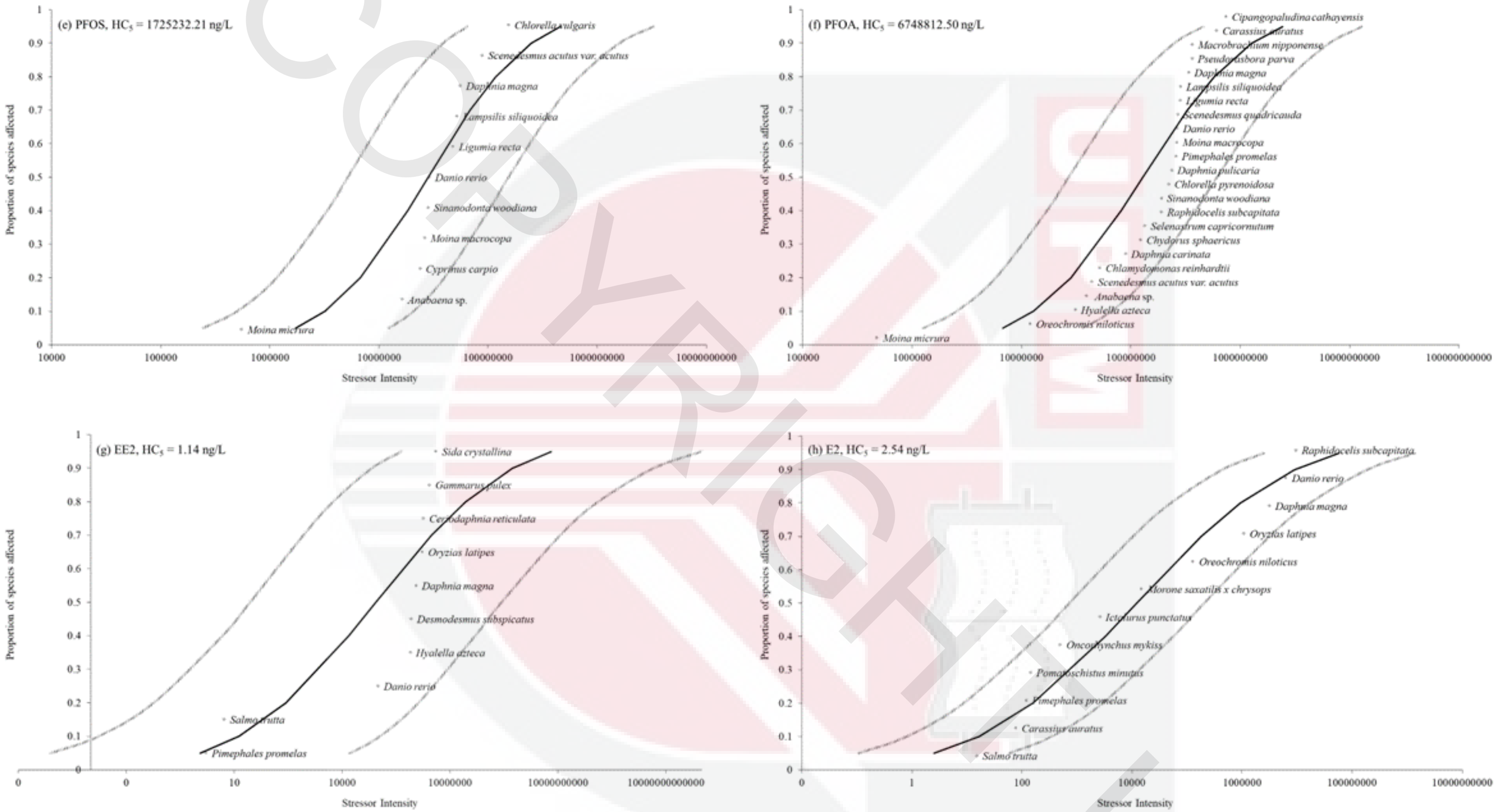

Figure 5.2: Continued

5.4 Conclusion

This study utilized an RQ approach to evaluate the potential ecological risk imposed by microplastics and EDCs within the Selangor River Basin (**Table 5.3**). These findings indicate that the Selangor River Basin currently carries a relatively low ecological risk associated with microplastics. However, ongoing anthropogenic activities that release microplastics may exacerbate their presence and distribution in riverine ecosystems, thereby increasing their potential impact on both ecological systems and human health. Therefore, it is crucial to stress the importance of continuous and comprehensive monitoring of microplastics, accompanied by future research that conducts thorough risk assessments to fully grasp the extent and potential hazards linked to microplastics in river environments.

Furthermore, ecological risk analysis of EDCs suggests that, except for EE2 and E2, all identified EDCs entail negligible risks. This observation implies a plausible connection between the hormone levels detected in the surface water of the Selangor River Basin and their implications for ecological systems and human health. Consequently, the preliminary prevalence of EDCs in river basins can offer valuable baseline data for future monitoring and comparative investigations. Moreover, it is essential to underscore the necessity for extensive research on the prevalence of EDCs across various environmental compartments, particularly within larger-scale river basins, while pinpointing the sources of pollution. This comprehensive approach is indispensable for gaining a holistic understanding of the origins, distribution, and fate of EDCs in the environment.

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Table 5.3: Summary of the research matrix for the ecological risk assessment of microplastics and EDCs

Objective	Activities	Findings/Outputs	Publications
3. To assess the potential ecological risk associated with the occurrence of microplastics and EDCs in the Selangor River Basin.	✓ Risk quotient calculation and interpretation for potential ecological risk. ✓ Data and descriptive analysis.	✓ Evaluating the potential ecological risks posed by microplastics and EDCs in the surface water of the Selangor River Basin. ✓ The exposure and risk characterization of microplastics and EDCs towards ecological risk. ✓ Characterizing risks to inform risk management and mitigation strategies. ✓ Identification of hotspot.	✓ Zaki, M.R.M., Zainuddin, A.H., Saifuddin, M.F.U., Razak, M.R., Isa, N.M., Ash'aari, Z.H., & Aris, A.Z. Microplastic contamination and ecological risk assessment in tropical riverine surface water of Selangor River Basin, Malaysia [ACS ES&T Water – Accepted, Q1, IF: 5.3, Top 15%]. ○ Zaki, M.R.M., Razak, M.R., Haron, D.E.M., Isa, N.M., Yusoff, F.M., & Aris, A.Z. Multiclass endocrine disrupting compounds in surface water from Selangor River Basin: Occurrence and ecological risk assessment [ACS ES&T Water – under review, Q1, IF: 5.3, Top 15%].

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CHAPTER 6

DAILY POLLUTION LOADS AND INFLUENCE OF DIFFERENT LAND-USE ACTIVITIES

6.1 Introduction

Microplastics and EDCs can be released into freshwater environments through anthropogenic discharge from industrial, residential, and commercial activities. Moreover, plastic debris and microplastics in freshwater environments containing plasticizers may desorb/leach into the surrounding water and sediment, thus contributing to the occurrence of EDCs in the environment (Hartman et al., 2017; Gulizia et al., 2023). Microplastics can also act as vectors for various emerging contaminants, including EDCs and heavy metals (Wagner and Lambert, 2018; Campanale et al., 2020). Therefore, microplastics and EDCs are of particular concern because of their persistence in freshwater environments and their ability to accumulate in aquatic organisms and humans.

This chapter is based on the papers:

a) Zaki, M.R.M., Sukatis, F.F., Roslan, M.Q.J., Isa, N.M., Yusoff, F.M., & Aris, A.Z. (2023). Microplastic pollution in freshwater sediments: Abundance and distribution in Selangor River Basin, Malaysia. Water, Air, & Soil Pollution, 234(7), 1–13. [Q2, IF: 2.9].
b) Zaki, M.R.M., Zainuddin, A.H., Saifuddin, M.F.U., Razak, M.R., Isa, N.M., Ash'aari, Z.H., & Aris, A.Z. Microplastic contamination and ecological risk assessment in tropical riverine surface water of Selangor River Basin, Malaysia [ACS ES&T Water – Accepted, Q1, IF: 5.3, Top 15%].
c) Zaki, M.R.M., Razak, M.R., Haron, D.E.M., Isa, N.M., Yusoff, F.M., & Aris, A.Z. Multiclass endocrine disrupting compounds in surface water from Selangor River Basin: Occurrence and ecological risk assessment [ACS ES&T Water – under review, Q1, IF: 5.3, Top 15%].
d) Zaki, M.R.M., Wee, S.Y., Haron, D.E.M., Isa, N.M., Yusoff, F.M., & Aris, A.Z. Occurrence and distribution of multiclass endocrine disrupting compounds in sediment of anthropogenically impacted Selangor River Basin, Malaysia [Preparing for journal submission].
e) M.R.M., Saifuddin, M.F.U., Ash'aari, Z.H., & Aris, A.Z. Microplastics and endocrine disrupting compounds in urbanized tropical riverine water: A comparative analysis, pollution load and land-use effect [Preparing for journal submission].

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Although research on microplastics and EDCs has primarily focused on marine environments, there is growing recognition of the need to study these contaminants in freshwater environments. This is because of their widespread ubiquity and potential impact on both ecosystems and human health, particularly in freshwater environments. Moreover, freshwater environments are often more fragile and susceptible to the impacts of environmental pollution (e.g., microplastics and EDCs) than marine environments. Consequently, pollutants in freshwater ecosystems can have a more significant and direct impact on both ecological systems and human health. River- and land-based sources have been identified as hotspots for microplastic origin and act as major pathways transporting microplastics to various environmental compartments, including marine ecosystems (Jambeck et al., 2015; Lebreton et al., 2017). Everaert et al. (2018) predicted that the input of floating microplastics into the marine environment would increase approximately up to 50-fold (from 2.5×10^7 to 1.3×10^8 tons) from 2010 to 2100. Similarly for EDCs loading, intense anthropogenic activities and economic development have resulted in the accumulation of EDCs in freshwater environments (Yan et al., 2022). As there has been little investigation of the emissions of microplastics and EDCs in freshwater environments (Kabir et al., 2021; Soltani et al., 2022; Yan et al., 2022), it is crucial to assess the pollution load and impact of various land-use practices on the distribution of emerging pollutants in rivers.

Therefore, this study aimed to advance the understanding of the impact of microplastics and EDCs in riverine environments. Specifically, this study sought to: (1) estimate the daily load of microplastics and EDCs through the river basin and (2) investigate the influence of different land-use sources on the distribution of these contaminants in the basin. The findings of this study will address further knowledge

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gaps regarding the fact and extent of microplastics and EDCs in freshwater ecosystems. Furthermore, this study contributes to our understanding of the potential impacts of these pollutants on aquatic organisms and human health.

6.2 Methodology

6.2.1 Study area

As detailed in Chapter 3, Section 3.2.2, Page 54.

6.2.2 Surface water sampling

6.2.2.1 Microplastics

As detailed in Chapter 3, Section 3.2.3, Page 58.

6.2.2.2 Endocrine disrupting compounds

As detailed in Chapter 4, Section 4.2.3, Page 105.

6.2.3 Preparation and extraction

6.2.3.1 Microplastics

As detailed in Chapter 3, Section 3.2.4.1, Page 63.

6.2.3.2 Endocrine disrupting compounds

As detailed in Chapter 4, Section 4.2.4.1, Page 105.

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6.2.4 Daily load of microplastics and endocrine disrupting compounds

Anthropogenic activities may have heavily impacted the Selangor River Basin with high loads of microplastics and EDCs, which are ubiquitous and abundant in the area and may also be transported to marine environments. Therefore, the daily load of microplastics and EDCs through the Selangor River Basin was calculated based on the study by Hossain et al. (2022), as shown in Eq. (3).

$$\text{Daily load of contaminants, DL} = \text{MEC} \times \text{WD} \tag{3}$$

where DL represents the daily load of microplastics (particles/day) and EDCs (ng/day). MEC is the measured (mean) environmental concentration of microplastics (particles/L) and EDCs (ng/L), and WD represents water discharge (m^3/s). The MEC of microplastics and EDCs used in this study were obtained from our previous research, while WD data for the Selangor River Basin were acquired from the National Hydrological Network Management System (SPRHiN) database.

6.2.5 Land-use in Selangor River Basin

Notably, the Selangor River Basin is a prominent river basin in Selangor that provides 60% of the water resources within Klang Valley (Santhi and Mustafa, 2013; Kusin et al., 2016a; Sakai et al., 2017a). The basin also provides water resources for primary land uses such as forested areas, industrial, residential, agriculture, and recreation activities. These land uses have been reported to contribute to various anthropogenic pollutants (e.g., microplastics, heavy metals, and EDCs) in riverine environments worldwide (Liu et al., 2017a; Giri, 2021). Therefore, it is compulsory to address the

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influence of land use in the Selangor River Basin that could contribute to water contamination, which may pose environmental health concerns. As depicted in **Figure 6.1**, the sampling points throughout the Selangor River Basin were classified based on the prevailing land use types in their surroundings, thus distinguishing between urban and rural areas. Urban areas are commonly referred to as regions that are heavily populated and encompass various land-use types such as industrial, residential, and commercial areas. Meanwhile, rural areas are associated with regions that are low population density, less developed, and mainly covered by forest, agricultural, and recreational areas. The present study focused on these areas within the Selangor River Basin to ascertain the potential impacts and fate of emerging contaminants, specifically microplastics and EDCs.

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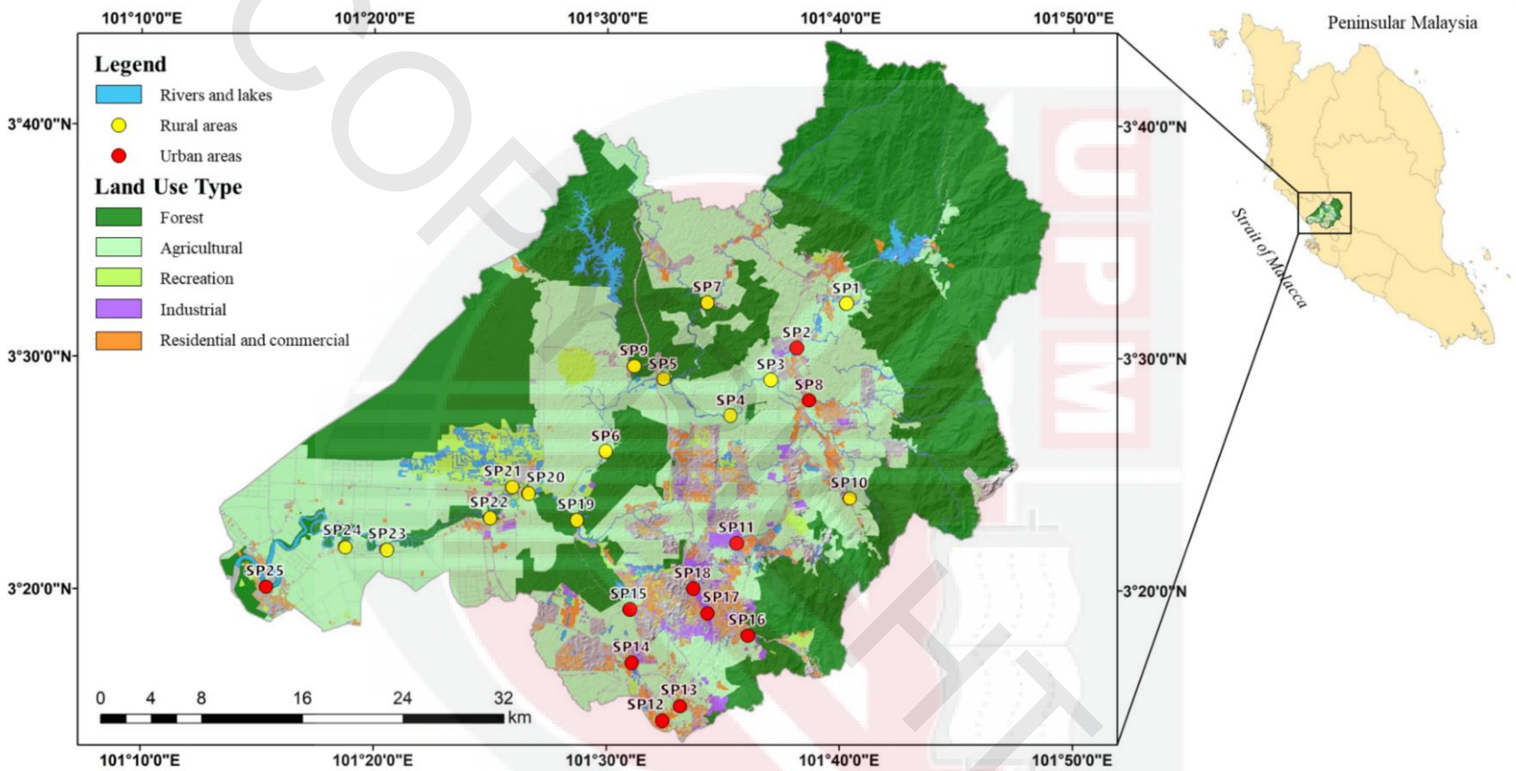

Figure 6.1: Spatial distribution of sampling points across the Selangor River Basin, Malaysia. The sampling points encompass various segments of the river, including upstream (SP1–SP6), tributaries (SP7–SP19), and downstream (SP20–SP25), classified into both rural and urban areas

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6.2.6 Data analysis

This study analyzed the correlation between the occurrence of microplastics and EDCs in the surface water and sediment of the Selangor River Basin to distinguish the potential association and relationship between these contaminants in riverine environments. Pearson correlation analysis was performed to ascertain the correlation between microplastics and EDCs in both matrices (e.g., surface water and sediment) of the Selangor River Basin. An independent sample *t*-test was used to examine the difference in contaminants between the surface water and sediment. Consequently, a *t*-test was also performed to elucidate the influence of different land uses (e.g., rural areas and urban areas) on microplastic and EDCs concentrations in both matrices. The differences were considered statistically significant at $p < 0.05$, and all statistical analyses were performed using IBM SPSS Statistics Software (IBM, USA) version 25.

6.3 Results and discussion

6.3.1 Estimation of daily load

6.3.1.1 Microplastics

Numerous discharge loads from anthropogenic activities have significantly impacted the ubiquity and distribution of microplastics and EDCs in the Selangor River Basin. The ubiquitous presence of these pollutants in the basin can be largely attributed to human sources and their associated activities. Moreover, previous studies have mainly focused on the estimation of pollution loads from wastewater treatment plants (Ye et al., 2012; Blair et al., 2019; Spataro et al., 2019; Cao et al., 2020; Chen et al., 2020; Nguyen et al., 2023), while there are limited studies that have been carried out in river

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and coastal ecosystems (Chinfak et al., 2021; Hossain et al., 2022). In fact, most previous studies have indicated that the contamination of microplastics and EDCs originated from terrestrial sources (Jambeck et al., 2015; Lebreton et al., 2017; Omar et al., 2018; Wee et al., 2019). Therefore, in this study, the river water discharge of the Selangor River Basin was acquired from the SPRHiN database to distinguish the pollution load of microplastics and EDCs into the basin and straits of Malacca. Based on the SPRHiN database, only three (n = 3) stations that were available in the Selangor River Basin to acquire river water discharge, which were located at SP1, SP5 and SP20.

Based on the average concentration of microplastics in the surface water from our previous findings (e.g., SP1, SP5, and SP20) and water discharge data, the daily loads of microplastics were calculated to be 2.70×10^9 particles/day, 1.17×10^{10} particles/day, and 7.90×10^9 particles/day, respectively (**Table 6.1**). Notably, the daily load of microplastics in the SP1 of Selangor River Basin was comparable to that in the Fengshan River system, where an estimated 1.00×10^9 particles/day entered the river (Tien et al., 2020). Although the catchment area of the Selangor River Basin (2200 km^2) is over 40 times larger than that of the Fengshan River system (53.85 km^2), the higher microplastic concentration in the Fengshan River system may be attributed to its heavy anthropogenic activities, primarily from domestic discharges (68.4%). In comparison, the Nakdong River in South Korea, which is larger than the Selangor River Basin with a catchment area of up to 9336 km^2, was estimated to have a much higher daily load of microplastics at approximately 2.00×10^{11} particles/day (Eo et al., 2019).

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Moreover, the highest daily load of microplastics was observed at SP5 (1.17×10^{10} particles/day), which had a high water discharge rate of 96.87 m^3/s compared to SP1 (24.02 m^3/s) and SP20 (83.08 m^3/s). A previous study by Hossain et al. (2022) reported that the daily load of microplastics entering the Bay of Bengal from the Karnafully River was 6.13×10^{10} particles/day with a discharge rate of 762.97 m^3/s. Chinfak et al. (2021) also estimated a similar trend, where a discharge rate of 600 m^3/s was associated with the introduction of 2.24×10^{10} particles/day into the Tapi-Phumduang River. This indicates that as the water discharge increases, the velocity and turbulence of the water are likely to intensify, leading to more powerful currents capable of carrying a greater volume of microplastics and other contaminants downstream.

6.3.1.2 Endocrine disrupting compounds

Similarly, for EDCs, the daily load was calculated based on the average concentration of EDCs in the surface water at SP1, SP5, and SP20 from our previous findings. However, it was not possible to compute the daily loads of EDCs if their concentrations were at SP1, SP5, and SP20 below the MDL. The daily loads of EDCs in the surface water of the Selangor River Basin ranged from 1.37×10^{10} ng/day to 1.19×10^{12} ng/day (**Table 6.1**). Among the EDCs analyzed, BPS had the lowest estimated daily load at SP20, which is located further downstream, whereas BPF had the highest evaluated daily load at SP5, which is situated in the middle stream of the Selangor River Basin. This suggests that the daily loads of EDCs may not be directly attributed to geographical and anthropogenic factors. Notably, the loading of EDCs in riverine environments can be influenced by various factors, including the discharge of wastewater, land-use practices, climate change, seasonal variability, and dilution of EDCs in water bodies (Wee and Aris, 2017b).

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Furthermore, limited research has been conducted on the daily loads of multiclass EDCs in riverine environments, posing challenges in comparing findings with previous studies and understanding their fate in riverine environments (Ozhan and Kocaman, 2019; Chakraborty et al., 2021). Most prior research has focused on analyzing the loads obtained from influent and effluent samples collected at wastewater treatment plants (Ryu et al., 2014; Archer et al., 2017; Spataro et al., 2019; Xue and Kannan, 2019; Kasprzyk-Hordern et al., 2021). In addition, the flow of river water diminishes the capacity of soil and sediment to absorb EDCs, contributing to higher concentrations of these compounds in surface water (Ismanto et al., 2022b). This reflects the findings of the present study, where the concentration of EDCs in sediments was relatively lower than that in the surface water.

Nevertheless, the presence of wastewater treatment plants, which involve physical, chemical, biological, advanced, and sludge treatment, may significantly reduce the loading of EDCs in the riverine environment. For instance, Archer et al. (2017) found a significant reduction of over 80% of EDCs (e.g., BPA, caffeine, atenolol, and ibuprofen) from wastewater treatment plants located in the Gauteng Province of South Africa. However, it is likely that these emerging contaminants can permeate through and be discharged into riverine environments. Meanwhile, in the Selangor River Basin, river pollution has caused multiple wastewater treatment plants (WTP), including Selangor River Phase 1 (SSP1), Selangor River Phase 2 (SSP2) WTP, and Selangor River Phase 3 (SSP3), to shut down due to high turbidity, fluoride, and ammonia content (Selangor Waters Management Authority, 2011; Loi et al., 2022). This is concerning, as the presence of operational wastewater treatment plants is essential to prevent the introduction of various contaminants into the riverine environment.

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Without such treatment, the discharge of untreated water can lead to severe environmental toxicity, posing serious threats to aquatic ecosystems and human health.

Table 6.1: Daily load of microplastics and multiclass EDCs in the surface water of three different sampling points along Selangor River Basin

Sampling point		SP1	SP5	SP20
Water discharge (m^3/s)		24.02	96.87	83.08
Daily load	Microplastics[a]	2.70E+09	1.17E+10	7.90E+09
	Bisphenol A[b]	6.75E+10	3.69E+11	3.86E+11
	Bisphenol F[b]	NA	1.19E+12	2.60E+11
	Bisphenol S[b]	NA	NA	1.37E+10
	Propranolol[b]	NA	NA	NA
	Perfluorooctane sulfonate[b]	NA	NA	4.59E+11
	Perfluorooctanoic acid[b]	NA	NA	NA
	Testosterone[b]	NA	NA	NA
	Progesterone[b]	NA	NA	NA
	17α-ethynylestradiol[b]	4.99E+10	2.00E+11	1.57E+11
	17β-estradiol[b]	2.18E+10	1.22E+11	1.26E+11

[a] particles/day; [b] ng/day; NA: Not available

6.3.2 Influence of different land-use

The ubiquity of microplastics and EDCs has been heavily influenced by various anthropogenic activities that encompass various terrestrial land uses. These activities, such as urbanization, agriculture, industry, and waste disposal, directly or indirectly introduce pollutants into ecosystems, thereby necessitating a comprehensive exploration of their influence. Therefore, the present study assessed the influence of different land uses that may contribute to microplastic and EDCs pollution in the freshwater environments of the Selangor River Basin.

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6.3.2.1 Microplastics

Microplastics are widely distributed in the Selangor River Basin and are present in both surface water and sediment. This study aimed to investigate the influence and impact of different land-use patterns, including rural areas (e.g., forests, agriculture, and recreation) and urban areas (e.g., industrial, residential, and commercial zones) on microplastic distribution in riverine environments. The results revealed that Microplastics were observed in all land use types within the Selangor River Basin. Urban areas emerged as the primary contributors to microplastic distribution in both surface water and sediment (**Figure 6.2**). However, statistical analysis indicated no significant difference between rural and urban areas regarding microplastic concentrations in the surface water and sediment of the Selangor River Basin. These findings suggest that while urban areas exhibit higher microplastic concentrations, the lack of statistical significance between rural and urban areas indicates that microplastic pollution in the Selangor River Basin is influenced by multiple sources. This implies that contributions from rural activities, such as agricultural runoff, land-use characteristics, hydrodynamic conditions, and sediment transport processes, also play a role in the overall distribution of microplastics in the basin.

Interestingly, the highest concentration of microplastics in both surface water and sediment was detected at SP25, which is located in an estuarine and urban area characterized by a high density of residential and commercial activities. This observation aligns with previous studies that have also reported high concentrations of microplastics in the vicinity of intense anthropogenic activities and diverse land-use types (Peters and Bratton, 2016; Chen et al., 2020; Zaki et al., 2021a; Soltani et al., 2022; Zainuddin et al., 2022). This study provides valuable insights into the

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distribution of microplastics in the Selangor River Basin, highlighting the dominant role of urban areas in microplastic pollution. However, it also emphasizes that the overall presence of microplastics cannot be solely attributed to rural or urban areas. The concentration of microplastics is influenced by their proximity to intense anthropogenic activities as well as various land-use patterns. These findings underscore the need for comprehensive mitigation strategies to address microplastic pollution in both rural and urban environments.

In contrast, the lowest concentrations of microplastics in both surface water and sediment were observed at SP22. This particular location is characterized by the presence of multiple water treatment facilities, such as the Rantau Panjang Water Treatment Plant and Selangor River Raw Water Intake Plant (SSP1–SSP3). The lower microplastic concentrations at this sampling point suggest that water treatment processes may contribute to the removal or reduction of microplastics in the rivers. These facilities primarily function to treat and supply raw water, which may lead to settling or filtration of microplastics before the water is discharged downstream. It is important to note that sewage systems are primary sources of microplastic pollution in rivers. This is due to the fact that sewage systems receive wastewater from a variety of sources, including industries, households, and residential and commercial establishments (Yonkos et al., 2014; Kiessling et al., 2019; Su et al., 2020). Furthermore, these sources can directly discharge microplastics into the environment, thereby increasing the risk of contamination and subsequent ingestion by aquatic organisms and humans. These findings highlight the significant influence of land use on the abundance of microplastics in riverine environments. Therefore, while the presence of water treatment facilities may explain the lower microplastic concentration

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observed at SP22, the overall influence of land-use activities, particularly urban and industrial discharges, must be considered when addressing microplastic pollution in riverine systems. Efforts should focus not only on reducing microplastic inputs from urban areas but also on implementing effective wastewater treatment processes to mitigate microplastic contamination from sewage systems.

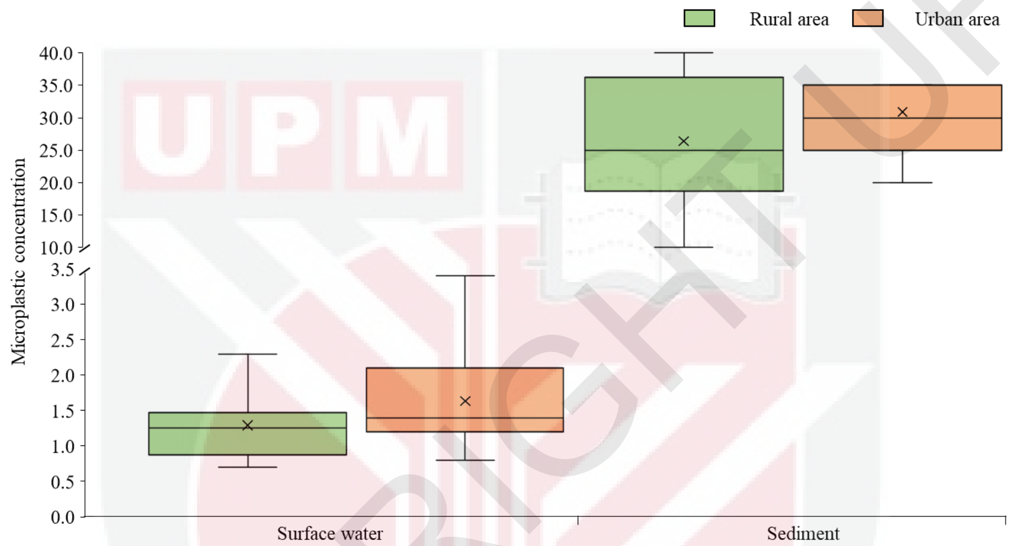

Figure 6.2: Comparison of microplastic abundance in different land-use areas from rural and urban areas of the Selangor River Basin. Microplastic concentration in surface water and sediment were indicated as particles/L and particles/kg, respectively

6.3.2.2 Endocrine disrupting compounds

A total of seven and nine multiclass EDCs were detected in the surface water and sediment of the Selangor River Basin, respectively. Similar to microplastics, these EDCs were observed across rural and urban areas, with the majority of EDCs in surface water being abundant in urban areas (**Figure 6.3a**). For instance, the mean concentrations of BPA, BPF, BPS, PFOA, and E2 in surface water were up to 4.8-fold higher in urban areas than in rural areas, with the exception of PFOS and EE2.

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However, only BPS was found to be statistically significantly higher in urban areas than in rural areas ($t(23) = -3.22$, $p < 0.01 = 0.004$). This is because BPS has been widely used as the primary substitute for BPA in various consumer and household products, which are notably less toxic than BPA. BPS was predominantly detected in various environmental compartments. The abundance or ubiquity of BPS may be associated with limited regulation, as BPS has not been subjected to the same level of regulatory scrutiny and monitoring programs as BPA, which has distinct health concerns. Moreover, previous studies have reported that the degradation rate of BPS is relatively lower than that of BPA under various laboratory conditions (Li et al., 2018a; Luo et al., 2020; Pei et al., 2022), which suggests its persistence and ubiquity in the environment.

Urban areas are typically characterized by higher population density and extensive industrial, residential, and commercial activities. These activities contribute to the release of EDCs into the environment through sources such as wastewater discharge, stormwater runoff, and industrial effluents. For instance, the maximum BPA concentration in rainfall runoff samples was found to be 5873 ng/L and was fourfold higher during wet seasons compared to the dry season in the urbanized Huizhou region, Dongjiang River basin (Zhao et al., 2021). These EDCs are eventually transported via rivers and accumulate in the marine environment, which also corresponds to high concentrations of EDCs in the downstream region of the Selangor River Basin. Zainuddin et al. (2023) indicated that high concentrations of bisphenol analogs (up to 1890.51 ng/L) in the Port Dickson coastline and Lukut estuary were contributed by industrial and recreational waste inputs from upstream and coastal regions. Therefore, the size and density of a population are commonly linked to the level of human activity

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and economic development, which have been observed to influence the distribution of EDCs.

In contrast to surface water, the levels of all EDCs in the sediment samples were comparatively higher in rural areas than in urban areas (**Figure 6.3b**). However, there were no significant differences in the average concentrations of EDCs between urban and rural areas. The presence of EDCs in surface water was predominantly detected in urban areas, which was attributed to heavy industrial activities and residential waste input. On the other hand, EDCs in the sediment were predominantly found in rural areas because of agricultural practices and the absence of adequate wastewater treatment infrastructure compared with urban regions. For instance, Yan et al. (2022) suggested that the presence of EDCs in rural counties within Taihu Lake Basin could be attributed to animal husbandry practices, along with the discharge of untreated domestic waste directly into the river connected to the lake. This is a major concern considering that the occurrence of emerging contaminants, such as EDCs, in untreated raw water can pose a potential risk to human health, particularly for those dependent on it as their daily drinking water supply (Wee et al., 2020).

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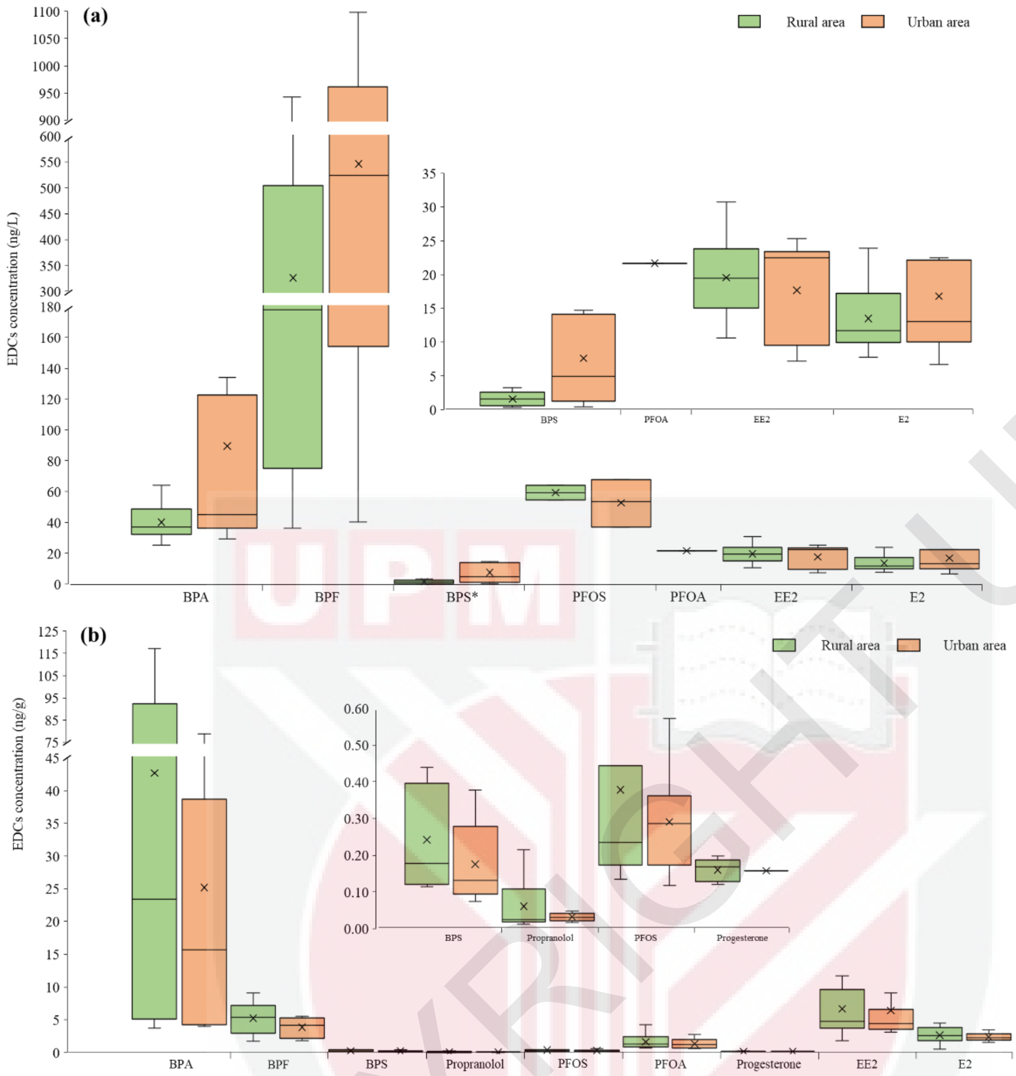

Figure 6.3: Comparison of the abundance of EDCs between rural and urban areas in (a) surface water and (b) sediment of Selangor River Basin. * Significant at $p < 0.01$

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6.4 Conclusion

A noteworthy observation emerging from this study is that the distribution patterns of these contaminants do not appear to be directly influenced by varying land-use types within the river basin (**Table 6.2**). Instead, the predominant factor influencing their presence and spread is the extensive input of anthropogenic waste, which varies between rural and urban regions. Urban areas, characterized by high population density, industrial activities, and inadequate wastewater treatment infrastructure, contribute significantly to the release of these contaminants into riverine systems. Conversely, rural regions, often dominated by agricultural practices and untreated domestic waste discharges, also play a role in the accumulation of EDCs in sediments. This finding underscores the critical importance of gaining a comprehensive understanding of how different land-use patterns shape contaminant pathways, fate, and persistence in aquatic environments. Future studies should adopt a holistic approach that expands the scope of monitoring to encompass a broader spectrum of environmental contaminants, as well as controlled experimental investigations. Additionally, these studies have shed light on the potential synergistic impacts that may arise from the co-occurrence of diverse contaminants. By acquiring a deeper understanding of these complexities, future studies can enhance their capacity to develop targeted and effective strategies to mitigate the adverse effects of these contaminants. Ultimately, this knowledge can serve as a cornerstone for protecting and preserving the health and well-being of both aquatic ecosystems and human populations facing environmental challenges.

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Table 6.2: Summary of the research matrix for the daily pollution loads, and the influence of different land-use activities of microplastics and EDCs

Objective	Activities	Findings/Outputs	Publications
4. To estimate the daily pollution loads and elucidate the influence of different land-use activities on the distribution of microplastics and EDCs within the Selangor River Basin.	o Riverine input calculations and their interpretation to identify potential emissions, pathways, and routes of microplastics and EDCs in Selangor River Basin. - Daily load of microplastics and EDCs. - Mean environmental concentration of microplastics and EDCs. - Water discharge of Selangor River Basin. o Sample collection. o Land-use mapping. o Descriptive and comparative analysis. o Statistical analysis: - An independent sample *t*-test elucidated the influences of different land-uses (e.g., rural areas and urban areas) on microplastics and EDCs concentrations in both	✓ Initial estimation of daily microplastics and EDCs pollution loads. ✓ Contamination levels by land-use type. ✓ Hotspot identification. ✓ Created spatial maps that vividly depict how land-use types actively correlate with microplastics and EDCs contamination. ✓ Anticipated factors or sources influencing the occurrence of microplastics in the riverine environment.	✓ Zaki, M.R.M., Zainuddin, A.H., Saifuddin, M.F.U., Razak, M.R., Isa, N.M., Ash'aari, Z.H., & Aris, A.Z. Microplastic contamination and ecological risk assessment in tropical riverine surface water of Selangor River Basin, Malaysia [ACS ES&T Water – Accepted, Q1, IF: 5.3, Top 15%]. o Zaki, M.R.M., Razak, M.R., Haron, D.E.M., Isa, N.M., Yusoff, F.M., & Aris, A.Z. Multiclass endocrine disrupting compounds in surface water from Selangor River Basin: Occurrence and ecological risk assessment [ACS ES&T Water – under review, Q1, IF: 5.3, Top 15%]. o Zaki, M.R.M., Saifuddin, M.F.U., Ash'aari, Z.H., & Aris, A.Z. Microplastics and

Table 6.2: Continued

	matrices.	endocrine disrupting compounds in urbanized tropical riverine water: A comparative analysis, pollution load and land-use effect [Preparing for journal submission].

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CHAPTER 7

SUMMARY, CONCLUSION AND RECOMMENDATIONS

7.1 Summary

The present study addresses the growing environmental concern regarding the occurrence of microplastics and EDCs within aquatic ecosystems, which have adverse effects on ecosystem and human health. Microplastics and EDCs are introduced through urban sources and anthropogenic activities and potentially accumulate in rivers and marine environments. Notably, there is a knowledge gap in our understanding of their co-occurrence, interactions, and effects on aquatic ecosystems. Existing studies have predominantly concentrated on individual pollutants, neglecting the intricate relationships between microplastics and EDCs in aquatic environments. Consequently, the main objective of this study was to elucidate the occurrence and spatial distribution of microplastics and multiclass EDCs in the riverine environment of the Selangor River Basin, with a particular emphasis on their comparative distribution in surface water and sediment. Follow-up objectives also include ecological risk assessment, estimation of daily pollution loads, and investigation of the influence of different land-use activities on the distribution of microplastics and EDCs in the Selangor River Basin.

The principal aim of this study is to advance the understanding of the occurrence and distribution of microplastics and EDCs within riverine environments, while concurrently investigating potential ecological ramifications for freshwater organisms. Through the analysis of their distribution patterns, potential synergistic relationships, and nature in the environment, this study provides a comprehensive assessment of their

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distribution, extent, and correlated risks to both aquatic organisms and broader ecosystems. Moreover, this study establishes vital baseline data and information for microplastics and EDCs occurrence in riverine ecosystems, particularly significant in urbanized and developing countries such as Malaysia, where rapid industrialization and land-use changes may exacerbate contaminant loads.

This study contributes to environmental sustainability and human health concerns, uncovering risks to both ecosystems and populations dependent on these resources. It also aids in raising awareness regarding microplastic pollution and responsible waste management. The outcome of this study can inform legislation and policies to mitigate microplastic and EDCs pollution risks, extending beyond knowledge of their presence to serve as a valuable tool for environmental monitoring and management. By utilizing the insights gained, effective measures can be implemented to safeguard ecosystems and human health. Ultimately, the study aligns with the broader goal of promoting sustainable and healthy coexistence with the natural environment.

7.2 Conclusion

The occurrence of microplastics and EDCs in aquatic environments has triggered profound environmental concerns owing to their extensive detrimental impacts on aquatic organisms and human health. Their widespread distribution has led to habitat degradation and poses significant threats to the well-being and survival of aquatic organisms and human populations. This assessment was undertaken to analyze the occurrence and spatial distribution of microplastics and multiclass EDCs in the freshwater environments of the Selangor River Basin. The abundance and distribution of microplastics and EDCs in riverine environments are crucial for understanding their

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potential sources, pathways, and extent in the environment. This study assessed the abundance and distribution of microplastics and EDCs in both the surface water and sediment of the Selangor River Basin. Subsequently, leveraging the data and findings derived from the microplastics and EDCs present in surface water, this study evaluated potential ecological risks, daily loads, and the impact of various land uses on the distribution of microplastics and EDCs within the riverine environment.

The observed microplastic abundance in surface water varied from 0.70 to 3.40 particles/L, with downstream areas heavily impacted by microplastic pollution. The predominance of microplastics in downstream areas is potentially associated with extensive anthropogenic discharge and input from upstream and tributaries. Therefore, the river basin could be a crucial source of microplastics entering the oceans. The present study revealed the largest proportion of microplastics (<1000 μm), fiber, transparency, and PE across the Selangor River Basin. Meanwhile, in sediment, microplastics exhibiting small size (<300 μm), bead-shaped morphology, and black color were found to be the most abundant, with concentrations ranging from 10 to 55 particles/kg. The dominant polymer composition in the sediment was identified as PP, followed by polyester and PE.

The concentration and spatial distribution of microplastics exhibited considerable variability within all sampling points in the surface water and sediment of the Selangor River Basin, with CV reaching up to 42.69% and 35.84%, respectively. Furthermore, the abundance and distribution of microplastics were found to be highly influenced by extensive urbanization as well as the presence of various anthropogenic activities, including industrial, commercial, residential, and tourism sources within the river

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basin. Notably, these types of microplastics exhibit a consistent predominance across various environmental compartments worldwide, indicating their widespread occurrence and posing potential risks to both ecological systems and human health. These findings emphasize the necessity for further monitoring assessments on a larger scale of rivers, encompassing different land-use patterns and seasonal variations. Such assessments are crucial to comprehensively evaluate the nature, extent, and fate of microplastics in the environment and effectively mitigate their adverse impacts on the environment, aquatic organisms, and human health.

The present study analyzed the abundance and distribution of ten multiclass EDCs (BPA, BPF, BPS, propranolol, PFOS, PFOA, testosterone, progesterone, EE2, and E2) in the surface water and sediment from 25 sampling points along the Selangor River Basin, Selangor, Malaysia. Plasticizers (e.g., BPA, BPF, and BPS) and hormones (e.g., EE2 and E2) are ubiquitous and frequently detected in surface water. Only propranolol, progesterone, and testosterone levels were below the detection limit. In the sediment, all EDCs were detected except for testosterone, which was below the MDL. Among the EDCs detected, BPA had the highest detection proportions (100%), ranging from 3.74 ng/g to 117.20 ng/g, followed by EE2 and BPF. The highest abundance of EDCs was found in the lower stream of the Selangor River Basin, indicating anthropogenic waste input from point and nonpoint sources originating from the upper stream and tributaries. Furthermore, EDC contamination in the Selangor River Basin indicates the influence of industrial, commercial, and residential waste sources. Thus, the present study provides baseline data that could fill the knowledge gaps regarding the occurrence of EDCs in surface water and sediment for better comparison in future studies. Moreover, spatial and temporal distribution in a larger-scale environment

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should be considered to ascertain the pathway and extent of EDCs in the environment. Addressing the accumulation and retention of EDCs in the environment is imperative, given that reported contamination has been linked to adverse effects on aquatic organisms and humans.

A key aspect of this study is the comparative analysis of microplastics and EDCs in surface water and sediment, as their environmental behaviors and fate differ significantly. Microplastics are predominantly detected in surface water because of their buoyant nature, which facilitates their transport across various sections of the river. In contrast, EDCs were found in both surface water and sediment, with notable variations between rural and urban areas. Urban areas exhibit higher concentrations of EDCs in surface water, mainly due to untreated wastewater discharge and industrial effluents. However, EDC concentrations in sediment were relatively higher in rural regions, likely influenced by agricultural activities, such as pesticides and livestock waste runoff, combined with the limited presence of wastewater treatment infrastructure. This variation highlights the different mechanisms governing the transport and accumulation of microplastics and EDCs in aquatic ecosystems.
The potential ecological risks of microplastics and EDCs were assessed using the RQ approach. These results indicate that the Selangor River Basin poses a low ecological risk. However, persistent microplastic discharge from anthropogenic activities can intensify its occurrence in riverine ecosystems, thereby increasing its implications for ecological and human health. Therefore, continuous microplastic monitoring and future investigations of ecological and human health risk assessments should be conducted comprehensively to address the extent and risk of microplastics in riverine environments. Meanwhile, all detected EDCs were estimated to have negligible risks,

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except for EE2 and E2, suggesting that hormone levels in the surface water of the Selangor River Basin are related to ecological and human health. Therefore, the preliminary prevalence of EDCs in river basins can be used for future monitoring and comparative investigations. Moreover, extensive research on the prevalence of EDCs in various environmental compartments, particularly the inclusion of large-scale river basins and point sources, is required to comprehensively evaluate their sources, extent, and fate in the environment.

The present study estimated significant daily loads of microplastics and EDCs, highlighting their widespread distribution in the Selangor River Basin. Although microplastic and EDC contamination were detected across all land-use types, the estimated daily loads at the three available monitoring points suggest relatively low pollution levels. The lowest microplastic daily load was recorded in the upstream region (SP1), whereas BPS exhibited the lowest estimated load in the downstream region (SP20). These findings suggest spatial distributions in pollution, likely influenced by various factors, including water discharge and anthropogenic inputs. Although urban areas exhibited higher microplastic and EDC concentrations, statistical analysis showed no significant differences between urban and rural regions, suggesting that land-use type alone does not dictate pollutant distribution. Instead, the primary drivers of contamination appear to be direct inputs of anthropogenic waste, wastewater discharge, and industrial activities. In rural areas, agricultural runoff and untreated domestic waste also contribute to EDC accumulation in sediments. These results emphasize the need for comprehensive mitigation strategies that address pollution sources rather than focusing solely on land-use categories. Enhanced

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wastewater treatment and pollution control measures are crucial for reducing microplastic and EDC contamination in riverine environments.

Overall, these findings emphasize the importance of understanding the co-occurrence, behavior, fate, and pathway of microplastics and EDCs to mitigate their environmental persistence and protect aquatic ecosystems and human health. Further studies should incorporate comprehensive monitoring, as well as experimental studies that encompass multiple contaminants in the environment. This could provide a better empirical understanding of their nature, interactions, fate, and combined effects on the environment, aquatic organisms, and human health. Such studies could provide valuable insights into the complex dynamics and potential synergistic effects of different contaminants, thereby facilitating more effective strategies for mitigating their adverse effects and safeguarding the well-being of both ecosystems and human populations.

7.3 Recommendation for future research

The present study recommends several areas for future research on microplastics and EDCs within aquatic environments. These recommendations are intended to enhance the broader and comprehensive understanding of the complex interactions between microplastics, EDCs, and their environmental implications. The suggested areas are as follows:

1. Establish standardized monitoring and analytical techniques that precisely ascertain and quantify the concentrations of microplastics and EDCs within

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various environmental compartments, including biota-rich samples in the aquatic environment.

2. Develop improved techniques for identifying and characterizing microplastics, including their size, shape, composition, and distribution with high sensitivity and precision. This can enhance the accuracy of assessing their prevalence, sources, and fate within the aquatic environment, given that microplastics can serve as carriers and sources of EDCs.
3. Prioritize comprehensive and extensive monitoring and comparative analysis of the co-occurrence of microplastics and EDCs in aquatic ecosystems to advance our understanding of their interactions, mechanisms, and associated risks.
4. Controlled laboratory experiments were performed to evaluate the toxicological effects of microplastics and EDCs on various aquatic organisms based on real conditions and concentrations in the environment to establish dose-response relationships. These investigations may consider the possible effects on biodiversity, trophic levels, and ecosystem stability.
5. An in-depth study of the synergistic effects of microplastics and EDCs in combination with other stressors such as pollution, climate change, and habitat destruction may provide a more comprehensive understanding of their overall impact.
6. Develop standardized methodologies to evaluate the ecological and human health risks of microplastics and EDCs associated with microplastics and EDCs in marine and freshwater environments.
7. Encourage the adoption of sustainable approaches, encompassing both environmental friendliness and economic viability, in designing systems that

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efficiently mitigate and remediate microplastics and EDCs within aquatic environments.

8. Engage in educational and outreach initiatives to increase public awareness regarding the risks associated with microplastics and EDCs in aquatic environments.
9. Develop comprehensive legislation that addresses the monitoring, management, and reduction of microplastics and EDCs in aquatic ecosystems, and establish clear guidelines for responsible waste management, pollution prevention, and sustainable practices to mitigate their adverse impacts on the environment and human health.

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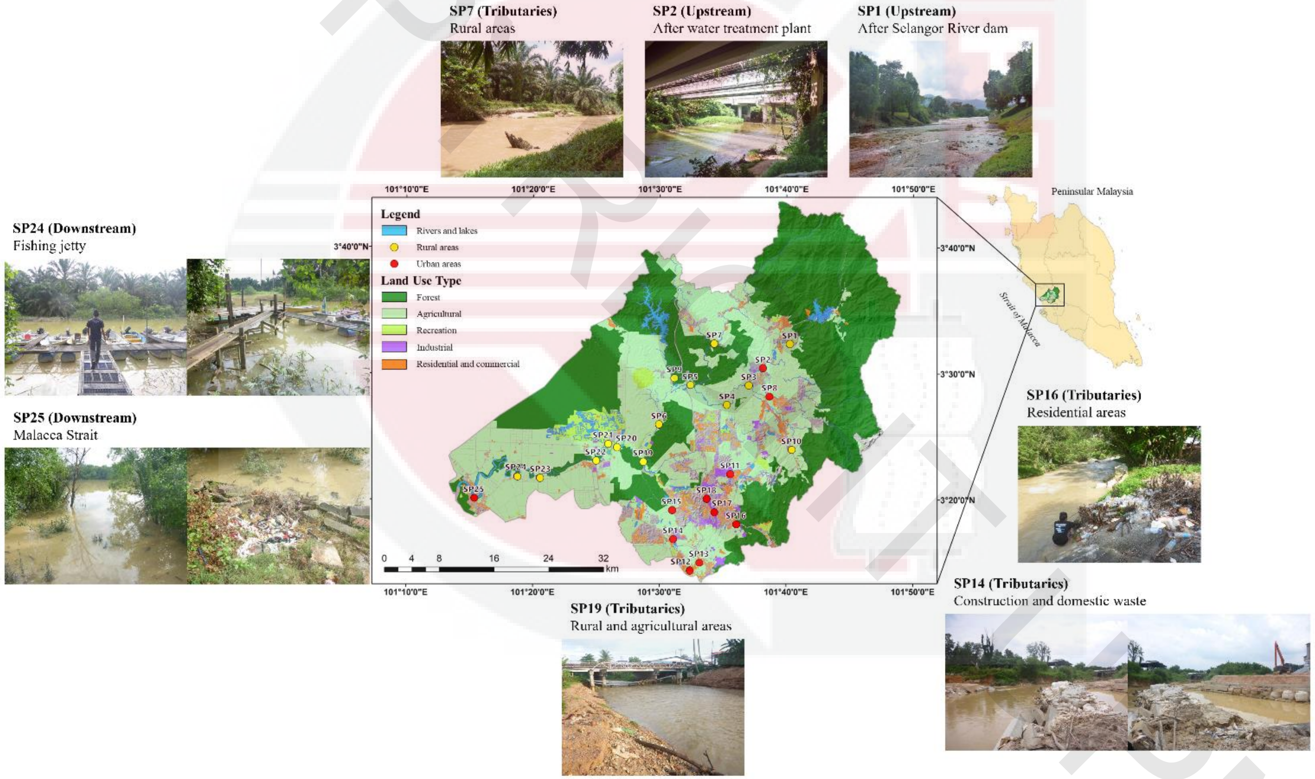
APPENDICES

Appendix 1: Sampling points for surface water and sediment in the Selangor River Basin



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Author: Muhammad Rozaimi Mohd Zaki,Syatirah Husna Mohamad Zaid,Azim Haziq Zainuddin,Ahmad Zaharin Aris

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Author: Muhammad Rozaimi Mohd Zaki et al

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Microplastic Pollution in Freshwater Sediments: Abundance and Distribution in Selangor River Basin, Malaysia

Author: Muhammad Rozaimi Mohd Zaki et al

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BIODATA OF STUDENT

Muhammad Rozaimi bin Mohd Zaki was born on March 26, 1996, in Kajang, Selangor. He attended primary and secondary schools at Sekolah Kebangsaan Sri Serdang, Sekolah Kebangsaan Gual Tinggi, Sekolah Menengah Kebangsaan Baroh Pial, and Sekolah Menengah Kebangsaan Pangkal Kalong. After completing his secondary education and obtaining his Sijil Pelajaran Malaysia (SPM), he pursued further studies at Kolej Matrikulasi Negeri Sembilan (KMNS). In 2018, he successfully completed his degree from Universiti Malaysia Terengganu, where his final year project focused on environmental pollution monitoring, specifically the assessment of heavy metal contamination in notched threadfin bream, *Nemipterus peronii* (Valenciennes 1830). Following the completion of his degree, he worked as an internship trainee at Pejabat Perikanan Negeri Kelantan. Recognizing his deep interest in environmental pollution, as well as the extensive knowledge and experience he gained during his undergraduate studies, he decided to enrol in the Master of Science program at the Faculty of Environmental Studies, Universiti Putra Malaysia. In his master's research, he concentrated on the occurrence of microplastics and endocrine disrupting compounds (EDCs) in estuarine and riverine environment. During his two-year master's program, he accomplished notable achievements, including the publication of two scientific articles in scholarly journals, namely *Marine Pollution Bulletin* (Q1, IF: 5.8, Top 25%) and *Environmental Geochemistry and Health* (Q2, IF: 4.2). Motivated by his passion and driven by his research accomplishments, he successfully converted his master's study into a Doctor of Philosophy program, specializing in the field of marine and freshwater environments. Throughout his doctoral studies, he continued to contribute to the scientific community by publishing

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several research and review articles in high-impact journals, with publications in Q1 and Q2 categories.

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LIST OF PUBLICATIONS

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Zaki, M.R.M., & Aris, A.Z. (2022). An overview of the effects of nanoplastics on marine organisms. Science of the Total Environment, 831, 154757. [Q1, IF: 9.8, Top 10%].

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Zaki, M.R.M., Razak, M.R., Haron, D.E.M., Isa, N.M., Yusoff, F.M., & Aris, A.Z. Multiclass endocrine disrupting compounds in surface water from Selangor River Basin: Occurrence and ecological risk assessment [ACS ES&T Water – under review, Q1, IF: 5.3, Top 15%].

Zaki, M.R.M., Wee, S.Y., Haron, D.E.M., Isa, N.M., Yusoff, F.M., & Aris, A.Z. Occurrence and distribution of multiclass endocrine disrupting compounds in sediment of anthropogenically impacted Selangor River Basin, Malaysia [Preparing for journal submission].

Zaki, M.R.M., Saifuddin, M.F.U., Ash'aari, Z.H., & Aris, A.Z. Microplastics and endocrine disrupting compounds in urbanized tropical riverine water: A comparative analysis, pollution load and land-use effect [Preparing for journal submission].

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