Elucidating Temporal Trends in Environmental Contamination with Microplastics and Organophosphate Flame Retardants

By

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Abstract

The relationship between microplastics (MPs) and organic pollutants such as organophosphate esters (OPEs), which are widely used as plastic additives is poorly understood. Given the potential implications for the bioavailability of such chemical additives, this is a significant research gap that should be addressed. Specifically, the impact on additive bioavailability of several factors including the type of plastic polymer and the concentration as well as the physicochemical properties of chemical additives. To further understand the interaction between microplastics and chemical plastic additives in freshwater sediments, the possibility of correlation between microplastic concentrations and the concentration of OPEs in the same sediment samples was investigated. Freshwater sediment samples were collecetd from a range of locations across the world and from 3 rivers and an urban canal in the West Midlands area of the UK on a monthly basis over a 12-month period. UK riverine samples were collected both upstream and downstream of WWTP discharge points.

Following extraction and quantification of microplastics using density separation and the Nile Red method, the mean number of MPs/kg (dw) for all four UK study locations ranged from 67-267 (Worcester and Birmingham canal), 133-283 (River Tame), 66.67-317 (River Severn), and 67-233 (River Sowe) per month. The highest mean number of MPs/kg was recorded in the River Tame. Overall, 57% of the mean number of MPs identified across all three locations over the 12 months period were detected downstream of the WWTPs while 43% were upstream. The most dominant morphological group in all the isolated MPs across all four study locations over the 12 months period were fragments (71%) and the least abundant fibres (9%). All target OPEs were detected at all four study locations following instrumental analysis on an Agilent 5975C GC coupled to an Agilent 5975C MSD fitted with a 30 m DB-5 MS column with detection frequencies of: (tris (chloroethyl) phosphate (TCEP), tris (2-chloroisopropyl) phosphate (TClPP), tris (1,3-dichloro-2 propyl) phosphate (TDClPP), tri-n-butyl phosphate (TNBP), tris (2-butoxyethyl) phosphate (TBOEP), 2-ethylhexyl diphenyl phosphate (EHDPP), tris (phenyl) phosphate (TPHP), and tri-m-tolyl phosphate (TmTP)) ranging from 58% to 100%. The concentration of Σ_8OPEs ranged from 107 ng/g (dw) (in the Worcester-Birmingham canal) to 52 ng/g (dw) in both Rivers Severn and Sowe). The highest $\sum_8$OPE concentrations for all study locations were recorded during autumn months (September, October, and November) and concentrations of $\sum_8$OPEs in sediment samples from the River Severn, River Tame, and River Sowe all varied inversely with the river level and flow rate. The potential risk

posed by target OPEs in all four study locations was found to be low, except for a moderate risk identified for EHDPP in the Worcester-Birmingham canal.

For sediment samples from Nepal, Greece, Poland, Swaziland, Spain, Mozambique, Switzerland, Canada, Malaysia, Republic of Korea, Chile, Nigeria, and India, $\sum_8$OPE concentrations ranged from 13 ng/g in the Republic of Korea to 95 ng/g (dw) in Nepal (with TDCIPP (62 ng/g (dw)) contributing substantially to the high concentration of $\sum_8$OPEs in Nepal). The detection frequency of all eight target OPEs in 11 out of the 14 countries was 100% while for the other three (Swaziland, Mozambique, and Republic of Korea), the detection frequencies were 80%.

The mean number of MPs/kg (dw) at the various countries from which samples were obtained ranged from 1217 MPs/kg (dw) in Spain (Francoli River and Cànoves stream) to 0 MPs/Kg in some locations (Thames Ontario (Kintore Creek West), Canoves in Spain and Kali Gandaki River in Nepal). The percentage of fragments per kg of sediment (dw) ranged from 100% in Nepal to 40% in the Republic of Korea. The highest percentage of fibres was recorded in South Korea (60%) and the lowest recorded in India (2%). Spheres were only identified in four countries (Greece, United Kingdom, Nigeria, and India) with percentage abundances of 10%, 3%, 20% and 3% respectively. The average length of MPs in all the countries where sediment samples were collected was < 1500 µm. In fact, for 6 out of the 14 countries studied, ave-age MP length was < 300 µm and was less than 1000 µm for all but one country.

Dedication

To my dearest mother, my unbroken heroine,
Thirty-plus Harmattans you bore, laden with struggles, steeped in pain,
In those tumultuous years, you crafted a foundation, solid and sovereign,
Upon which my existence now proudly stands, ever strong and humane.

Your toil, unspoken yet palpable, shone through the darkest days,
Your sacrifices, unseen yet monumental, etched in my heart always.
Your unwavering belief in me, a steadfast beacon guiding my way,
Your courageous fight for me, the shield against life's disarray.

You bore my turbulent childhood, with fortitude so grand,
Carved a pathway through impossibility, with your tender, nurturing hand.
Through each daunting milestone that my journey now spans,
I yearn for your presence, for you to see the fruits of your toil.

Oh, how I wish you were here, in this moment sublime,
To witness the echoes of gratitude, in this heart of mine.
Your legacy endures, as the stars in their celestial array,
In your enduring spirit, I find my way each day.

Acknowledgement

But for the grace of God Almighty, I would not be here today; for that, I am eternally grateful. I am also eternally grateful to my lovely wife Nkemakolem Onoja, who through tough times has not only stood by me but has been much-needed and reliable support. My Lovely Kids Cheryl Onoja and Jason Onoja who have both had to deal with my absence while enduring the sounds of me typing away behind closed doors. I say Thank you. To my late mother (Racheal Onoja), my late father (Stephen Onoja), my elder brother Ibrahim M. Onoja (who has assumed the role of a father by supporting me through university and all subsequent endeavours) as well as my other siblings whose love and support cheered me through impossible situations, I say thank you very much.

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CHAPTER 1

1.1 Introduction

This chapter contains some material taken verbatim from the following peer-reviewed publication: Onoja, S., Nel, H.A., Abdallah, M.A.E. and Harrad, S., 2022. Microplastics in freshwater sediments: Analytical methods, temporal trends, and risk of associated organophosphate esters as exemplar plastics additives. Environmental Research, 203, p.111830.

1.2 Background

The littering effect of human activities on water bodies can be traced back over hundred and forty years ago (Verne, 1998). Today the concern has mounted as the list of harmful substances threatening the hydrosphere (which sustains nearly half of global primary production and serves as habitat to millions of aquatic species) continues to increase (Dümichen et al., 2015, Field et al., 1998, Ibe and Kullenberg, 1995). These substances are associated with materials including medical waste, cigarette filters, fishing nets, and plastics which have recently become the focus of increased environmental/health concerns. It is estimated that about 280 million tonnes of plastics are produced annually, of which approximately 10% end up in water bodies (including oceans) (Cole et al., 2011). Moreover, it has been projected that at current rates of production, an additional 33 billion tonnes will be produced by 2050 (Rochman et al., 2013). The Great Lakes alone have been reported to receive around 10,000 tonnes of plastics annually (Hoffman and Hittinger, 2017), making the problem of plastic pollution in freshwater systems as serious as in marine environments. In fact, it has been reported that rivers are responsible for transporting between 1.15 to 2.41 million tonnes of plastics into the marine environment each year (Lebreton et al., 2017).

Plastics enter the aquatic environment from a wide range of sources and after a while, they degrade through photolytic, mechanical, and biological processes into sizes smaller than 5.0

mm and are then referred to as microplastics (MPs) (Arthur et al., 2009). MPs that originate from the degradation of originally larger plastic particles are referred to as secondary MPs while those released directly into the environment in the micro size (longest dimension < 5 mm) are described as primary MPs (Boucher and Friot, 2017a).

Primary MPs have continued to find their way in increasing amounts into the aquatic environment since their initial use over fifty years ago for personal care products such as toothpaste and cleansers. Such emissions of primary MPs continue to be augmented substantially by those of secondary MPs with land-based anthropogenic activities such as wastewater disposal accounting for most of these pollutants (Horton et al., 2017c, Dris et al., 2015).

Wastewater treatment plants (WWTPs) have been acknowledged as one of the primary pathways through which MPs infiltrate aquatic ecosystems (Dris et al., 2015, Harley-Nyang et al., 2022, Grbić et al., 2020) and research indicates that approximately 25% of MPs that enter marine environments originate from WWTP effluents (Boucher and Friot, 2017b). Single use of everyday products such as exfoliants and toothpaste can release something in the range of 4,000 to 95,500 microbeads into the wastewater system. Moreover, a washing machine containing a 5 to 6 kg load of synthetic fabrics can release upwards of 6 million fibers in a single cycle (Napper and Thompson, 2016, Carr et al., 2016, Boucher and Friot, 2017b). This is particularly disturbing because a substantial proportion of these MPs ultimately end up in the environment through the final effluent and sludge products (Ziajahromi et al., 2017, Sun et al., 2019, Gies et al., 2018). In fact, it was reported that a WWTP in Scotland discharges approximately 65 million MPs into the aquatic environment daily despite serving only 650,000 inhabitants (Murphy et al., 2016). Another investigation in Italy revealed a daily discharge of approximately 160 million MPs from a WWTP catering to 1,200,000 inhabitants (Magni et al., 2019). Both studies highlight a daily release of MPs exceeding 100 times the population serviced by the respective WWTPs, thus emphasizing the need for further research in this area.

1.2.1 Risks associated with Microplastics in aquatic environments.

The risks associated with MPs in the aquatic environment have been grouped by Lambert and Wagner according to their effect on aquatic organisms. These include behavioural effects, morphological effects, physiological effects and life cycle effects. (Lambert and Wagner, 2016). Specifically, these risks include disruption of the food chain because of the effect of MPs on algae photosynthesis, as well as growth reduction in some aquatic organisms (Zhang et al., 2022a, Yin et al., 2018, Huang et al., 2021). Other toxicological impacts associated with MPs include intestinal damage (Lei et al., 2018), accumulation in vital organs and tissues (Wang et al., 2021), disruption of metabolism (Huang et al., 2021, Wan et al., 2019) and serving as vectors for harmful pathogens (Bellas et al., 2016). However, an accurate quantitative assessment of these risks is complex given that the risks are due not only to MPs but also to associated chemical additives. Thus, differentiating the impacts of MPs from those of additives present in those MPs, as well as those of additives leached from MPs and larger plastic waste into the environment, is an important research goal.

The risk of microplastics transporting plastic chemical additives as well as other persistent, bio-accumulative, and toxic (PBT) chemicals into the food chain upon ingestion by aquatic (and terrestrial) organisms has become a subject of great concern (Lambert and Wagner, 2016). Whether primary or secondary, MPs in aquatic environments will either float or accumulate along with other sediments (depending on their density) (Woodall et al., 2014) and in any case exhibit a greater tendency towards absorbing and desorbing toxic chemicals/additives (compared to larger size plastic particles) due to their smaller sizes and corresponding larger surface areas per unit mass/volume (Mason et al., 2016).

A wide range of chemical additives such as plasticisers, pigments, fillers/extenders, and flame retardants are often incorporated into plastic polymers during manufacture to impart specific desirable properties (Table 1). The problem however is that some of these chemical additives have been found to be hazardous to health and harmful to the environment (Pantelaki and Voutsa, 2019, Lai et al., 2015).

Table 1. 1. Plastic Additives and their function (Herrera et al., 2003, Bpf.co.uk, 2021, Hahladakis et al., 2018)

ADDITIVE CLASS	**FUNCTION**	**EXAMPLES (Although some have now been banned)**
Flame Retardants	To prevent the start of fire or reduce the spread	Chlorinated organophosphate esters (TCEP, TDCIPP)
Antimicrobials/Biostabilisers	To prevent deterioration of plastic materials because of bacterial attack.	Silver, copper
Antioxidants	To prevent the polymer from reacting with oxygen	Tetrakismethylene-(3,5-di-t-butyl-4-hydroxyhydrocinnamate) methane, and bisphenolics such as Cyanox 2246 and 425, Tris-nonyl-phenyl phosphate (TNPP)
Antistatic Agents	To prevent static electric charge	Cationic compounds such as quaternary ammonium salts and anionic compounds such as compounds containing phosphorus
Biodegradable Plasticisers	Used as an environmentally more friendly plasticiser and in some cases as degradation initiators.	Dioplex,
Blowing Agents	Used in the manufacture of foam materials	Azodicarbonamide powder
External Lubricants	To prevent damage during processing	Parafin wax, Synthetic wax, Stearic Acid
Fillers/Extenders	To improve strength and in some cases lower the cost of the plastic	Glass Fibre, asbestos, carbon black and silicate
Fragrances	For fragrance	
Heat Stabilisers	To prevent decomposition during processing	Cadmium and Lead compounds; Nonylphenol (barium and calcium salts).
Impact Modifiers	To provide shock absorbing effect for plastic products	Elastomer or rubber
Internal Lubricants	To lower viscosity and ease the processing of plastics	glyceryl monostearate
Light Stabilisers	To prevent chemical degradation because of exposure to UV light.	Hindered Amine Light Stabilizers (HALS)
Pigments	For coloration	Cobalt (II) diacetate., Cadmium compounds; Chromium compounds; Lead compounds.
Plasticisers	To improve flexibility and softness	Chloroethyl phosphate (TCEP); dicyclohexyl phthalate (DCHP), butyl benzyl phthalate (BBP)
Process Aids	To ease processing	Aromatic Process Oil
Reinforcements	To improve strength and stiffness	Finely divided silica, carbon black, talc, mica, and calcium carbonate
Mould release agents	To ease release of plastic from manufacturing mould	Silicone oils, graphite, zinc stearate, and molybdenum disulfide
Surfactants	To reduce surface tension	Sodium stearate

1.2.2 Organophosphate esters as plastic additives.

Organophosphate esters (OPEs) which are used both as flame retardants and as plasticisers, are among the most important and widely used plastic additives (Wang, 2000b, Herrera et al., 2003). Chlorinated OPEs such as tris(2-chloroethyl) phosphate (TCEP), tris(2-chloroisopropyl) phosphate (TCIPP), and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) are mostly used as flame retardants, while the non-chlorinated alkyl-, aryl- phosphates are often used as industrial lubricants and as plasticisers (Zeng et al., 2018) (Table 2).

Due to the highly combustible nature of most plastic materials and their diverse applications in electrical, industrial, transportation and household materials, measures are often taken during manufacture to reduce their flammability. Flame retardants are chemical additives that prevent the start of fire or reduce its spread once started and are often used at higher concentration than other additives during the manufacture of most plastic products (Wang, 2000a). Approximately 85% of all manufactured flame retardants are used as plastic additives (Flameretardants-online, 2014). Some flame retardants are classified as "additive" because they do not chemically bond with the parent polymer while others are classified as "reactive" because they bond with polymer units in a chemical reaction (Wang, 2000). Since additive flame retardants do not react chemically with the polymer, they can be released into the environment by mechanisms such as dissolution, volatilisation, or abrasion (Pantelaki and Voutsa, 2019, Lai et al., 2015, Marklund et al., 2005, Kovačić and Mrklić, 2002). Organophosphorus flame retardants (OPFRs) which are among the most used flame retardants (accounting for 18% of worldwide consumption of flame retardants) often exist in additive rather than reactive forms in most polymers (Marklund et al., 2005). Others include aluminium hydroxide (accounting for 38% of worldwide consumption of flame retardants), halogenated flame retardants (accounting for 31% of global consumption), organophosphorous flame retardants (18 %) and others (13%) (Figure 1) (IHS, 2017)

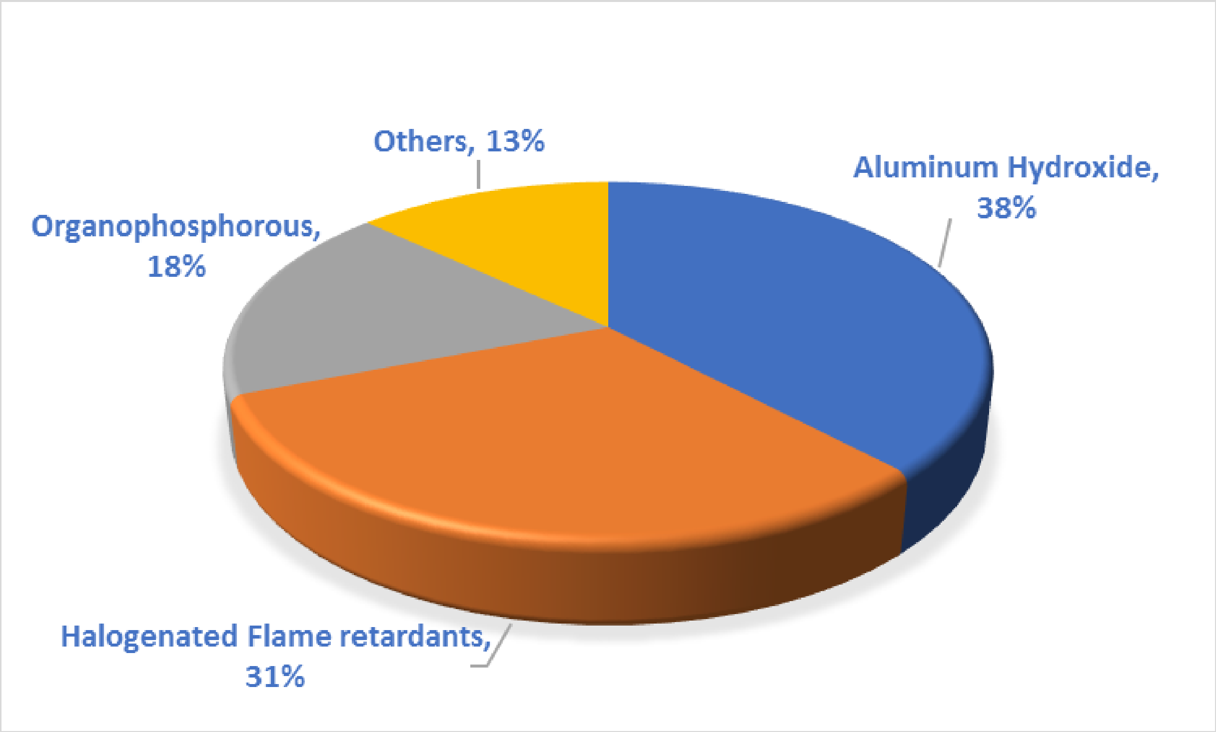


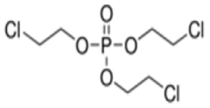
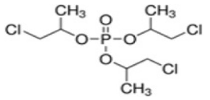
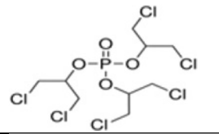
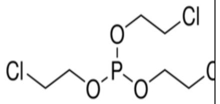
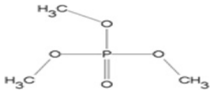
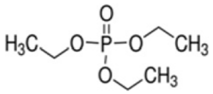
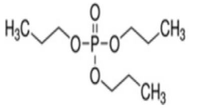
Figure 1.1. Global Flame Retardants Market by Chemistry, 2016 (Source: IHS Markit).

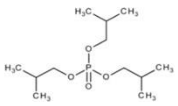
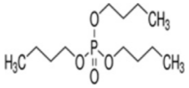
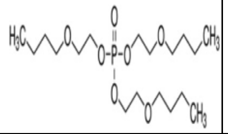
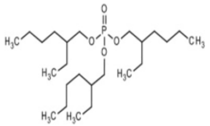
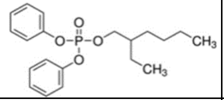
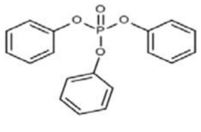
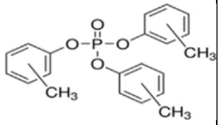
The European Flame Retardants Association (EFRA) has reported that the estimated consumption of flame retardants in Europe for 2006 was 46,500 t and that 20 % of these flame retardants were OPFRs (EFRA, 2017). This is an expected trend considering the restrictions and bans on the legacy flame retardants hexabromocyclododecane (HBCDD) and polybrominated diphenyl ethers (PBDEs) due to their adverse environmental and health impacts (Wei et al., 2015). However, mounting evidence suggests OPFRs and other organophosphate esters (OPEs) which have found several uses in recent times might not be a safer substitute after all (Hou et al., 2016). This perhaps explains the reported occurrence of OPEs in several matrices including dust, soil, air, water, plants and sediments as well as their accumulation in human blood, milk and urine (Castro-Jiménez et al., 2014, Cristale and Lacorte, 2013, Marklund et al., 2005, Andresen et al., 2004, Li et al., 2019b, Shah et al., 2006, Kim et al., 2011). Studies have identified leaching from plastics as one major way these OPEs find their way into the environment (Van der Veen and de Boer, 2012) and this is expected considering the role OPEs play in the manufacture of plastics. In a study that investigated OPEs (mainly TCEP and TCPP) in MPs collected from 20 coastal beaches of the Bohai and Yellow Sea in north China, it was reported that the maximum concentration of all 4 OPEs ($\sum_4$OPEs) was 85,000 ng/g (Zhang et al., 2018). An increase in OPE concentrations in a rural river (Kurose River, Higashi-Hiroshima) was also linked to the leaching of TCP from agricultural plastic films used for a greenhouse (Cho et al., 1996). Similarly, wastewater treatment plants (WWTPs) have been identified as a major source of OPEs in surface waters as high concentrations of OPEs have been associated with some WWTPs (Fries and Püttmann, 2003, Truong, 2016, Bester, 2005, Marklund et al., 2005). Laundry wastewater has also been identified as a substantial pathway of OPEs from indoor to outdoor environments (Schreder and La Guardia, 2014, Saini et al., 2016).

1.2.3 Toxicity of Organophosphate Esters.

OPEs have been associated with serious environmental and health concerns (Table 1.2). Beyond environmental persistence, the possibility of some OPEs (mostly the chlorinated alkyl phosphates) being carcinogenic as well as posing a risk of other such serious health concerns as infertility and neurotoxicity have been reported (Kawagoshi et al., 2002, Shi et al., 2018, Hou et al., 2016, Hoffman et al., 2017, WHO, 1993, WHO, 1993b, Van der Veen and de Boer, 2012, Stapleton et al., 2009). Decreased semen quality and other fertility problems have been associated with TDCIPP and TPHP (Stapleton et al., 2009), while tris(methyl-phenyl) phosphate (TMPP) and triphenyl phosphate (TPHP) have both displayed neurotoxic potential in zebra fish larvae (Shi et al., 2018). TCEP has been classified as a category 2 reproductive toxicant and category 2 carcinogen (EU, 2009). Another area of concern is the effect of the alcohols released as a result of hydrolysis of some of these OPEs, as it has been confirmed that butoxyethanol produced by the hydrolysis of tributoxy ethyl phosphate (TBEP), is not just a mutagen but also an endocrine disruptor (Boatman et al., 2004). Overall, the ecotoxic effects of several chemical plastic additives including organophosphate flame retardants and plasticisers are well-documented in the literature. Therefore, the enhanced release of these hazardous chemical additives to the aquatic environment from MPs due to their large surface area to mass/volume ratios and subsequent toxic impacts on aquatic organisms is highly concerning.

Table 1. 2. Names, abbreviations, Structures, CAS Numbers, molecular formula, and application of some OPEs.

Compound	**Abbr.**	**Structure**	**Cas no.**	**Molecular formula**	**Applications**	**Environmental/ health hazard**	**Reference for applications and environmental/ health hazard**
Tris (chloroethyl) phosphate	TCEP		115-96-8	$C_6H_{12}Cl_3O_4P$	Flame Retardant, Plasticizer, Lacquer Paint, Glue, Industrial processes	carcinogenic, highly toxic, environmentally persistent, and cytotoxic in high concentration,	(Van der Veen and de Boer, 2012, Wei et al., 2015, Reemtsma et al., 2008, Matthews et al., 1993, Föllmann and Wober, 2006), Sigma-Aldrich, 2019
Tris (2-chloroisopropyl) phosphate	TClPP		13674-84-5	$C_9H_{18}Cl_3O_4P$	Flame Retardant and Plasticizer	suspected carcinogen cytotoxic in high concentration,	(Van der Veen and de Boer, 2012, Wei et al., 2015, Reemtsma et al., 2008, Föllmann and Wober, 2006, WHO, 1998)
Tris (1,3-dichloro-2-propyl) phosphate	TDClPP		13674-87-8	$C_9H_{15}Cl_6O_4P$	Flame Retardant, Plasticizer, Lacquer paint and glue	Carcinogenic, Developmental, and reproductive toxicity, endocrine disruption,	(Van der Veen and de Boer, 2012, Wei et al., 2015, Reemtsma et al., 2008, WHO, 1998, Rhyu et al., 2019, Lu et al., 2017)
Tris(2-chloro-ethyl) phosphite	CLP1		140-08-9	$C_6H_{12}Cl_3O_3P$	Flame retardant, Stabilizer and Hydraulic fluids, and industrial processes.	Suspected carcinogen, Reproductive toxicity	(Van der Veen and de Boer, 2012, Wei et al., 2015, Reemtsma et al., 2008, (Beth-Hübner, 1999)
Trimethyl phosphate	TMP		512-56-1	$C_3H_9O_4P$	Industrial Processes	Genotoxic, possible reproductive toxicity,	(Van der Veen and de Boer, 2012, Wei et al., 2015, OECD,1996, Reemtsma et al., 2008)
Triethyl phosphate	TEP		78-40-0	$C_6H_{15}O_4P$	Plasticizer, Catalyst, ethylating agent, Raw material for insecticides	Possible developmental and reproductive toxicity	(Gumbmann et al., 1968, Van der Veen and de Boer, 2012, Wei et al., 2015, Reemtsma et al., 2008)
Tripropyl phosphate	TPP		513-08-06	$C_9H_{21}O_4P$	Flame Retardant, Plasticizer, Hydraulic fluids, Lacquer paint, glue, Cosmetic products, and industrial processes		(Van der Veen and de Boer, 2012, Wei et al., 2015, Reemtsma et al., 2008), Bennett et al., 1984, Cadogan and Howick, 2000, Toy, 1976

Tris (isobutyl) phosphate	TIBP		126-71-6	$C_{12}H_{27}O_4P$	Plasticisers, Hydraulic fluid, Floor finish, wax, lacquer paint, glue, Anti-foam agent and industrial processes		(Van der Veen and de Boer, 2012, Wei et al., 2015, Reemtsma et al., 2008, Hawley and Lewis Sr, 2001,
Tri-n-butyl phosphate	TNBP		126-73-8	$C_{12}H_{27}O_4P$	Flame retardants, Hydraulic fluid, Plasticizer,	Neurotoxic, suspected carcinogen	(Van der Veen and de Boer, 2012, Wei et al., 2015, Reemtsma et al., 2008,(Van Esch and Organization, 2000)
Tris (2-butoxyethyl) phosphate	TBOEP		78-51-3	$C_{18}H_{39}O_7P$	Flame Retardant, Plasticizer, floor finish, wax, lacquer paint, glue, anti-foam agent	Suspected carcinogen, developmental toxicity	(Van der Veen and de Boer, 2012, Wei et al., 2015, Reemtsma et al., 2008, World Health Organization, 2000a, (Guan et al., 2016)
Tris (2-ethylhexyl) phosphate	TEHP		78-42-2	$C_{24}H_{51}O_4P$	Flame Retardant, Plasticizer and fungus resistant	Not considered to be carcinogenic in humans, Reproductive and developmental toxicity needs to be investigated.	(Van der Veen and de Boer, 2012, Wei et al., 2015, Reemtsma et al., 2008,(Van Esch and Organization, 2000)
2-Ethylhexyl diphenyl phosphate	EHDPP		1241-94-7	$C_{20}H_{27}O_4P$	Flame retardants, Plasticizer, certain food packaging applications		(Van der Veen and de Boer, 2012, Wei et al., 2015, Reemtsma et al., 2008,
Tris (phenyl) phosphate	TPHP		115-86-6	$C_{18}H_{15}O_4P$	Flame retardants and Plasticisers	Developmental and reproductive toxicity, Neurotoxicity, genotoxicity, endocrine effect, Bioaccumulation and very toxic to aquatic life,	(Van der Veen and de Boer, 2012, Wei et al., 2015, (Stapleton et al., 2009, Svara et al., 2000, Mendelsohn et al., 2016, Du et al., 2016, Hou et al., 2016, OR, 2015, Organization, 1991)
Tris (methylphenyl) phosphate (or Tricresyl phosphate)	TMPP		1330-78-5	$C_{21}H_{21}O_4P$	Plasticizer, flame-retardant, Industrial processes, non-flammable fluid in hydraulic systems and as stabilizers	Neurotoxic, acute toxicity, Hazardous to the aquatic environment,	(Van der Veen and de Boer, 2012, Wei et al., 2015, Reemtsma et al., 2008,(O'Neill, 2006, Wyman et al., 1993) (ECHA), 2020)

1.2.4 Sediments as sink, as well as indicator of spatial and temporal trends in contamination of MPs and OPEs

Sediments have been identified as a major sink for microplastics (Peng et al., 2018b, Claessens et al., 2011b, Chen et al., 2020, Scherer et al., 2020b) with one study reporting a 600,000-fold higher concentration of MPs in sediment (mean: 3,350,000 particles m^{-3}, 125–5000 μm MP) than in water (mean: 5.57 particles m^{-3}, 150–5000 μm MP) (Scherer et al., 2020a). This includes low density polymers such as polyethylene (PE), polypropylene (PP) which are often described as buoyant polymers. As a result of biofouling, heteroaggregation and other similar processes, these low-density polymers often sink to the riverbed and are well represented in sediment samples (Corcoran, 2015, Chubarenko et al., 2016). Furthermore, there have been reports of a more diverse range of polymers including: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polymethyl methacrylate (PMMA), and dye particles in sediments (in comparison to water) (Horton et al., 2017a, Klein et al., 2015, Tibbetts et al., 2018, Scherer et al., 2020b).

OPEs have been reported in sediments by several studies (Castro-Jiménez and Ratola, 2020, Liao et al., 2020, Ren et al., 2019, Wang et al., 2018b) and in one such study, the ΣOPE concentrations in lower depth sections of a sediment core were significantly higher than those in surface sediments. This study which also determined the trend of OPE concentration over the decade, further confirms that sediments are major sinks for pollutants and a good source of information on historic trends (Liao et al., 2020). This is particularly interesting because even pollutants that are now being regulated or that have been banned can still be investigated using sediment MPs. Such findings will probably prompt regulatory bodies to ensure new chemicals or substances are thoroughly investigated before being used as replacements for ones that have been found to be ecotoxic.

1.2.5 Occurrence of MPs in freshwater sediment

There are indications of increasing interest in the study of MPs in freshwater ecosystems with significant improvements in the understanding and measurement of MPs in freshwater sediments between 2015 and the present (Onoja et al., 2021). Vaughan et al., (2017) used microscopy to determine the abundance and distribution of MPs in what was described as "the first assessment of microplastics concentration in the sediments of either a small or an urban lake and the first for any lake in the UK". The study reported relatively low concentrations (25–30 particles per 100 g (dw)) of MPs in Edgbaston Pool, Birmingham, UK but acknowledged the fact that more recent methods of identification and characterization (e.g. Fourier-transform infrared spectroscopy (FT-IR) and Raman spectroscopy) were not available (Vaughan et al., 2017). Compared to other studies across the globe (Fischer et al., 2016, Su et al., 2016), Yuan et al. (2019a) described Poyang Lake in China as having higher levels (54–506 items/kg dw) and biological risks of MPs (Yuan et al., 2019b). Research has also shown that a combination of one or more methods can enhance the quality of results, as one method can compensate for the disadvantages of another. This is illustrated by the work of Hidalgo-Ruiz et al. (2012), where previously established methods such as visual microscopy were combined with Raman spectroscopy for more accurate analyses of MPs (Hidalgo-Ruz et al., 2012). It is clear from the foregoing that 2017 to 2018 marked an upturn of interest in the study of MPs in freshwater sediments. Therefore, an overview of studies between January 2016 and December 2021 provides a more recent and focused perspective of the current trends in the study of microplastics in freshwater sediments (Table 1.3). Furthermore, our focus on recent publications provides insights into the current challenge arising from the lack of harmonization in reporting formats and sampling designs that has been identified as a major impediment to elucidating temporal trends in environmental pollution with MPs (Browne et al., 2015, Mai et al., 2018, Hanvey et al., 2017).

Table 1. 3. Overview of studies of microplastics in freshwater environments between 2016 and 2021.

Reference	**Country/region**	**Size range**	**Concentration of MPs**	**Trend identified**	**Gaps identified**
(Li et al., 2021)	China	<300 µm >1000 µm	573.84–2878.97 MPs/kg	Certain polymers such as Rayon (RA) and nylon (PA) were more in urban areas while polypropylene (PP) and polyethylene (PE) were associated typical agricultural areas.	Further studies on microplastic pollution characteristics in freshwater environments with different regional functions
(Scherer et al., 2020a)	Europe	20–5000 µm	Median concentration of 7.57 × 105 p m^{-3}	High MP pollution comparable to the high levels reported in some Asian countries.	However, such comparisons are hampered by the diversity of analysis and methods used in monitoring studies
(Jian et al., 2020)	China	0.03-0.1 mm	821 ± 100 MPs/kg to 1936 ± 121 MPs/kg	Compared to an earlier study on the same study location (Yuan et al., 2019), A higher concentration of MPs (approximately 4–30 times higher) was reported.	further long-term and systematic studies of the environmental fate of smaller size MPs (<0.1 mm) in freshwater systems.
(Turner et al., 2019)	United Kingdom (London)	1 µm to 500 µm	Approximately 539 particles per kilogram of dried sediment	Relatively low number of MPs were found in older sediments.	Improvements in contaminant reduction measures are highly recommended for future stratigraphic work. Use of more effective microplastic detection methods along with future paleolimnological work to allow a more diverse quantification of historical flux of microplastic wastes in terrestrial, freshwater, and marine environments.
(Wang et al., 2017)		50 – 5000 µm.	1660 ± 639 to 8925 ± 1591 number of particles per cubic meter of water (n/m^3)	High heterogeneity was discovered in the concentration of MPs across the study areas. A decrease in the concentration of MPs with increasing distance from the urban centre was discovered. The plastic levels recorded for the rivers were less than for the lakes, likely because of enhanced loss of MPs from rivers because of stronger hydrodynamics.	Comprehensive monitoring of the abundance of microplastics in inland freshwaters is recommended. Further research on accurate determination of MP sources and pollution control strategies is needed. A need for studies of the effect of color on the rate of ingestion of MPs is highlighted.

				Fibrous (52.9 – 95.6%), coloured (50.4 – 86.9%) and small (MPs less than 2 mm accounting for more than 80%) MPs were the most dominant.	
(Wang et al., 2019c)	Lake Ulansuhai in China	Six categories: < 0.5 mm, 0.5 – 1 mm, 1–2 mm, 2–3 mm, 3–4 mm and 4–5 mm	1760 ± 710 to 10,120 ± 4090 number of particles per cubic meter of water (n/m^3)	Spatial distribution of MPs was such that higher levels of MP were recorded at the entrance of a farmland drainage canal with a downward trend towards the end of the drainage canal of the lake	Further studies of the toxic hazard MPs pose to plants and aquatic animals is recommended
(Yuan et al., 2019a)	Poyang Lake China	<0.5 mm	54 – 506 number of particles per kilogram (dw)		More accurate and effective studies should be carried out on the impacts and fate of microplastics in freshwater ecosystems. Also, the potential impacts of MP consumption by human beings should be studied
(Wang et al., 2019a)	South Yellow Sea, China	<0.5 mm	560–4205 MPs/kg (dw)		
(Wen et al., 2018)	Changsha, China Surface sediments	<1mm	Ranged from 270.17 ± 48.23 items/kg to 866.59 ± 37.96 MPs/kg (dw)		More research on understanding the environmental behaviour of microplastics in urban waters
(Jiang et al., 2018)	West Dongting Lake and South Dongting Lake, both in China	macroplastics (>25 mm) and mesoplastics (5–25 mm)	From 617 to 2217 items/m3 and 717 to 2317 items/m^{-3} in surface water of West Dongting Lake and South Dongting Lake, respectively while the lakeshore sediment for both study locations ranged from 320 to 480 items/m3 and 200–1150 items/m^3, respectively.		Further work is encouraged on the effect of hydrodynamic conditions on the distribution of microplastics

(Matsuguma et al., 2017)	Africa	315 µm–5 mm (Although most were between 315 µm – 1 mm)	Japan: 1900 pieces/kg (dw) Thailand: 100 pieces/kg dry sediment	For all study locations, there has been an increasing trend in the abundance of MPs from the 1950s to the 2000s. Sediment is an important sink for MPs as more MPs were found in sediments than the surface water of Tokyo Bay. There is a consistency between the trend of microplastic abundance in sediments and the global trend of plastic production.	A more detailed analysis of microplastic pollution with time using more of the core is recommended. To enable a better understanding of the controlling factors behind the difference in polymer composition between the different study areas, Analysis of more samples is recommended.
(Ballent et al., 2016)	Lake Ontario Canada	< 2 mm	Approximately 760 particles per kg dry sediment.	It is likely that fibres are transported through suspension for greater distances than fragments. Some of the previously identified methodological problems in microplastic research have been resolved. However, standardization of operational protocol will still require more research.	Thorough analysis of microplastic morphology and composition may be used to shed more light on the role played by the type of microplastic on the depth to which they are transported in an aquatic environment. Future studies aimed at understanding how microplastic abundance in sediment vary with distance from outfall are recommended. This should be done using studies directly adjacent to storm waters, wastewater treatment plants and sewer outfalls. Future monitoring of microplastic in the sediments of Lake Ontario is also recommended
(Mason et al., 2016)	Lake Michigan, USA	< 1 mm	An average plastic abundance of ~17,000 particles/km^2 lake surface area	The relative abundance of fragments shows that secondary sources of microplastics are more significant to this study area than primary sources. The order of abundance of plastic types (polyethylene- mostly high-density being the most abundant followed by polypropylene) is consistent with global trends in the mass production of plastics.	

1.3 Analysis of MPs in sediment

The analysis of MPs in sediments requires strict adherence to standard procedures. This is particularly important considering the possibility of post-sampling contamination (Woodall et al., 2015). Based on published methodology, we address such impacts according to the following sub-headings: 1. sampling, 2. extraction, 3. identification / quantification, and 4. quality assurance/quality control.

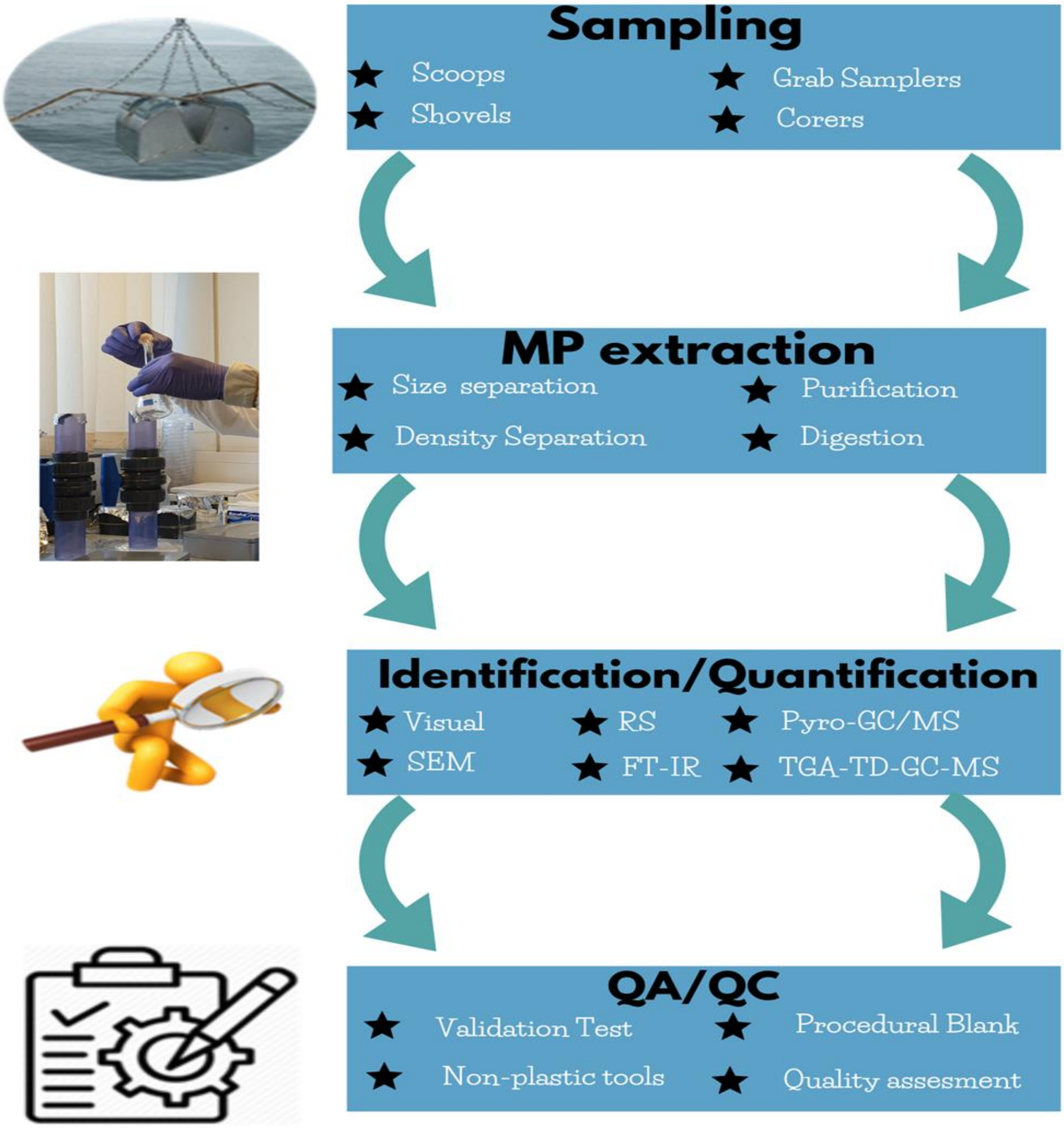


Figure 1.2. Flow chart of the procedures followed for analysis of MPs in sediments (Pyro-GC/MS: Pyrolysis Gas Chromatography/Mass Spectrometry, TGA-TD-GC–MS: Thermogravimetric analysis-thermal desorption-gas chromatography–mass spectroscopy, FTIR: Fourier transform infrared, SEM: scanning electron microscope, RS: Raman spectroscopy).

1.3.1 Sampling

Several tools and approaches have been employed to sample sediment most of which are dictated by the objectives of the research and the peculiarities of the study location. However, previous reviews on the methods of measuring MPs in sediments (Mai et al., 2018, Hanvey et al., 2017) identified a lack of standardized procedures in sediment sampling, leading to the grareporting of data in a variety of units. These units include MP pieces per m^2 (do Sul and Costa, 2014, Wessel et al., 2016), pieces per volume (mL or L) (Woodall et al., 2015, Browne et al., 2011), or weight (g or kg) (Ng and Obbard, 2006, Alomar et al., 2016, Klein et al., 2018) of sediment. This has made it difficult to compare the results of different studies quantifying microplastic contamination across the world, and is further complicated by the density estimation required for conversion between such units that can lead to errors (Cauwenberghe et al., 2015).

To get a more focused view of this subject as it relates to the most recent studies, the keywords "Sediment sampling for microplastics" were used to search the entire database of the ISI Web of Science for relevant studies between 2018 and 2022. The result was then narrowed down to 18 publications from different regions of the world using such criteria as year of publication and relevance to the topic of interest. Seven of the sixteen publications discussed the sampling tools utilized. For sampling below surface sediment, nine of the studies used Van Veen or other grab samplers (Khan et al., 2022, Baldwin et al., 2020, Crew et al., 2020, Eo et al., 2019, Ramirez et al., 2019, Scherer et al., 2020a, Shruti et al., 2019, Xu et al., 2020) and one used a box corer (Pagter et al., 2020). For surface sediment sampling, metal hand scoop (Schessl et al., 2019), stainless-steel shovel (Jiang et al., 2018, Peng et al., 2018a), metal pail shovel (Deng et al., 2020, Ramírez-Álvarez et al., 2020a), stainless steel split spoon or Ekman dredge (Eo et al., 2019, Wilkens et al., 2020), garden spade (Mani et al., 2019, Peller et al., 2019) and stainless-steel spatula (Toumi et al., 2019) were used. This further confirms the lack of standardization between sampling methods while showing that the box corer seems the most common approach to depth sampling. It is clear though, that the choice of equipment and sampling procedure is often governed largely by the nature of sediments to be sampled and the accessibility of the sampling site. A further consideration is that secondary contamination by MPs can be avoided by use of non-plastic sampling tools (Wen et al., 2018, Jiang et al., 2018).

1.3.2 Extraction

Once sediment samples are collected and transported to a laboratory for analysis, the next objective is to separate the MPs from a complex matrix of other organic and inorganic materials. A standard method for doing this is yet to be established (Horton et al., 2017b) but most recent studies seem to employ a combination of size and density separation techniques (Vaughan et al., 2017). The use of fluidization/elutriation (another density-based technique) has also been reported in some recent publications (Claessens et al., 2011a, Nuelle et al., 2014, Kedzierski et al., 2016, Zhu, 2015, Jarosz et al., 2022).

Most of the reviewed studies employed varying mesh sizes including: 8 µm to 47 mm (Zhang et al., 2019, Khan et al., 2022), and 90 µm to 4.75 mm (Pan et al., 2019b); it is however recommended that the choice of mesh size should be such that it allows the collection of a wide size fraction, while minimizing clogging of mesh holes (Mai et al., 2018).

The second approach is density separation, this approach takes advantage of the low density of most plastic materials (0.9 -1.55 g/cm^3). In a review of 48 studies from 18 different countries, Hanvey et al., (2017) reported the application of density separation in 84% of studies. This is partly because recovery rates as high as 100% have been previously reported (Mai et al., 2018). The basic idea behind this approach is allowing the MPs to float in a high-density solution thereby permitting the MPs to be filtered out. Four main steps have been identified, specifically: (1) introduction of an aqueous solvent of known density, (2) manual or mechanical shaking/stirring for a specific period, (3) allowing time for settling and equilibration, and (4) filtration (Hanvey et al., 2017). The aim of shaking or stirring after introducing the solution is to ensure that the MPs are properly separated from other sediment particles. This can either be done manually,(Wang et al., 2019c, Zhang et al., 2019) or mechanically using a vortex mixer (Woodall et al., 2014), or mechanical shaker (Ng and Obbard, 2006). The time allowed for equilibration and settling after shaking can be as short as five minutes (Corcoran et al., 2015), or up to 12 hours (overnight) (Stolte et al., 2015). There are a variety of high-density solutions that can be used for the first step and a recovery rate of 80 – 100% has been reported for NaCl solution (Fries et al., 2013), 96 to 100% for $ZnCl_2$ solution (Imhof et al., 2012) and 94 – 98% for NaI solution (Claessens et al., 2013a). Turner et al., 2019 used sodium polytungstate (SPT) solution to achieve a density as high as 2.2 g/cm3, suitable for the extraction of high-density plastics such as polytetrafluoroethylene (PTFE) (2.08 - 2.17 g/cm^3) and polyvinylidene fluoride or polyvinylidene difluoride (PVDF) (1.79 g/cm^3) (Turner et al., 2019a). While attempting to mitigate the limitations of most density separation techniques (expensive reagents that might be hazardous and limited density range), Jarosz et al, reported a recovery rate of up to 90% using potassium formate water solution (H_2O/KCOOH) (Jarosz et al., 2022). Attempts have also been made to use water for density separation but the recovery rate reported was less than 70% (Quinn et al., 2017).

As an alternative to the density based techniques, an oil extraction protocol has been reported (Crichton et al., 2017). This cost-effective alternative had a reported recovery rate of 92.7% ± 4.3% for fibres and 99% ± 1.4% for particles. In recent times, the use of techniques capable of providing recovery rates as high as 100% has also been reported. These include the use of pressurized fluid extraction (which has provided recovery rates between 101 and 111%) (Fuller and Gautam, 2016) and air-induced overflow (91 – 99%) (Nuelle et al., 2014).

Digestion of organic matter may improve MP detection accuracy and remove interference during polymer identification (see section 1.2.3). Following the removal of all or most of the organic materials, the next step is the filtration or wet sieving of the mixture. This is meant to separate suspended low density microplastics from other components of the solution. This separation is principally achieved by sieving or vacuum filtration and for most studies, the use of stainless steel sieves or glass fibre filters is the norm (Mai et al., 2018). Just as for dry sieving, the choice of mesh size should be such as allows the collection of a wide range of size fractions and minimizes clogging of mesh holes.

An important part of the microplastic extraction phase is the purification of extracts to eliminate organic matter to avoid interference during final quantification/identification steps. This is often achieved by a process sometimes referred to as digestion (Prata et al., 2019a) with several methods used by previous researchers (Table 1.4). In a review of over 60 publications on methods for sampling and detecting microplastics in water and sediment, Prata et al (2019) reported that 60% did not carry out any form of purification, 35% used H_2O_2 and 15% used Fenton's reagent (a solution of H_2O_2 with ferrous iron as a catalyst). Two other reviews (Mai et al., 2018) and (Hanvey et al., 2017) concluded a solution of 30% H_2O_2 to be the method of choice for this process. This is because it is very effective in dissolving higher molecular weight organic matter, while leaving the physical properties of the MPs intact (although it has been speculated that changes in the colour of MPs are possible) (Mai et al., 2018). Sodium hypochlorite (NaOCl) has recently been used with some reports of improvement (Bottone et al., 2021).

Table 1. 4. Methods for separating sediment MPs from organic and inorganic matter.

Method	Description	Disadvantage	Reference
Acid digestion	Use of acids such as nitric acid (55% HNO3) and hydrochloric acid (37% HCl) to degrade organic matter	Risk of digesting some low resistance MPs such as nylon and polyethylene terephthalate at high acid concentration and temperature	(Qiu et al., 2016)
Alkali digestion	Use of alkali such as potassium hydroxide (10% KOH) and sodium hydroxide (20 -50% NaOH) for the digestion process	Risk of damage or discoloration of MPs	(Mai et al., 2018, Qiu et al., 2016)
Oxidizing agents	Use of oxidizing agents such as hydrogen peroxide (35% H2O2). Sometimes combined with NaOH and HCl for better performance.	Shrinkage of certain plastics has been observed.	(Prata et al., 2019b)
Enzymatic digestion	This involves the use of enzymes such as proteinase K (500 mg/mL) for organic matter digestion.	No known risk to the integrity of MPs associated with this method yet.	(Karlsson et al., 2017a, Karlsson et al., 2017b)
Other Methods	Use of microwaves and ultrasonication	High risk of damage to the MPs.	(Karlsson et al., 2017b, Yiying et al., 2009)

1.3.3 Identification/ quantification

The identification and quantification of MPs often begin with their isolation from non-plastic particles based on physical properties that are unique to plastics. The process of identification based on physical properties such as morphology and colour are called visual inspection or visual sorting. This approach - conducted using a microscope - has proven rather ineffective, as reports exist of over or underestimation (Lenz et al., 2015, Löder et al., 2015, Cheung et al., 2016). For example, in one reported instance, 32% of particles and 25% of fibres were wrongly identified using visual inspection (Lenz et al., 2015). Another study (Eriksen et al., 2013b), reported that 20% of particles identified as MPs by visual sorting, were later identified as aluminium silicate after viewing with a scanning electronic microscope. Other more effective approaches have been reported. These include: Fourier-transform infrared spectroscopy (

FTIR) (Peng et al., 2017), Raman spectroscopy (Käppler et al., 2016a), scanning electron microscopy (Eriksen et al., 2013a), pyrolysis-gas chromatography-mass spectrometry (PyroGC-MS) (Fischer and Scholz-Böttcher, 2017) and thermal desorption gas chromatography-mass spectrometry (TD-GC/MS) (Altmann et al., 2019).

1.3.4 Raman Spectroscopy

Raman spectroscopy is non-destructive and can be used for both identification and quantification of MPs (Hanvey et al., 2017b). It has been used successfully to identify and quantify MPs down to as small as 1 μm (Pan et al., 2019a, Lenz et al., 2015). Other advantages of this method include the ability to generate information on the chemical composition of the MPs and other associated organic and inorganic matter (Klein et al., 2018).

1.3.5 Fourier Transform Infrared Spectroscopy (FTIR)

According to Hanvey et al. (2017b), 23 out of 43 studies reviewed used FTIR. The use of FTIR has also been reported in other forms such as micro-FTIR (Vianello et al., 2013) (which is basically a combination of optical microscopy and FTIR) and attenuated total reflectance (ATR) FTIR (Cheung et al., 2016). The advantages of this method include its high throughput efficiency and non-destructive nature. It is however limited to measuring particles of sizes around 25 μm and thickness < 100 μm. There is also the possibility of an overlap of polymer bands which make it difficult to distinguish between identified polymers (Käppler et al., 2016b, Käppler et al., 2018).

1.3.6 Scanning Electron Microscopy (SEM)

The use of scanning electron microscopy for identification of MPs has been very effective. Among other advantages, SEM provides clear images of vital physical properties of the particle (Hanvey et al., 2017b). The laborious preparatory processes involved in using this method is however a major disadvantage (Cao et al., 2017). It is good for showing physical degradation such as pits and cracks among other advantages and has been reported to be useful in differentiating between MPs and other organic/inorganic components (Crawford and Quinn, 2017). It has also been combined with energy-dispersive X-ray spectroscopy (SEM-EDS) to examine the composition as well as additive content of plastics (Crawford and Quinn, 2017, Fries et al., 2013).

1.3.7 Pyrolysis-gas chromatography - mass spectrometry (PyroGC-MS)

The basic principle of this method involves thermal decomposition of the particle of interest, followed by identification of the cryo-trapped evolved gases using mass spectrometry (Shim et al., 2017, Dümichen et al., 2017). This method is not only useful in identifying MPs but very effective for determining the polymer type, as well as associated organic matter (Mai et al., 2018). However, it is time consuming and destructive (Mai et al., 2018), and has difficulty correctly identifying polymers with polar sub-units (Dekiff et al., 2014a, Käppler et al., 2018, Fries et al., 2013).

1.3.8 Thermogravimetric Analysis coupled with Thermal desorption gas chromatography with mass spectrometric detection (TGA-TD-GC/MS)

TGA-TD-GC/MS works basically by interfacing thermo-gravimetric analysis connected to a solid-phase sorbent, with thermal desorption GC/MS (Dümichen et al., 2017, Elert et al., 2017). It has been successfully applied to measure MPs with the notable advantages of being more efficient even with complex samples. However, the method is destructive and time consuming (Dümichen et al., 2015)

1.3.9 Atomic force microscopy based infrared spectroscopy (AFM-IR)

This method is an advanced microscopy based technique that uses the tip of an AFM probe to detect thermal expansion in a sample after absorption of infrared radiation (Dazzi and Prater, 2017). It can be used to analyse nanosized particles as it produces high resolution images of up to 0.3 nm and can produce 3D images of polymer surface structure (Mariano et al., 2021). A major limitation though is that errors might arise from possible damage caused by contact with sample (Mariano et al., 2021).

1.4 Concerns over identification/quantification of MPs

Following the successful identification and quantification (sometimes by counting or weighing) of MPs using the previously discussed procedures, the results are often presented in varying units. A major concern is the lack of harmonization in the units of previous reports on the quantity of MPs in sediment (Hanvey et al., 2017). As a way of checking if this trend has changed since the Hanvey review, we conducted a search of the ISI web of knowledge database with the keywords "sediment" and "microplastics", restricted to papers published between 2019 and January 2022. This revealed that the inconsistency in result reporting remains a concern, although there are tentative indications of a movement towards a consensus on the use of items or particles per kg of dried sediment. A total of 38 publications met our search criteria and of these, only 7 quantified the measured MPs in terms of some form of units. Four of these reported the concentrations of MPs in items or particles per kg or g dry sediment (Yuan et al., 2019b, Mu et al., 2019, Zhang et al., 2019); two reported as particles or items per kg (without specifying whether dry weight or wet weight of sediment) (Bordós et al., 2019, Li et al., 2019a), while the remaining study reported as items/km of river (Xiong et al., 2019). It is recommended that items per kg be adopted as a standard as some recent studies have further classified MPs into fibres, particles, fragments, filaments, microbeads, foams etc. based on their shape (Bertoldi et al., 2021, Kumar et al., 2021, Sang et al., 2021). Also, the use of both weight and volume is recommended where possible.

Another concern from the foregoing is the cost and efficiency of the methods discussed above. The need for "a cheaper, faster and more easily applied method" is glaring, especially if sufficient data from lower income regions of the world are to be obtained in order to establish temporal and spatial trends on a global scale (Maes et al., 2017). One positive step in this direction, as suggested by (Andrady, 2011) is the use of lipophilic fluorescent dyes such as Nile Red to enhance visual identification/quantification of MPs under microscopes. Nile Red is a solvatochromic fluorescent dye that fluoresces with varying emission spectrum depending on the polarity of its environment (Maes et al., 2017). It has been applied successfully to the identification and quantification of microplastics in environmental samples (Maes et al., 2017, Erni-Cassola et al., 2017, Shim et al., 2017), Overall, this approach in combination with fluorescence microscopy and image analysis software is - according to Erni-Cassola et al. (2017) - a fast, reliable and cost-effective method of detecting, quantifying and sizing polyethylene (PE), polypropylene (PP), polystyrene (PS), and Nylon-6 particles in the size range of 20 µm to 1 mm. Shim et al. (2016) described the Nile Red staining approach as "straight forward and quick for identifying/quantifying polymer particles in laboratory-controlled samples" and reported a recovery rate of 98% for polyethylene (100-300 µm). In comparing the recovery rates of LDPE particles (in the size range of 100-300 µm) using the Nile red staining method and FTIR, Shim et al. (2016) reported 96±7% for FTIR and 98±3% for the Nile Red method. Furthermore, the Nile Red method gave on average a 1.4 times higher particle count than FTIR, following comparison of the quantitative analysis of field samples using both methods. This shows that the fluorescent dye staining method, which is a lot cheaper and faster, is also efficient. Despite its limitation of staining other non-plastic organic materials, it provides (among other advantages) a good solution to the difficulty in visual identification of transparent and white MPs (Shim et al., 2016). It is also highly unlikely that Nile Red would pose any risk of secondary contamination or interference with further analysis of additives present in the MPs (e.g., phthalates or OPEs) (Ramirez et al., 2019, Onoja et al., 2021). More serious limitations of the Nile red method include poor staining of certain polymers such as PVC, PA, and PES, as well as not being able to identify chemical composition of polymers except when used in combination with other techniques such as FT-IR (Erni-Cassola et al., 2017, Shim et al., 2016, Liu et al., 2022). Counting stained polymers using an automated technique is also recommended as this will reduce errors due to human judgment (Liu et al., 2022).

1.5 Quality assurance/quality control (QA/QC)

Considering the ubiquity of MPs, it is extremely important to put measures in place to prevent contamination of samples and ensure data accuracy. It is also important to guard against over- and under-estimation (Dekiff et al., 2014b). The level of such accuracy largely depends on the extent to which standard QA/QC measures are adhered to (Ibe and Kullenberg, 1995). Rinsing all apparatus 3 or 4 times with distilled water (Wen et al., 2018, Jiang et al., 2018, Yuan et al., 2019b) and then cleaning the workspace with 70% alcohol (Wen et al, 2018) has been reported. A strict use of 100% cotton lab coats as well as nitrile gloves, in addition to ensuring that all equipment and glass containers are covered by aluminium foil has also been reported (Wang et al., 2019b). Some researchers pre-filter all liquids before use (Wang et al, 2019, Yuan et al 2019b). Other quality assurance and quality control measures that are recommended include validation studies and procedural blanks. Validation studies are laboratory tests performed to verify that a technique or procedure is accurate and reproducible, specifically highlighting any limitations. Hanvey et al., (2017) reported that only 7 out of the 43 reviewed studies conducted some form of validation test. Some more recent studies have used procedural blanks as QA/QC measures (Wang et al., 2019; Yuan et al., 2019b, Jiang et al., 2018). Other steps that have been undertaken in previous studies to ensure the integrity of the results of MPs studies, include the use of non-plastic sampling, storage, and processing tools (Hanke et al., 2013).

1.6 Possible effect of MP extraction methods on the analysis of associated additives

The bioavailability of additives such as OPEs that are present in sediments, will very likely vary substantially depending on whether they are sorbed to MPs or to naturally occurring sediment organic matter. It is therefore important to be able to differentiate between OPEs bound to MPs present in sediment and those associated with the sediment itself. Moreover, in order to achieve such differentiation, it is crucial to understand the impact exerted on the determination of OPEs by the procedures used to isolate MPs from sediments - e.g., will the use of Nile Red dye chemically affect OPEs and/or introduce chemical interferences? Although not much has been done in freshwater environments, a few studies have looked at the presence of plastic additives in MPs isolated from marine environments. (Hirai et al., 2011, Fries et al., 2013, Rani et al., 2015) identified MP types and associated organic plastic additives (OPAs) following their extraction from sediments using the density separation approach. QA/QC measures such as the use of metal spoons, covering all materials with aluminium foil after each step and rinsing of equipment with ultra-pure water were reported, but no mention was made of the possible effect of MP extraction procedures on the analysis of OPAs associated with MPs. In view of this, we have examined a cross section of recent studies on microplastic extraction from sediments with a view to identifying possible sources of OPE contamination to isolated MPs subsequently analysed for these and/or related additives (Table 1.5).

Table 1. 5. Examples of procedures adopted to measure MPs in sediment and possible impacts on the analysis of OPEs and/or related MP additives.

Reference	**Sampling method**	**Extraction method**	**Clean-up**	**Identification**	**QA/QC**	**Possible source of OPEs/other additives**
(Wen et al., 2018)	A shovel was used to collect samples into aluminium foil and kept at 5 °C.	Density Separation	Digestion with 30% H_2O_2 and Fe (II) solution (catalyst)	micro-Raman spectroscopy	The workspace was cleaned with 70% alcohol before. All pieces of apparatus were rinsed three times with distilled water and covered with aluminium foil. Blank tests were used.	No obvious source was identified but it is highly recommended that procedural blanks are incorporated as there is a possibility of contamination from indoor air.
(He et al., 2018b, Zhou et al., 2018, Liu et al., 2018a)	Clean stainless-steel shovels and spoons were used.	Air flotation and density separation using saturated sodium chloride solution	Use of 30% H_2O_2 for 72 h at 50 °C was the most favoured digestion method.	Visual identification under an optical microscope and confirmation of MPs using micro-Fourier transform infrared (μ-FT-IR) and Raman spectroscopy	All materials thoroughly cleaned and covered with aluminium foil. Lab coats always worn during analysis. Blank tests conducted for MPs.	All studies only used blanks to monitor background contamination by MPs. A total of 33 fibres were extracted from one of the blanks and another study excluded fibres from the study entirely as they acknowledged that atmospheric contamination of samples with fibres from the air was unavoidable.
Jiang et al., 2018	Sediment (0–2 cm) from lakeshore line was collected with a stainless-steel shovel.	Density separation was used	H_2O_2 (30%, v/v) catalysed with ferrous sulfate.	microscopic identification and Raman Spectroscopy	All equipment was pre-cleaned three times by ultrapure water and wrapped in aluminium foil when not in use. Blank tests were used to determine the background values of contamination of MPs from the laboratory	No obvious source was identified but it is strongly recommended procedural blanks be incorporated as there is a possibility of contamination from indoor air.
Wang et al., 2019	For surface sediments stainless-steel shovel was used. A plexiglass tube was used for vertical core.	Sediments were dried at 60 °C overnight. NaI solution used followed by sieving with a steel sieve. The process was repeated three times.	30% H_2O_2 and 65% HNO_3 (1:3, v/v) for two days	FTIR analysis SEM	All liquids used were filtered with 0.45 μm membrane filters. All glass containers were covered by aluminium foil, and a cotton laboratory coat and nitrile gloves were worn. Moreover, the entire process of analysis and identification was conducted by one person in a closed room, with blank experiments conducted to detect any ambient microplastic contamination	No obvious source was identified but it is highly recommended procedural blanks be incorporated as there is a possibility of contamination from indoor air.
(Yuan et al., 2019b)	Sediment was collected from boat using a Van	A density separation was employed	Digestion using 30%	Visual identification as well as Stereoscopic and	Cleaning of tools with filtered pure water before sampling.	No obvious source was identified but it is highly recommended procedural blanks be incorporated

	Veen grab sampler.		H_2O_2 overnight. The digested solution was diluted and filtered using a 0.45 µm filter.	Raman analyses were employed for identification/ Quantification	Nitrile gloves and a cotton lab gown worn. All solutions filtered through a 0.45 µm filter before use. Method blank tests were carried out in the laboratory. The experiment was repeated three times.	as there is a possibility of contamination from indoor air.

1.7 Analysis of OPEs in sediment.

OPEs have been studied extensively in several environmental media but only few studies have looked at OPEs in sediments. This is despite the fact that sediments are generally seen as likely sinks for organic pollutants (Yadav and Devi, 2020). One major source of these OPEs to the aquatic environment is wastewater treatment plants and the fact that the same is the case with microplastics makes an investigation into possible correlation between OPE concentration and microplastic count in sediments a potential research interest (Chokwe and Mporetji, 2019, Woudneh et al., 2015, Horton et al., 2017a, Dris et al., 2015).

Different methods have been employed in the analysis of sediment samples for OPEs (Wang et al., 2018b, Liu et al., 2018b). GC-MS was used by Yadav and Devi (2000) to analyse sediment samples for 8 OPEs (TCEP, TCIPPs, TDCIPP, TNBP, TEHP, TPHP, EHDPHP and TMPPs) and the concentrations obtained are as shown on Table 1.6. Samples were Soxhlet extracted using dichloromethane (DCM) for 24 hours and target OPEs were eluted with 20 mL ethyl acetate. The QA/QC measures include soaking all glassware in 5% KOH and 95% ethanol solution before washing them with Milli-Q water and then DCM and hexane. Use of field and laboratory blanks as well as a recovery of 108-124 % was reported (Yadav and Devi, 2020).

In what was described as "the first study on organophosphate ester (OPEs) flame retardants and plasticisers in the sediment of the Great Lakes", concentrations of 14 OPEs (TMP, TEP, TCEP, TPrP, TCPP, TDCP, TiBP, TPHP, TNBP, CDPP, TBEP, TCrP, EHDPP, TEHP) were reported for Lakes Ontario, Michigan, and Superior. The samples were analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) following extraction by ultrasonication and clean-up using solid phase extraction cartridges (Cao et al., 2017). The concentration of total OPEs (Σ_{14}OPEs) averaged 2.2, 4.7, and 16.6 ng/g (dw) in Lakes Superior, Michigan, and Ontario, respectively. QA/QC involved the analysis of field blanks and procedural blanks with every batch of samples (Cao et al., 2017).

Wang et al., reported a total concentration (∑OPEs) of 2.82–47.5 ng/g dw following analysis of sediment samples from Taihu Lake for eleven (11) OPEs (tri(isopropyl) phosphate (TIPP); TNBP and tri(isobutyl) phosphate (TIBP), which are two isomers of tributyl phosphate (TBP); TBOEP; tris(chloroethyl) phosphate (TCEP); TCIPP; tris(1,3-dichloropropyl) phosphate (TDCIPP); triphenyl phosphate (TPHP); 2-ethylhexyl diphenyl phosphate (EHDPP); tris(2-ethylhexyl) phosphate (TEHP); and tri(methylphenyl) phosphate (mixture of isomers) (TMPP)) (Wang et al., 2018b). Based on the work of Yang, samples were analysed using an ultraperformance liquid chromatography-tandem electrospray-triple quadrupole mass spectrometry system (UPLC-MS/MS) (Yang et al., 2014). QA/QC measures employed include the use of multi-level calibration curves, use of method blanks and solvent blanks in every batch of twelve samples and calculation of recoveries (74.4–124%). Furthermore, the method detection limits (MDLs) for the target compounds in sediment was in the range of 0.004–0.8 ng/g dw (Wang et al., 2018b).

Giulivo et al., 2017 analysed sediment samples from three European rivers (the Evrotas (Greece), the Adige (Italy) and the Sava (Slovenia, Croatia, Bosnia and Herzegovina and Serbia)) using gas chromatography, coupled to tandem mass spectrometry (GC–MS-MS). The levels of OPFRs in sediment samples ranged between 0.31 and 549 ng/g dw, and the target OPFRs were (EHDP, IDPP, TBP, THP, TPHP, TPPO, TBOEP, TCEP, TClPP, TDCPP, TEHP, IPPP, TMCP) (Giulivo et al., 2017).

Table 1. 6. Concentrations of target OPEs reported in previous studies.

Study			**Xing et al., 2018**	**Wang et al., 2018b, Liu et al., 2018b**	**Giulivo et al., 2017**	**Giulivo et al. 2017**	**Giulivo et al. 2017**	**Cao et al., 2017**	**Cao et al., 2017**	**Cao et al., 2017**	**Yadav and Devi, 2020**
Location			**Luoma Lake, China**	**Lake Taihu, China**	**Evrotas River (Greece), the**	**Adige River (Italy)**	**Sava River (Slovenia, Croatia, Bosnia and Herzegovina and Serbia)**	**Lakes Superior**	**Lake Michigan.**	**Lake Ontario**	**Kathmandu valley, Nepal.**
	Target OPEs and Concentration (ng/g dw)										
	TNBP	Minimum	<LOD	0	ND	NQ	ND	NQ	ND	0.22	5.04
		Midian	0.02	0	1.7	2.25	6.835	0.15	0.4	1.17	O
		Average	0.02	0	2.39	5.52	7.65	0.34	0.52	1.43	76.41
		Maximum	0.05	0	5.54	5.05	14.2	1.36	1.86	7.22	319.93
	TCEP	Minimum	0.01	<LOD	ND	0.33	ND	ND	ND	ND	10.96
		Midian	0.17	1.8	1.76	1.62	0.56	ND	ND	ND	16.5
		Average	0.38	3	1.78	2.51	0.79	0.15	ND	ND	18.46
		Maximum	1.72	2.8	2.27	3.59	1.59	1.9	NQ	NQ	38.3
	TCIPP	Minimum	<LOD	0.27	ND	0.53	0.24	ND	ND	ND	1.69
		Midian	0.07	0.45	4.44	7.75	5.66	NQ	ND	0.48	38.57
		Average	0.06	1.2	4.59	14.93	6.6	0.15	0.39	0.71	112.59
		Maximum	0.11	10	7.62	53.7	14.7	1.1	1.6	3.37	883.48
	TBOEP	Minimum	<LOD	0	ND	NQ	NQ	ND	ND	ND	-
		Midian	0.001	0	1.3	0.94	2.26	ND	0.48	4.66	-
		Average	0.001	0	1.47	2.36	3.16	0.03	1.52	7.29	-
		Maximum	0.001	<LOD	3.35	9.98	11	0.46	9.1	23.74	-
	EHDPP	Minimum	<LOD	<LOD	ND	4.27	1.86	ND	ND	ND	33.93

		Midian	0.06	0.32	4.54	13.65	4.36	ND	ND	0.24	131.25
		Average	0.06	0.38	4.82	37.31	4.76	0.01	0.03	0.31	135.64
		Maximum	0.14	0.94	6.39	288	8.48	0.19	0.32	1.44	417.6
	TMTP1	Minimum	<LOD	<LOD	ND	4.27	1.86	ND	ND	ND	-
		Midian	0.06	0.32	4.54	13.65	4.36	ND	ND	0.24	-
		Average	0.06	0.38	4.82	37.31	4.76	0.01	0.03	0.31	-
		Maximum	0.14	0.94	6.39	288	8.48	0.19	0.32	1.44	-
	TPHP	Minimum	<LOD	<LOD	ND	NQ	ND	-	ND	ND	3.33
		Midian	0.01	6.6	0.3	0.47	ND	ND	NQ	1.38	20.02
		Average	0.01	1.7	0.36	0.86	ND	0.04	0.17	2.62	39.59
		Maximum	0.03	55	0.67	9.69	ND	1.04	0.91	9.03	130.09
	TDCIPP	Minimum	-	<LOD	ND	NQ	ND	-	-	-	3.89
		Midian	-	0	1.41	1.18	0.36	-	-	-	5.31
		Average	-	0.095	1.63	2.31	0.36	-	-	-	5.66
		Maximum	-	4.3	2.96	0.39	0.39	-	-	-	8.93

<LOD: Below Limit of Detection
NQ: Detected but not quantified
ND: Not detected

1.8 Data and research gaps.

The role of MPs as conduits of chemical pollutants, widely used as plastic additives, to the freshwater environment is poorly understood. While there seems to be an apparent correlation between concentrations of MPs and chemical pollution in the few studies that have addressed this topic (Foshtomi et al., 2019, Forgione et al., 2023), it is not clear if MPs act as a source or a sink of lipophilic chemicals to the aquatic environment. In particular, nothing is known about the partitioning of chemical plastics additives in sediments between sediment particles and the MPs themselves. Furthermore, no research has looked specifically at possible correlation between MPs and OPEs.

Further studies are required to fully understand the role of WWTPs in environmental pollution, especially long-term studies that would also take into consideration the role of spatiotemporal factors in the distribution and characteristics of these MPs and associated chemical additives. The relationship between MPs and organic pollutants such as OPEs which are widely used as plastic additives is poorly understood. Given the potential implications of this for the bioavailability of such chemical additives, this is a significant research gap that should be addressed. Specifically, the impact on additive bioavailability of several factors including the type of plastic polymer, the concentration and physicochemical properties of chemical additives, the organic content of sediment and the temperature, is unknown. Studies are urgently required to understand the fate and behaviour of hazardous chemical additives present in MPs and the factors influencing their release to the freshwater environment. This also means that while working on biodegradable plastics and other ways of promoting “Reduction, Re-use and Recycling” of plastics; sources of microplastic pollution and the use of chemical additives should be carefully considered because the facilitated degradation of these biodegradable plastics may provide a further source of chemical pollution to the environment. The potential impact of colour on the rate of ingestion of MPs by aquatic organisms, as recommended by

Wang et al. (2017), is an area of particular interest. If it is proven that microplastics of certain colour are ingested more than others, then production of plastics with the least favoured colour could reduce ingestion of MPs by aquatic organisms and consequently their magnification through the food chain.

Finally, more studies on temporal/spatial trends of MPs (and associated additives such as OPEs) are required, to enable modelling and prediction of future trends and associated risks to the aquatic environment. This will also provide valuable information for shaping pollution control strategies and the effect of hydrodynamic conditions on the distribution of MPs in freshwater systems.

1.9 Hypotheses, project aim and objectives.

The overriding hypothesis to be tested by this study is that there is a correlation between MPs abundance and concentrations of OPEs in sediment samples upstream and downstream of WWTPs as well as in sediment samples from different parts of the world. It is expected that such correlation will be established in form of both seasonal and spatial trends. As well as measuring MPs and OPEs in freshwater sediments from a range of locations across the world; this study also quantifies MPs and OPEs in freshwater sediment samples from 3 rivers and an urban canal in the UK West Midlands on a monthly basis over a 12-month period. UK riverine samples were collected both upstream and downstream of WWTP discharge points. The objectives of this thesis are thus to:

1. Develop and validate analytical methodology for measuring the concentrations of OPEs in sediments.

2. Develop and validate analytical methodology for quantifying and characterising MPs in sediments.

3. Quantify and characterise MPs in sediment samples from 64 locations around the world and 4 freshwater systems within the UK. These sediment samples will also be analysed for OPEs.

4. To compare the concentrations of OPEs as well as the abundance and characteristics of MPs in all study locations and,

5. To identify and discuss any possible correlations and trends.

CHAPTER 2

2.1 Materials and Methods

To test the hypotheses outlined in the previous chapter, sediment samples were collected and analysed for MPs and OPEs. The surface sediment samples were collected from 14 countries using traditional methods and the analytical methodology for quantification and classification of MPs consists of three main steps (sampling, extraction, and identification / quantification). The determination of OPEs consists of four major steps (sampling, extraction, clean-up, and analysis). This chapter covers a detailed discussion of the materials and methods used for collecting sediment samples, extracting microplastics from the sediments and analysing the sediments for OPEs (Figure 2.1). It presents the validation of the applied analytical procedures and the quality assurance/ quality control measures employed to guarantee the reliability of generated data.

2.1.1 Chemicals

All solvents used during this study (n-hexane (HEX, 95%), ethyl acetate (ETAC, 99.8%), dichloromethane (DCM, 99.8%), Toluene (TOL, 99.5%) and acetone (ACE, 99.8 %)) were HPLC grade and were purchased from Fisher Scientific (Loughborough, UK) and Sigma-Aldrich (St Louis, MO, USA). Individual chemical standards of 99.9% purity for eight native OPEs (TNBP, TCEP, TCIPP, TDCIPP, TBOEP, TPHP, EHDPP and TMTP), recovery determination standard (RDS): 2,3,4,6- tetrachlorobiphenyl (PCB-62) and isotope-labelled internal standards (TCEP-d_{12}, TDCIPP-d_{12}, and TPHP-d_{15}) were purchased from Wellington laboratories, (Guelph, ON, Canada). Standard reference material SRM 1944 (New York/New Jersey waterway sediment) was purchased from the US National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Hypersep Florisil® SPE cartridges were purchased from Thermo Scientific (Rockwood, USA), and the nitrogen gas used for solvent evaporation was purchased from BOC gases, United Kingdom. Chemicals used for MP analysis: Zinc chloride, (reagent grade, ≥98%), Hydrogen peroxide solution 30 % (w/w) and Nile red ≥97.0% (HPLC grade) were all purchased from Sigma- Aldrich.

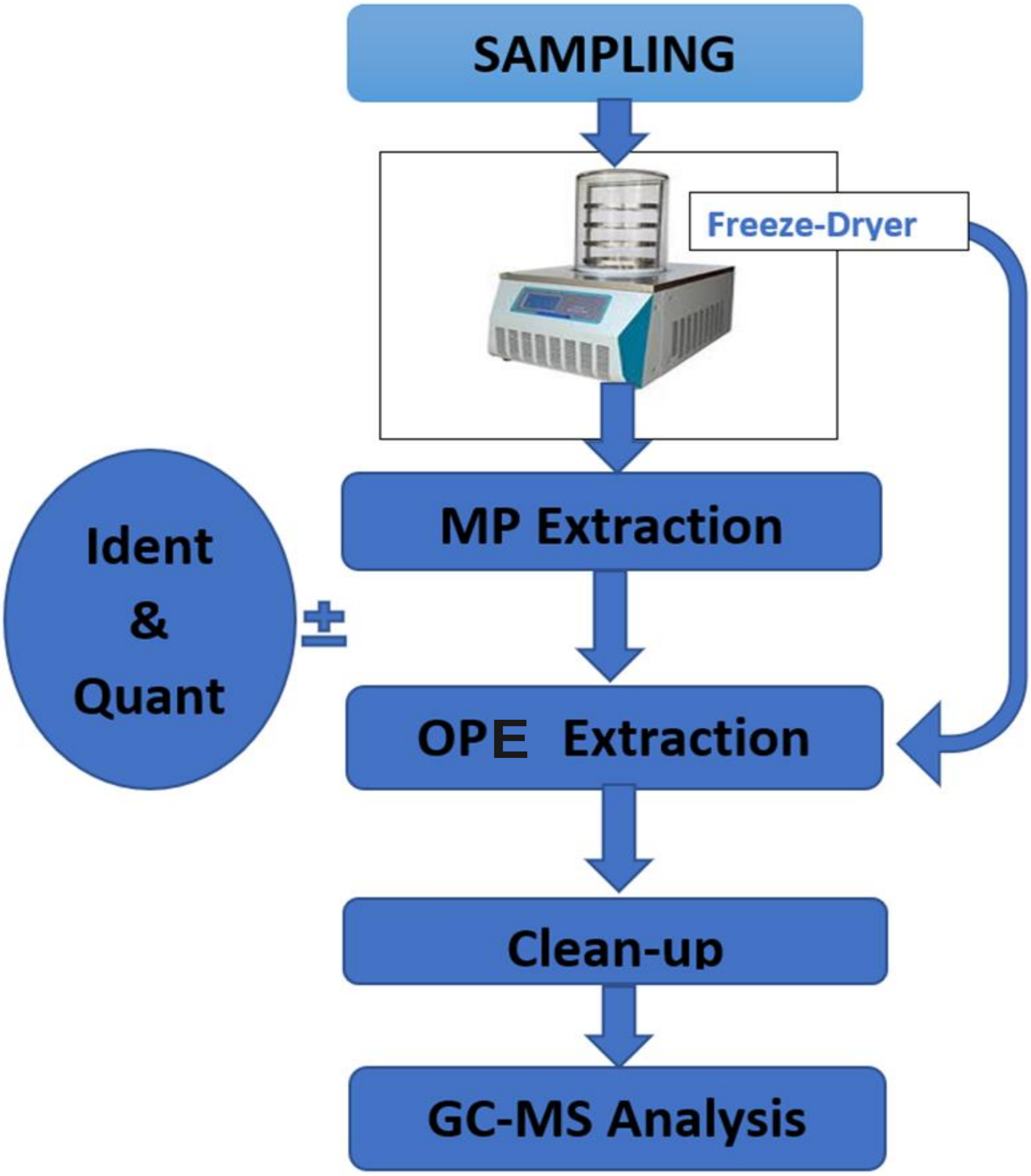


Figure 2. 1. Flow chart of the procedures for analysis of sediment samples for MPs and OPEs Flow chart of the procedures for analysis of sediment samples for MPs and OPEs.

2.1.2 Sampling

Three sets of sediment samples were collected and analysed during this study, comprising:

1. Monthly samples from the Worcester and Birmingham Canal at University rail station in the West Midlands.
2. Monthly samples from upstream and downstream of WWTPs along the River Tame in Water Orton, the River Severn in Worcester, and the River Sowe in Coventry (all in the West Midlands)
3. International samples from 64 sampling points in 14 countries (Nepal, Greece, Poland, Swaziland, Spain, Mozambique, Switzerland, Canada, Malaysia, Republic of Korea, United Kingdom, Chile, Nigeria, and India).

2.1.2.1. Sampling Locations

Sediment samples were collected upstream and downstream of the effluent discharge points of 3 WWTPs in the West Midlands region of England and from the Worcester Birmingham Canal near the University Station in Birmingham (Table.2.0). For reasons of confidentiality, the WWTPs will be referred to as WWTP1, WWTP2 and WWTP3 as shown in Figure 2.2. WWTP1 serves a population of 2.5 million people within the Birmingham area, WWTP2 serves a population of 430,470 people within the Coventry area, while WWTP3 serves a population of 50,000 to 200,000 people within the Worcester area. The river levels range between 0.03 m and 2.84 m for WWTP1, 0.06 m and 1.44 m for WWTP2 and 0.55 m and 3.35 m for WWTP3 (Riverlevelsuk, 2022) (Figure 2.3) and the sampling points were all located within urban areas. Accessibility and distance from the effluent discharge points were among the key factors that informed the choice of sampling point and as much as possible, samples were collected from the same point all year round.

The international sediment samples were all (except for samples from Nigeria) kindly provided by the Leverhulme Trust-funded 100 Plastic Rivers project at the University of Birmingham, UK (https://www.birmingham.ac.uk/research/water-sciences/projects/plastic-rivers.aspx). The samples were collected from different locations across 5 continents (Africa, Asia, Europe, North America, and South America) and from 29 points along the Ganges River from Devprayag to Noorpur. The GPS coordinates and other details of the sampling points are as enumerated on Table 2.1.

Table 2. 1. GPS coordinates of monthly sample locations.

Site name	**Latitude**	**Longitude**
River Severn (Upstream)	52°10'36.501"N	2°13'31.603"W
River Severn (Downstream)	52°10'34.061"N	2°13'28.86"W
River Tame (Upstream)	52°31'13.273"N	1°44'42.534"W
River Tame (Downstream)	52°31'14.212"N	1°44'38.485"W
River Sowe (Upstream)	52°21'50.151"N	1°30'53.143"W
River Sowe (Downstream)	52°21'30.223"N	1°30'50.768"W
Worcester and Birmingham Canal	52°27'3.58"N	1°56'11.91"W

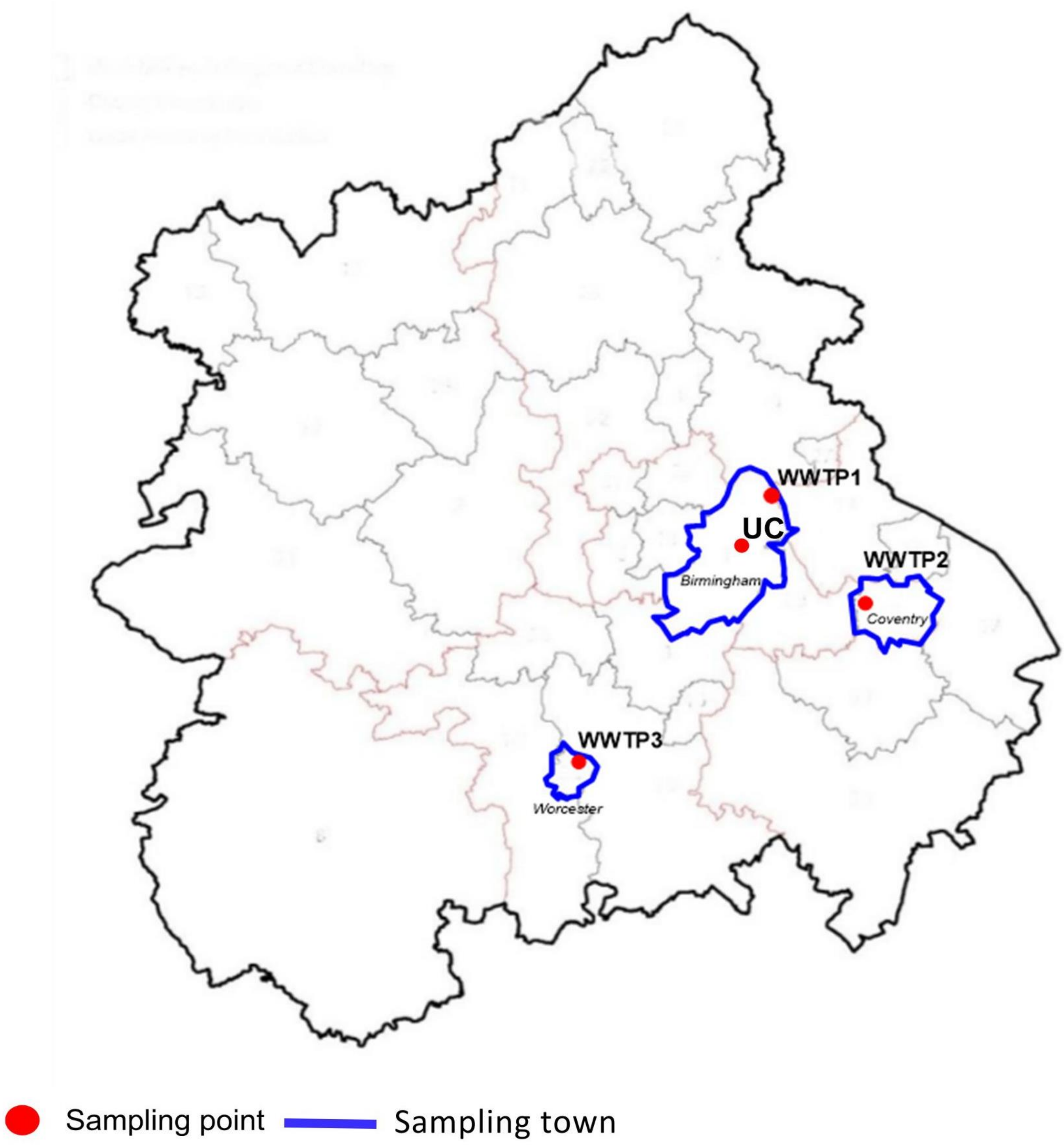


Figure 2. 2. Map of West Midlands showing sampling locations.

Table 2. 2. GPS coordinates of international samples locations.

Continent	Country	River	Year of sampling	Latitude	Longitude
Africa	**Mozambique**	Limpopo River	2021	22°40'37.9"S	31°49'14.9"E
		Mkomati River	2021	25°11'40.2"S	32°30'20.3"E
	Nigeria	River Niger UPP	2021	7° 46' 46.61"N	6° 44' 37.24"E
		River Benue UPP	2021	7°45’37.56N”	6°45’13.26E”
		River Benue Down	2021	7°44’47.93N”	6°45’37.66E”
	Swaziland	Mbuluzi River	2021	26°10'43.8"S	32°02'58.0"E
Asia	**Malaysia**	Klang River1	2019	3°02'41.2"N	101°36'22.0"E
		Klang River2	2019	3°02'48.4"N	01°24'42.4"E
	Nepal	Koshi River	2020	26°06'41.8"N	86°29'37.6"E
		Kali Gandaki River1	2020	28°30'09.6"N	83°39'24.8"E
		Kali Gandaki River2	2020	28°36'05.1"N	83°38'54.8"E
	South Korea	Geum River	2019	36°01'20.5"N	26°45'31.2"E
	India	Ganges River UPP 1	2019	28°14’47.40N”	78°22’9.48E”
		Ganges River UPP 2	2019	29°56’32.35N”	78°10’5.05E”
		Ganges River UPP 3	2019	30°9’6.80N”	78°35’59.03E”
		Ganges River UPP 4	2019	30°7’21.29N”	78°18’40.90E”
		Ganges River UPP 5	2019	26°52’57.32N”	80°6’6.55E”
		Ganges River UPP 6	2019	27°24’3.67N”	79°37’39.90E”
		Ganges River UPP 7	2019	29°22’19.42N”	78°229.26E”
		Ganges River UPP 8	2019	28°45’18.86N”	78°8’47.29E”
		Ganges River UPP 9	2019	30°8’41.14N”	78°35’52.22E”
		Ganges River UPP 10	2019	30°4’23.09N”	78°30’5.33 E”
		Ganges River MID 1	2019	25°22’3.94N”	85°59’49.42 E”
		Ganges River MID 2	2019	25°36’51.05N”	85°12’8.24 E”
		Ganges River MID 3	2019	25°39’33.48N”	84°56’0.71 E”
		Ganges River MID 4	2019	25°34’26.36N”	83°34’37.45 E”
		Ganges River MID 5	2019	25°36’51.05N”	85°12’8.24 E”
		Ganges River MID 6	2019	25°40’3.58N”	85°9’37.55 E”
		Ganges River MID 7	2019	25°41’18.10N”	85°11’25.19 E”
		Ganges River MID 8	2019	25°39’3.53N”	85°4’44.18 E”
		Ganges River MID 9	2019	25°14’52.48N”	84°39’15.48 E”
		Ganges River MID 10	2019	25°41’41.14N”	85°11’46.36 E”
		Ganges River Low 1	2019	25°25’29.75N”	87°3’34.92 E”
		Ganges River Low 2	2019	24°50’2.00N”	87°56’45.49 E”
		Ganges River Low 3	2019	25°22’6.60N”	86°32’35.84 E”
		Ganges river Low 4	2019	24°41’10.18N”	87°57’22.32 E”
		Ganges River Low 5	2019	25°20’25.37N”	87°36’56.45 E”
		Ganges River Low 6	2019	25°17’26.77N”	86°19’36.98 E”
		Ganges River Low 7	2019	24°43’5.23N”	87°54’51.41 E”
		Ganges River Low 8	2019	24°6’10.80N”	88°14’42.86 E”
		Ganges River Low Low 9	2019	25°19’25.46N”	87°42’49.18 E”
		Ganges River Low 10	2019	25°2’56.65N”	87°55’24.64 E”
Europe	**Switzerland**	Thur River 1	2020	47°11'16.1"N	9°16'09.4"E
		Thur River 2	2020	47°14'42.9"N	9°10'25.1"E

		Thur River 3	2020	47°17'54.3"N	9°05'22.7"E
		Thur River 4	2020	47°21'28.5"N	9°05'03.3"E
		Thur River 5	2020	47°26'49.4"N	9°03'50.1"E
	Poland	Vistula River 1	2020	52°23'17.8"N	20°24'33.7"E
		Vistula River 2	2020	53°15'17.1"N	18°17'06.9"E
		Vistula River 3	2020	53°54'11.5"N	18°52'54.8"E
	Greece	Kifisos River 1	2019	37°59'52.5"N	23°41'47.4"E
		Kifisos River 2	2019	37°57'24.9"N	23°40'28.4"E
	Spain	Canoves		41°40'55.05"N	2°21'19.76"E
		Francoli River	2020	41°17'06.1"N	1°11'36.6"E
	UK	River Irwell C	2019	53°35'59.30"N	2°18'12.80"W
		River Itchen	2019	50°59'24"N	1°20'20.24"W
		River Irwell Site B	2019	53°36'34.30"N	2°18'30.30"W
		River Irwell Site A	2019	53°36'34.49"N	2°18'0.5"W
		Chicheley Brook Site 1	2019	52°4'45.41"N	0°37'40.12"W
		Chicheley Brook Site 2	2019	52°437'47.32"N	0°37'39.79"W
South America	**Chile**	Tres Bazos	2020	53°16'20.71"S	70°56'40.99"W
North America	**Canada**	Thames Ontario (Kintore Creek East)	2020	43°9'46.30"N	81°1'26.11"W
		Thames Ontario (Dingman Creek)	2020	42°54'21.09"N	81°16'13.69"W
		Thames Ontario (Kintore Creek West)	2020	43°9'29.19"N	81°3'14.29"W

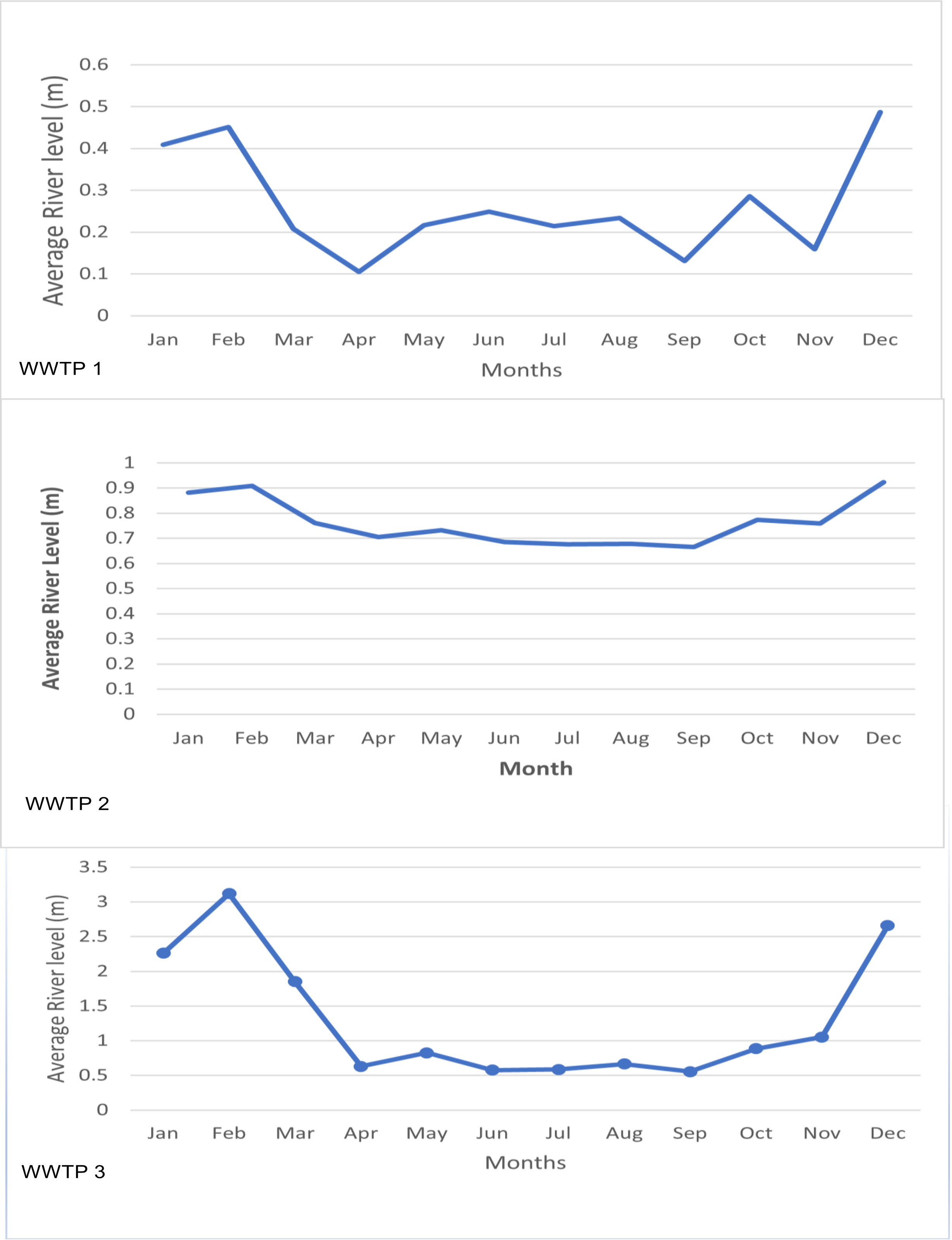


Figure 2. 3. Monthly River level at UK sampling sites (Riverlevelsuk, 2022).

2.2 Monthly samples from Worcester-Birmingham canal, River Tame, River Severn, and River Sowe.

Sediment samples were collected before and after the effluent discharge points for all 3 WWTPs and at a point near the University station over 12 months (December 2019, January 2020, February 2020, March 2020, April 2021, May 2021, June 2021, and July to November 2020). The April, May, and June sampling events were moved to 2021 because of a COVID 19 lockdown and this then gave us an opportunity to compare the 3 months before lockdown to the 3 months after. At the sampling points, a stainless-steel extensible soil auger was used to collect sediment samples up to a sediment depth of 6 cm. The sediment samples were then stored in pre-rinsed glass jars and covered with aluminium foil for transportation to the laboratory at the University of Birmingham for analysis. During the sampling campaign, use of synthetic materials was avoided where possible and specific note was made of any relevant feature such as the presence of visible plastics and relevant human activities. Also, field blanks (a pre-rinsed jar left open while the sediment sample is being collected) were collected at each sampling point.

Figure 2. 4. Sampling at the Severn and Sowe Rivers.

2.3 International Sediment samples from Africa, Asia, Europe, North America, and South America.

Sediment samples were collected at all locations following a guideline that was prepared and sent to different partners in the locations of interest. According to the guideline, sediment samples were collected using metal spoons, homemade metal corer (tin can with both ends open) or any metal (non-plastic) shovel from the top 5 cm. Samples were stored in 225 mL glass jars and covered tightly with a parafilm awaiting transportation to the laboratory for analysis. Polyester and other plastic polymer textiles as well as synthetic clothing were avoided during sampling campaigns. Field blanks were collected, and this involves a pre-rinsed jar left open while the sediment sample is being collected in other to account for potential atmospheric contamination.

2.4 Extraction of Microplastics from sediments.

Sediment samples were freeze dried for 48 hours while the international samples were oven dried at 50 ˚C for 72 hours. The freeze drier and oven were both cleaned, and the glass jars were all covered with clean aluminium foils. Microplastic extraction from sediments was carried out using the density separation method which takes advantage of the low density of most plastic materials (0.9 -1.55 g/cm^3) to separate them from other sediment components. Previous studies using this method have reported recovery rates as high as 100% (Mai et al., 2018). All reagents including $ZnCl_2$ solution and distilled water were first filtered through a 0.7 µm glass fibre filter (GF/F, Whatman) before use to prevent microplastic contamination.

2.4.1 Density separation using the SMI unit.

The density separation phase of the extraction was carried out using the Sediment Microplastic Isolation (SMI) unit (Figure 2.5) (Coppock et al., 2017) with slight modification based on the work of Nel et al, 2019 (Nel et al., 2019). The SMI units were washed and rinsed three times with distilled water and then filled with 700mL of Zinc chloride solution ($ZnCl_2$; 1.5 g cm^{-3}) (Nel et al., 2019). This was followed by the addition of 30 to 50 g of dried sediment samples along with a magnetic stirring bead. The SMI unit was then covered with a clean aluminium foil and placed on a magnetic stirrer and allowed to stir for 10 minutes at a rotation frequency of 5 to 8 revolutions per minute (rpm). Thereafter, the unit was left to settle for 7 to 12 hours depending on the sediment sample. This is because sediments with high proportion of fine particles took longer to settle. Once the denser particles settle out, the supernatant was poured over a 63 µm stainless steel mesh into a beaker for filtration and re-use. The upper chamber of the SMI unit was also removed and rinsed over the mesh to ensure that all particles clinging to the surface of the equipment are collected.

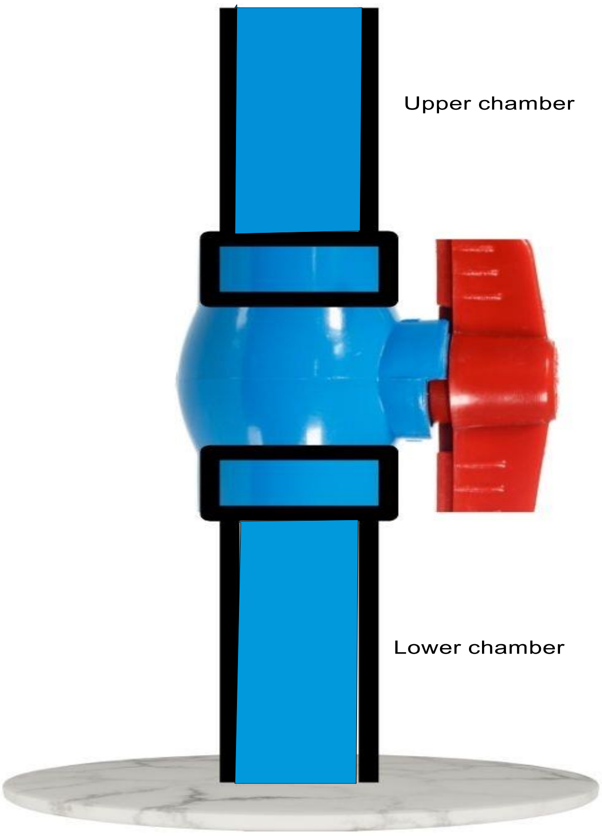


Figure 2. 5. An SMI unit showing lower and upper chambers.

2.4.2 Digestion of organic components

The organic components in the filtered samples were eliminated using the digestion method of (Claessens et al., 2013).The mesh was rinsed with 30 % hydrogen peroxide (H_2O_2) into 250 mL glass beakers pre-washed with distilled water. The beakers were then covered with aluminium foil and left in an oven at 60 ˚C for 48 hours. Upon expiration of 48 hours, the content of the beaker was poured over a 63 µm stainless steel mesh and the beakers properly rinsed over the mesh to ensure that all particles clinging to its surface are collected. The microplastics on the mesh were carefully rinsed into clean petri dishes and placed in the oven to dry in preparation for the identification and quantification phase.

Figure 2. 6. Close up photos of some of the steps involved in density separation using the SMI unit.

2.4.3 Identification and Quantification of Microplastics

Identification and quantification were done using the Nile Red method (Shim et al., 2016, Erni-Cassola et al., 2017) with slight modification according to Nel et al, 2019 and fluorescent microscopy. 1 mg mL^{-1} Nile Red (NR; Cas No: 7385-67-3) stock solution was prepared by adding 20 mg of the extract to a small quantity of acetone (Cas No: 67-64-1) in a 20 mL volumetric flask and completing to the mark with acetone. The solution was then filtered using a sterile 0.22 µm Polyethersulfone (PES) syringe filter into a clean glass screw-top vial. 100 µL of NR stock solution (1 mg mL^{-1}) was used per 20 mL deionised water to make a final concentration of 5 µg mL^{-1}, which was used to stain the microplastics in a petri dish before leaving the petri dish in an oven at 50 degrees to dry.

Once the microplastics were dry, a Nikon SMZ-1000 stereo microscope fitted with a Nikon Intensilight C-HGF1 long-life mercury light source and a Nikon DS-Fi1 5-megapixel digital microscope camera head suitable for both brightfield and fluorescence applications was then used to Identify and quantify the microplastics (Figure 2.7).

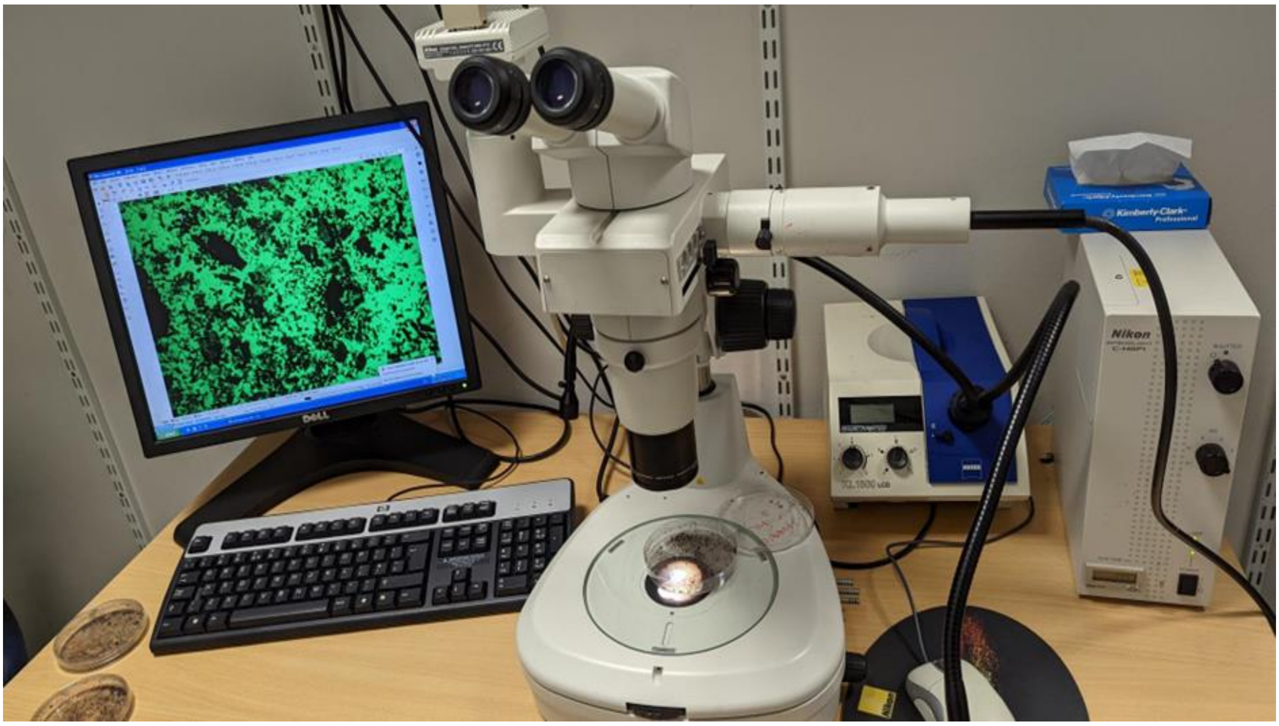


Figure 2. 7. Nikon SMZ-1000 stereo microscope fitted with a Nikon Intensilight C-HGF1 long-life mercury light source and a Nikon DS-Fi1 5-megapixel digital microscope camera head.

2.4.4 Quality Assurance/Quality Control (QA/QC).

QA and QC measures employed for this phase of the study include a strict use of non-plastic sampling, storage, and processing tools (Hanke et al., 2013). Use of 100% cotton lab coats as well as nitrile gloves was also ensured. All apparatus was rinsed 3 or 4 times with distilled water (Wen et al., 2018, Jiang et al., 2018, Yuan et al., 2019), the workspace was cleaned with 70% alcohol (Wen et al, 2018) and all liquids and solutions were filtered through 0.45 µm filters before use (Wang et al., 2019, Yuan et al., 2019) All equipment and glass containers were covered with aluminium foil (Wang et al, 2019). Procedural blanks (using pure or de-ionized water) as well as field blanks were analysed alongside sample batches to ensure that all sources of background contamination and interferences are properly evaluated.

2.5 Analysis of OPEs in Sediments.

During this study, sediment samples were analysed for 8 OPEs (TCEP, TCIPPs, TDCIPP, TNBP, TEHP, TPHP, EHDPHP, and TMPPs) following the steps outlined in Figure 2.8. The sediment, samples were freeze-dried, ground into powder, and filtered through a stainless-steel sieve of 5 mm mesh size (Hu et al., 2019). Chemicals used were HPLC grade acetone, hexane, ethyl acetate, iso-octane, florisil, and glass wool. TCEP d12, TDCPP d12 and TPHPd15 were used as internal standards.

2.5.1 Sample extraction.

The extraction was done using a method developed by (Brommer et al., 2012) with slight modifications. 1 g of sediment was mixed in a clean dry test tube with 1 g of copper powder and then spiked with 10 ng of internal standard mixture (d_{12}-TCEP, d_{12}-TDCPP and d_{15}-TPHP). The samples were then extracted with 5 mL of hexane:acetone (1:1 v/v) and vortexing for 1 minute before ultrasonicating for 10 minutes at 30 °C. The samples were centrifuged at 3500 rpm for 3 min and the supernatant collected in a clean dry test tube. The steps from extraction with hexane:acetone to collection of supernatant were repeated twice and the combined extracts was evaporated under a gentle stream of nitrogen to approximately 1 mL.

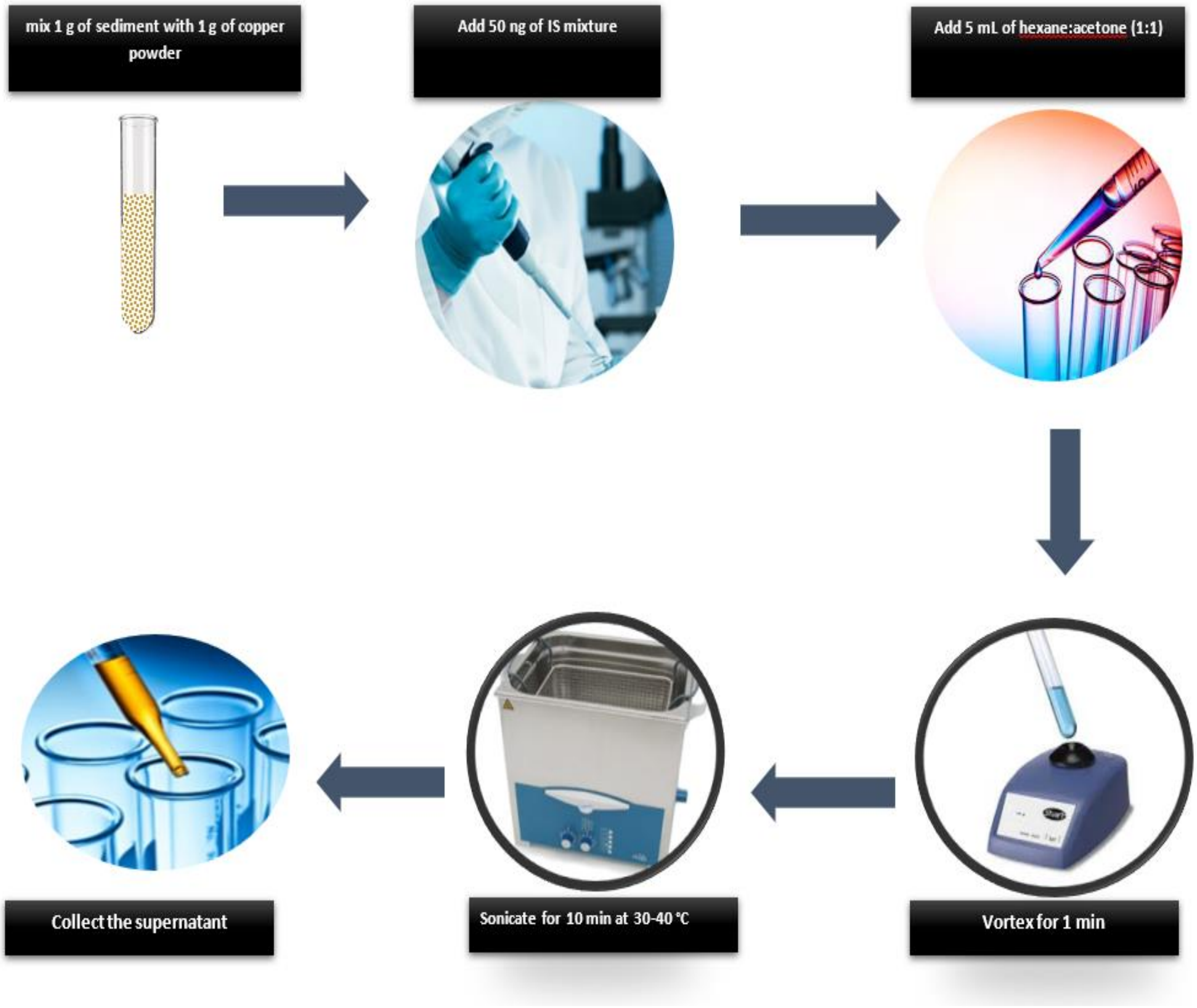


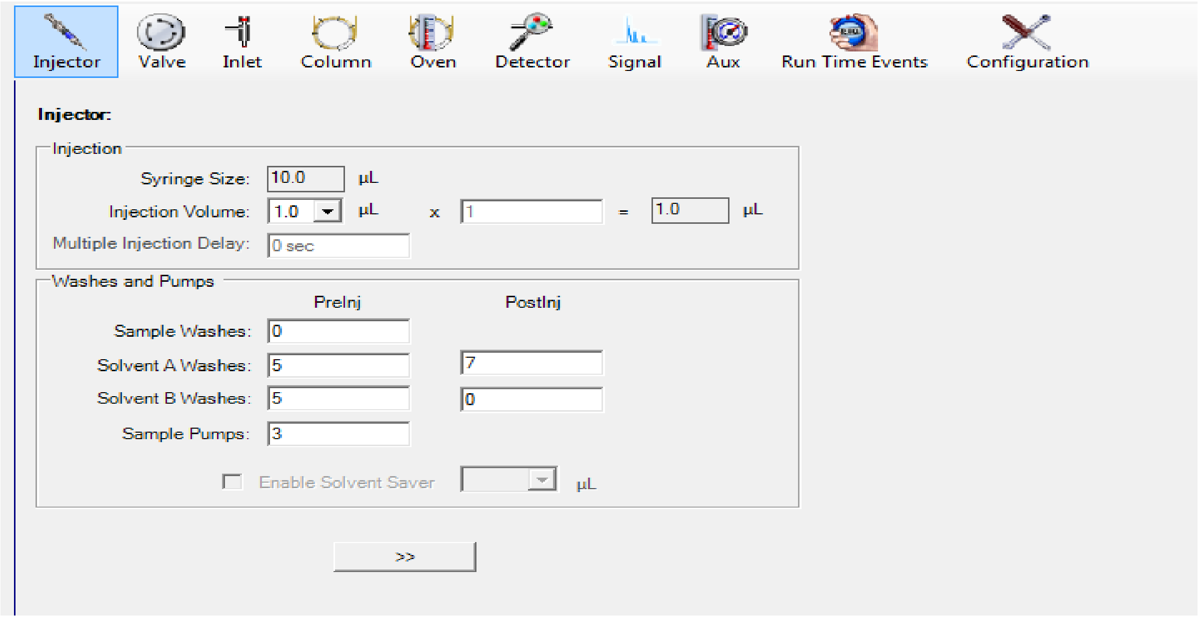
Figure 2. 8. Steps in OPE analysis in sediments.

2.5.2 Extract purification (Clean-Up).

Florisil cartridges were conditioned with 2 x 3 mL of hexane and the crude extracts loaded onto the conditioned florisil cartridges. The cartridge was washed with 10 mL of hexane (to remove interfering lipophilic chemicals) before eluting the target OPEs with 8 mL of ethyl acetate. The eluate was then collected in a clean dry test tube and evaporated under a gentle stream of nitrogen until incipient dryness. The extract was reconstituted in 100 µL of toluene containing 250 pg/µL of PCB 62 as recovery determination standard (RDS) before transferring into a vial with insert and stored in a freezer awaiting injection into the GC-MS.

2.5.3 GC-MS Analysis of OPEs.

Analysis for OPEs was conducted on an Agilent 5975C GC coupled to an Agilent 5975C MSD fitted with a 30 m DB-5 MS column (0.25 mm ID, 0.25 µm film thickness) and operated in electron ionisation mode (EI) (Restek, USA). The carrier gas was helium at a constant flow rate of 1.0 mL/min. The injector temperature was set at 290 °C in split-less mode and the MS operated with a solvent delay of 5 minutes. The temperatures of the ion source, quadrupole and interface were set at: 230 °C, 150 °C and 300 °C respectively. The GC temperature programme was 65 °C, hold for 0.75 min, ramp 20 °C/min to 250 °C, hold for 1 min, ramp 5 °C/min to 260 °C, hold for 0 min, ramp 30 °C/min to 305 °C, and hold for 1 min (Figures 2.8, 2.9 and 2.10). TNBP, TCEP and TCIPP were quantified against d27TNBP, TDCIPP, TPHP, EHDPP and TMPP against d_{12}TPHP while TDCIPP was quantified against d-12-TDCPP. The dwell time for each ion was 15 minutes. Illustrative chromatograms for a mixed internal standard (500pg/uL) and procedural blank are shown in Figures 2.9 and 2.13:



Injector:

Injection

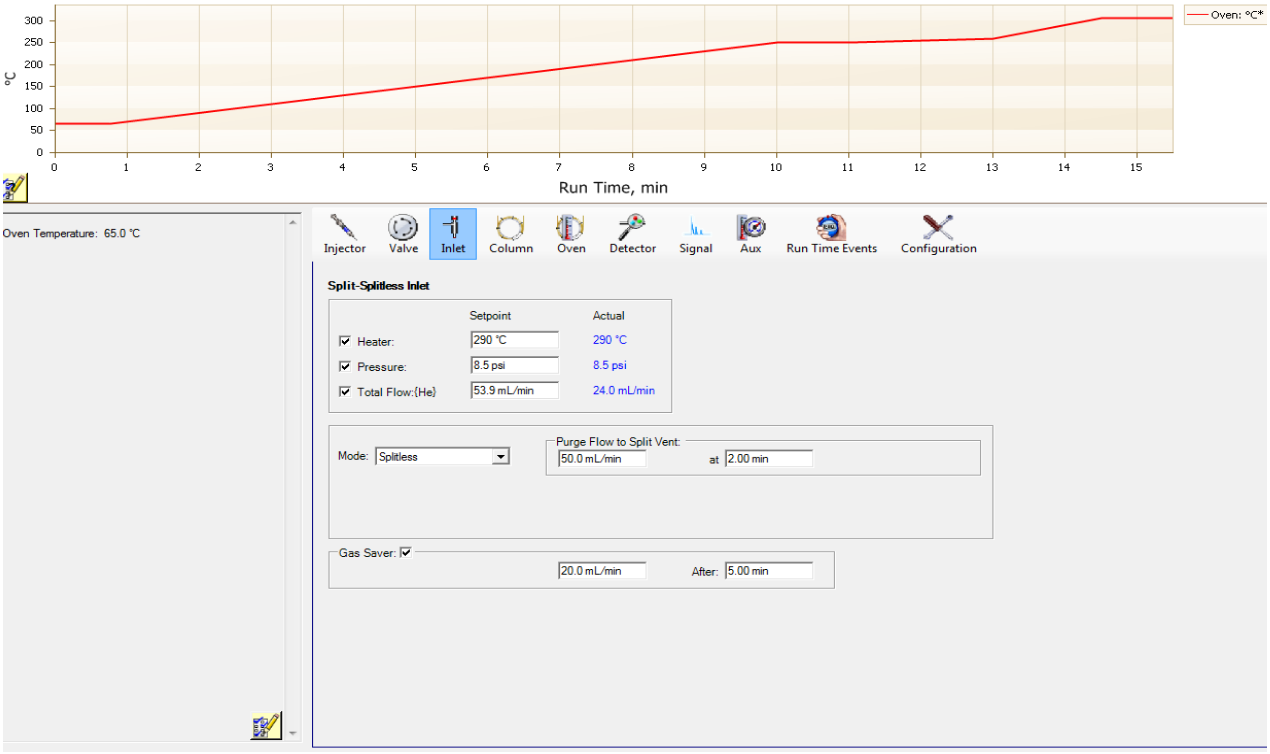
Syringe Size: 10.0 µL
Injection Volume: 1.0 µL x 1 = 1.0 µL
Multiple Injection Delay: 0 sec

Washes and Pumps

	PreInj	PostInj
Sample Washes:	0	
Solvent A Washes:	5	7
Solvent B Washes:	5	0
Sample Pumps:	3	

☐ Enable Solvent Saver µL

Figure 2. 9. OPE extraction method showing injector settings.



Oven Temperature: 65.0 °C

Split-Splitless Inlet

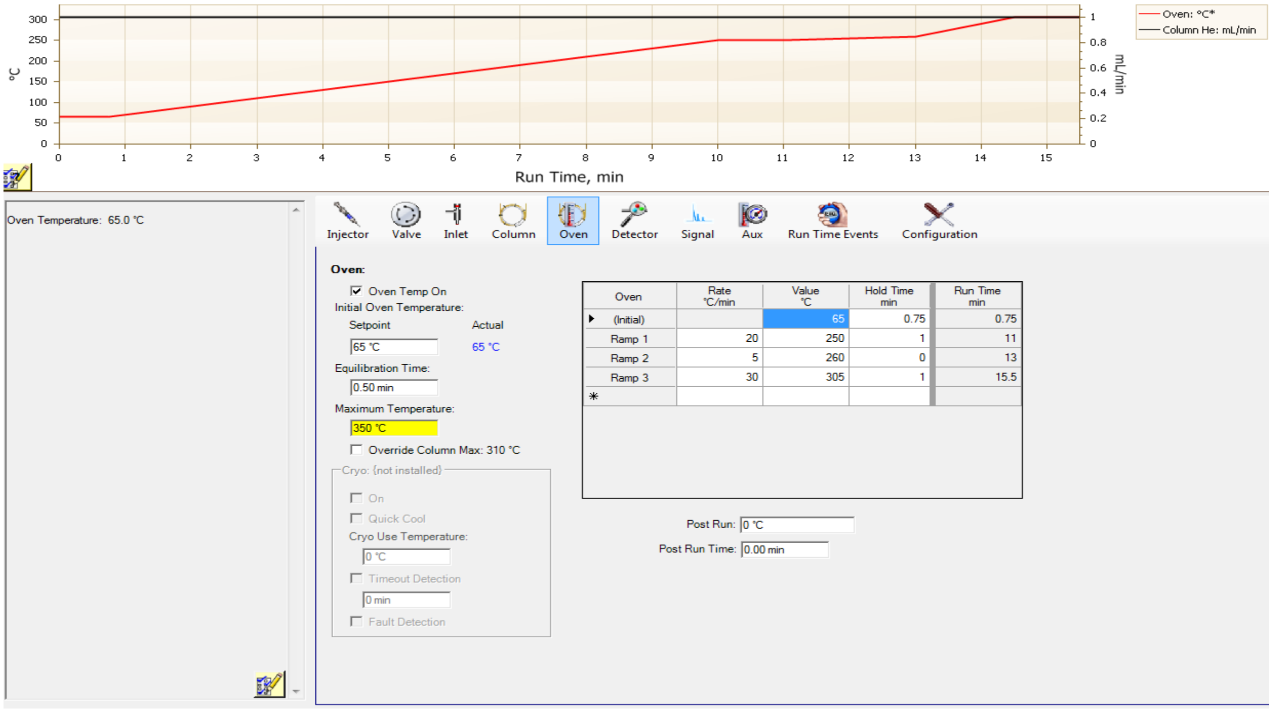
	Setpoint	Actual
☑ Heater:	290 °C	290 °C
☑ Pressure:	8.5 psi	8.5 psi
☑ Total Flow:{He}	53.9 mL/min	24.0 mL/min

Mode: Splitless

Purge Flow to Split Vent: 50.0 mL/min at 2.00 min

Gas Saver: ☑ 20.0 mL/min After: 5.00 min

Figure 2. 10. OPE extraction method showing inlet settings.



Oven Temperature: 65.0 °C

Oven:

☑ Oven Temp On

Initial Oven Temperature:

Setpoint: 65 °C Actual: 65 °C

Equilibration Time: 0.50 min

Maximum Temperature: 350 °C

☐ Override Column Max: 310 °C

Cryo: {not installed}

☐ On

☐ Quick Cool

Cryo Use Temperature: 0 °C

☐ Timeout Detection

0 min

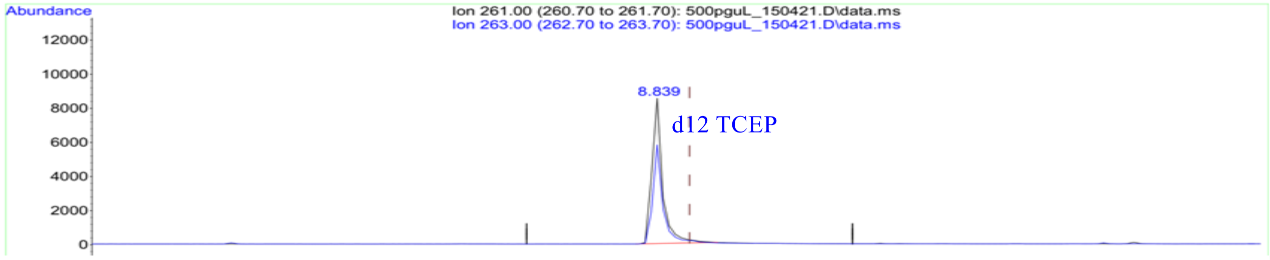
☐ Fault Detection

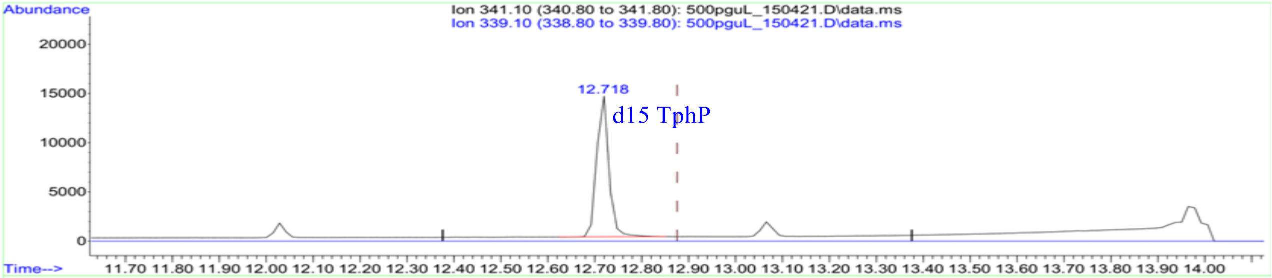
Oven	Rate °C/min	Value °C	Hold Time min	Run Time min
(Initial)		65	0.75	0.75
Ramp 1	20	250	1	11
Ramp 2	5	260	0	13
Ramp 3	30	305	1	15.5
*				

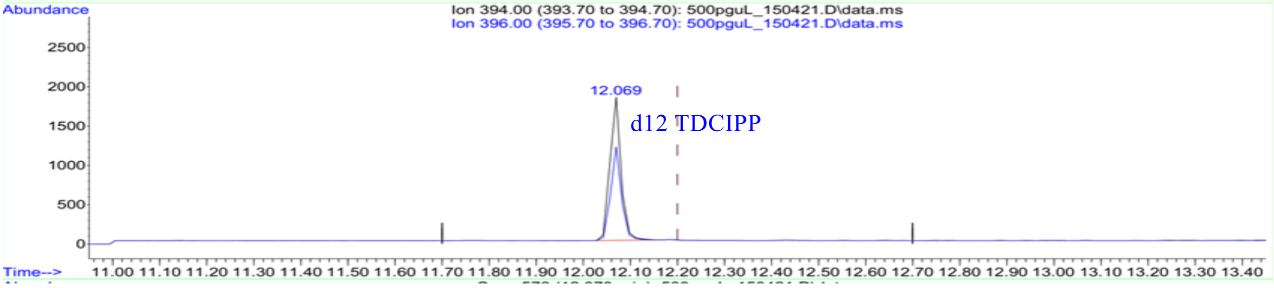
Post Run: 0 °C

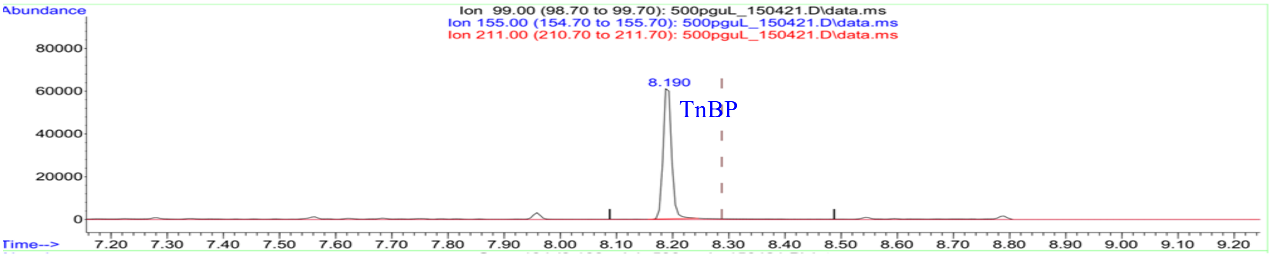
Post Run Time: 0.00 min

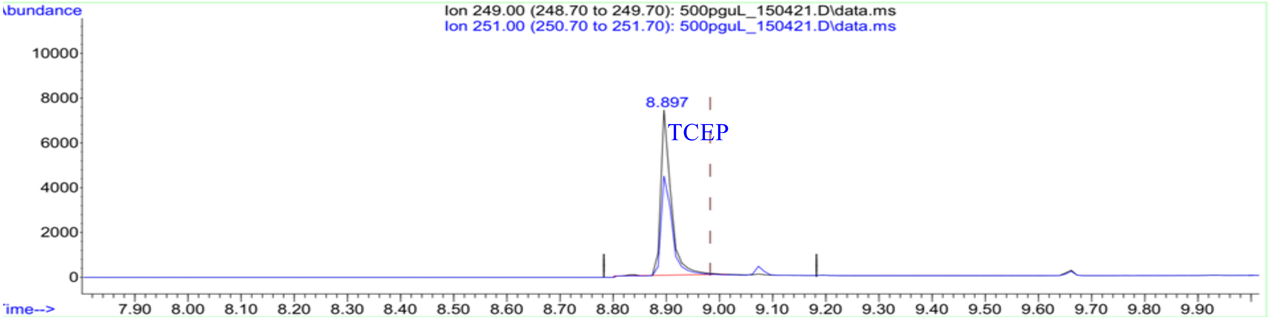
Figure 2. 11. OPE extraction method showing oven settings.

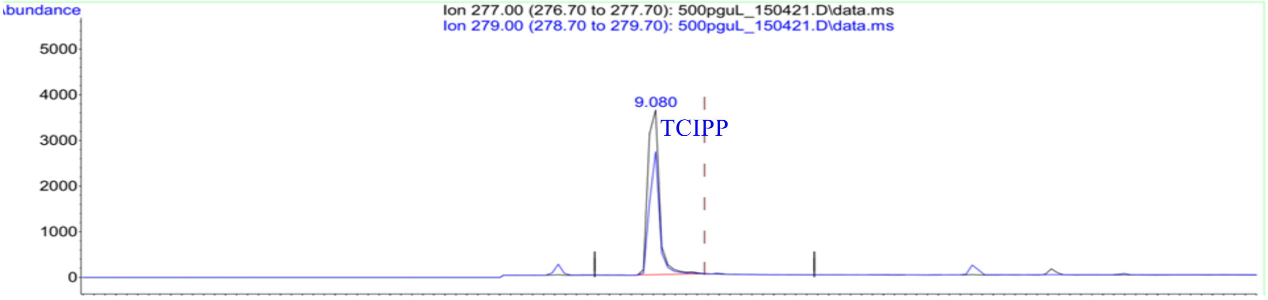

Abundance
Ion 261.00 (260.70 to 261.70): 500pguL_150421.D\data.ms
Ion 263.00 (262.70 to 263.70): 500pguL_150421.D\data.ms
12000
10000
8000
6000
4000
2000
0
8.839
d12 TCEP


Abundance
Ion 341.10 (340.80 to 341.80): 500pguL_150421.D\data.ms
Ion 339.10 (338.80 to 339.80): 500pguL_150421.D\data.ms
20000
15000
10000
5000
0
12.718
d15 TphP
Time-->
11.70 11.80 11.90 12.00 12.10 12.20 12.30 12.40 12.50 12.60 12.70 12.80 12.90 13.00 13.10 13.20 13.30 13.40 13.50 13.60 13.70 13.80 13.90 14.00


Abundance
Ion 394.00 (393.70 to 394.70): 500pguL_150421.D\data.ms
Ion 396.00 (395.70 to 396.70): 500pguL_150421.D\data.ms
2500
2000
1500
1000
500
0
12.069
d12 TDCIPP
Time-->
11.00 11.10 11.20 11.30 11.40 11.50 11.60 11.70 11.80 11.90 12.00 12.10 12.20 12.30 12.40 12.50 12.60 12.70 12.80 12.90 13.00 13.10 13.20 13.30 13.40


Abundance
Ion 99.00 (98.70 to 99.70): 500pguL_150421.D\data.ms
Ion 155.00 (154.70 to 155.70): 500pguL_150421.D\data.ms
Ion 211.00 (210.70 to 211.70): 500pguL_150421.D\data.ms
80000
60000
40000
20000
0
8.190
TnBP
Time-->
7.20 7.30 7.40 7.50 7.60 7.70 7.80 7.90 8.00 8.10 8.20 8.30 8.40 8.50 8.60 8.70 8.80 8.90 9.00 9.10 9.20


Abundance
Ion 249.00 (248.70 to 249.70): 500pguL_150421.D\data.ms
Ion 251.00 (250.70 to 251.70): 500pguL_150421.D\data.ms
10000
8000
6000
4000
2000
0
8.897
TCEP
Time-->
7.90 8.00 8.10 8.20 8.30 8.40 8.50 8.60 8.70 8.80 8.90 9.00 9.10 9.20 9.30 9.40 9.50 9.60 9.70 9.80 9.90


Abundance
Ion 277.00 (276.70 to 277.70): 500pguL_150421.D\data.ms
Ion 279.00 (278.70 to 279.70): 500pguL_150421.D\data.ms
5000
4000
3000
2000
1000
0
9.080
TCIPP

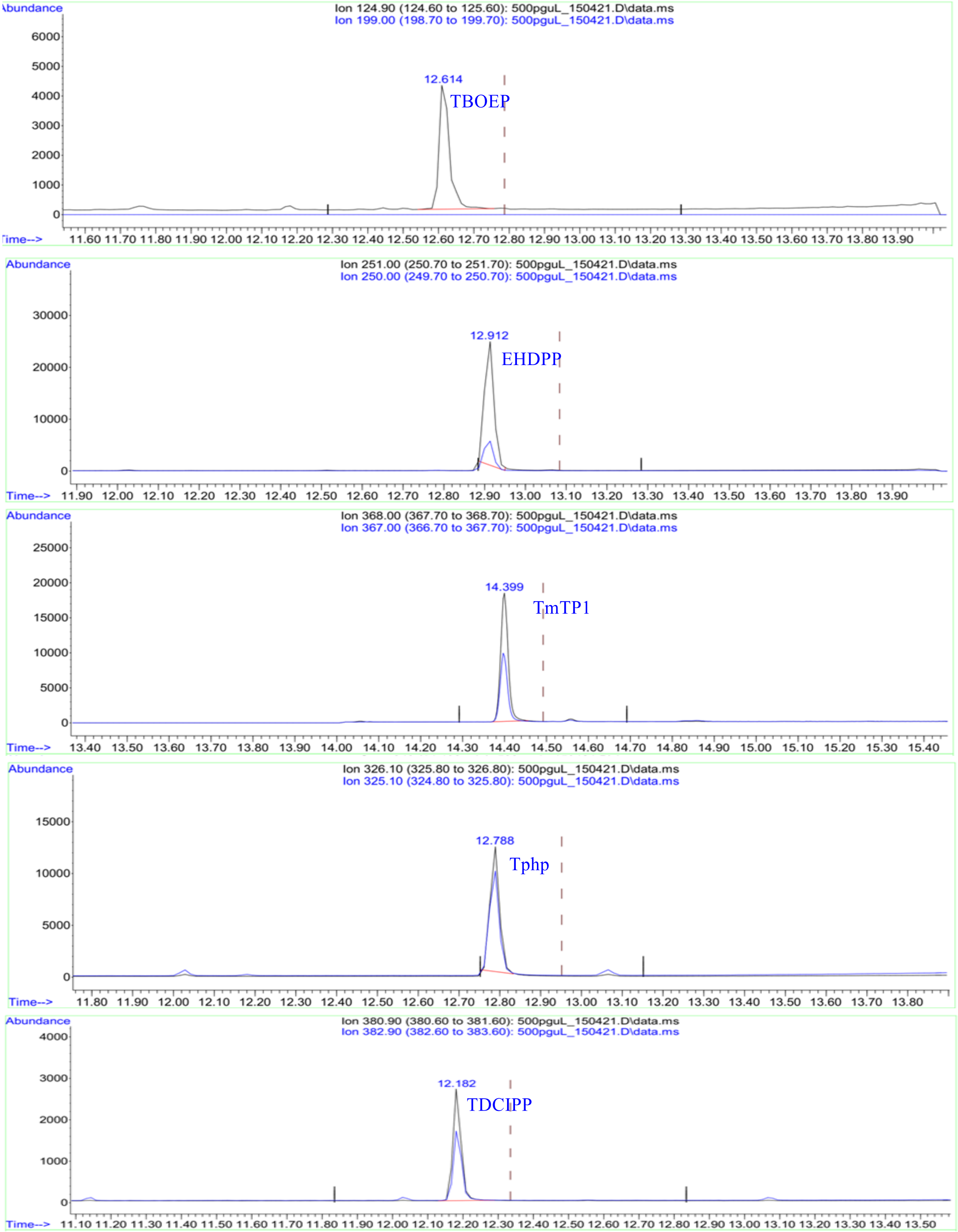


Figure 2. 12. GC-MS chromatograms of calibration standard (500 pg/µL).

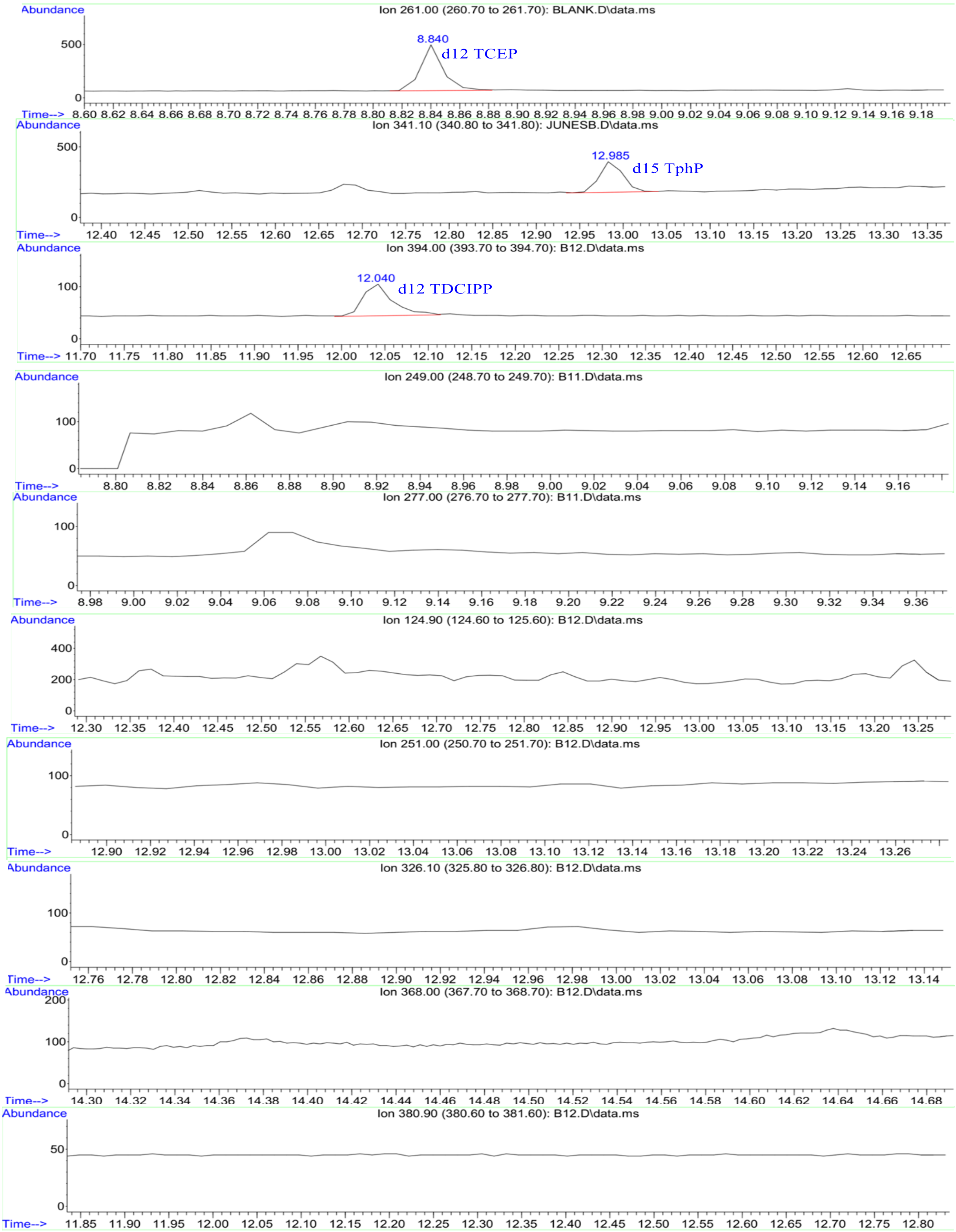


Figure 2. 13. GC-MS chromatograms of blank with 10 ng of IS mixture (d12-TCEP, d12-TDCPP, and d15-TPHP).

2.5.4 Quality Assurance/Quality Control.

To ensure that the analytical data generated during this study are precise and accurate, the following measures were taken.

2.5.5 Instrument calibration

Full five-point calibration comprising concentrations of 50, 100, 250, 500 and 1000 pg/µL was conducted to assess the linearity of the GC-MS response which was indicated with an R2 > 0.996. The calibration standards contained the target OPEs (native analytes), internal standard (IS) as well as the recovery determination standard (RDS) and was run on the GC-MS before and along with every batch of sample injected. This was done to capture possible instrumental drift during analysis. The relative response factor (RRF) which is defined as the instrument response for a unit amount of target pollutant relative to the instrument response obtained for the same amount of the internal standard (IS) was then calculated for each target compound using equation 2.1.

$$RRF = \frac{A_{NAT}}{A_{IS}} \, x \, \frac{C_{IS}}{C_{NAT}} \text{ .... Equation 2.1}$$

Where A_{NAT} is the peak area for the native compounds, A_{IS} is the peak area of the internal standard, C_{IS} is the concentration of internal standard, and C_{NAT} is the concentration of the native compound in the standard. The percentage relative standard deviation (% RSD) of the RRFs calculated for each target compound at each concentration point of the calibration plot did not exceed 5%.

2.5.6 Determination of internal standard recoveries.

To enable assessment of total IS loss at the extraction and clean up phases of sample analysis, RDS (PCB-62) was added to the sample extracts before injecting to the GC-MS. This was then used to calculate the recoveries of IS using equation 2.2.

$$\%\text{IS Recovery} = \left[\left(\frac{A_{IS}}{A_{RDS}}\right)_S \times \left(\frac{A_{RDS}}{A_{IS}}\right)_{STD} \times \left(\frac{C_{IS}}{C_{RDS}}\right)_{STD} \times \left(\frac{C_{RDS}}{C_{IS}}\right)_S\right] \text{x100...........Equation 2.2}$$

where $(A_{IS}/A_{RDS})_S$ is the ratio of internal standard peak area to recovery determination standard peak area in the sample; $(A_{RDS}/A_{IS})_{STD}$ is the ratio of recovery determination standard peak area to internal standard peak area in the calibration standard (the average of values obtained for both calibration standards run before and after this batch of samples); $(C_{IS}/C_{RDS})_{STD}$ is the ratio of concentration of internal standard to concentration of recovery determination standard in the calibration standard; and $(C_{RDS}/C_{IS})_S$ is the ratio of concentration of recovery determination standard to concentration of internal standard in the sample.

2.5.7 Method Accuracy and precision.

The accuracy of the method used for sample analysis was determined using a standard reference material (SRM 1944 - New York/New Jersey Waterway Sediment) from the National institute of Standards and Technology (NIST). Ten (10) replicate analyses of SRM 1944 were conducted before commencement of analysis of the sediment samples. The mean concentration and standard deviation values for the replicates are provided in Table 2.4. The data from the SRM analysis was compared to previously conducted and published studies on the target OPEs (Table 2.3) and the concentrations obtained indicate the precision of the method used for this study. Furthermore, to ensure that accuracy is constantly measured and maintained, an aliquot of the SRM was analysed with every 20 sediment samples. The concentration from this analysis is then compared to ensure it is within 30% of the average concentrations obtained from the initial 10 replicates of SRM.

Table 2. 3. Average concentration of target OPEs in SRM 1944 compared with average values reported in literature.

Study	**TNBP (ng/g)**	**TCEP (ng/g)**	**TCIPP (ng/g)**	**TBOEP (ng/g)**	**EHDP (ng/g)**	**TMTP (ng/g)**	**TPHP (ng/g)**	**TDCIPP (ng/g)**
(Xing et al. 2018)	0.1	0.4	0.2	0.1	0.2	0.2	0.1	-
(Liu et al. 2018)	0.0	3.0	1.2	0.0	0.4	0.4	1.7	0.2
(Giulivo et al. 2017)	2.4	1.8	4.6	1.5	4.8	4.8	0.4	1.6
(Giulivo et al. 2017)	5.5	2.5	14.9	2.4	37.3	37.3	0.9	2.3
(Giulivo et al. 2017)	7.7	0.8	6.6	3.2	4.8	4.8	ND	0.4
(Cao et al. 2017)	0.3	0.2	0.2	0.1	0.1	0.1	0.1	-
(Cao et al. 2017)	0.5	ND	0.4	1.5	0.1	0.1	0.2	-
(Cao et al. 2017)	1.4	ND	0.7	7.3	0.3	0.3	2.6	-
(Yadav and Devi, 2020)	76.4	18.5	112.6	-	135.6	-	39.6	5.7
Average SRM Concentrations for this study	8.7	15.9	33.5	321.1	11.0	15.5	11.4	5.3

Table 2. 4. Concentrations (ng/g) of OPEs in SRM 1944 (n=10).

OPE	**TNBP**	**TCEP**	**TCIPP**	**TBOEP**	**EHDPP**	**TMTP**	**TPHP**	**TDCIPP**
C1	10.0	13.7	35.7	396.0	8.6	14.9	7.8	4.8
C2	8.4	14.7	34.8	315.0	9.9	16.9	8.5	4.4
C3	13.2	19.5	39.8	361.0	11.9	16.45	10.5	4.2
C4	9.8	24.5	35.1	444.0	14.1	21.3		4.9
C5	8.4	14.6	38.1	432.0	12.9	11.05	15.6	5.2
Blank	0.0	12.7	0.0	90.4	0.0	0	0.0	0.0
RSD	19.7	26.3	5.9	13.6	19.4	23.1	33.2	8.5
C6	6.4	9.8	23.5	210.0	8.9	10.8	4.4	3.9
C7	6.4	19.0	32.6	263.0	8.5	12.9	12.7	5.7
C8	9.6	19.0	38.5	248.6	7.0	15	19.0	8.4
C9	7.9	11.3	22.6	265.0	14.7	18.15	17.9	6.0
C10	6.8	13.5	33.9	276.7	13.7	17.35	6.4	5.3
Blank	0.0	7.7	0.0	157.7	0.3	0.4	0.6	0.4
RSD	18.4	29.6	22.9	10.2	32.3	21.45	54.5	27.8
RSD all	23.9	27.9	17.7	25.6	25.1	22.5	45.4	24.2
AVERAGE all	8.7	15.9	33.5	321.1	11.0	15.5	11.4	5.3
SD all	2.1	4.4	5.9	82.1	2.8	3.5	5.2	1.3

2.5.8 Determination of Blank Concentrations

Another QA/QC measure employed was the use of reagent blanks in every batch of 5 samples injected unto the GC-MS. The reagent blanks which were used to access the concentration of target compounds present in an analysis where the sample is omitted, but internal standards where added was prepared using 1g of pre-baked Na_2SO_4 extracted and cleaned as a sample. It was ensured that the concentration of target pollutants in the blanks for each batch of samples was less than 5 % of the concentration in a sample from that batch, in cases where it was > than 5%, the blank concentrations were subtracted from that in the sample. (n =17)

Table 2.5 shows the summary of reagent blank concentration for analysis of OPEs in sediment (ng/g). Also, Table 2.6 shows the summary of procedural blanks for MP extraction.

Table 2. 5. Summary of reagent blank concentration for analysis of OPEs in sediment (ng/g)

Reagent Blank (n=17)	**TNBP**	**TCEP**	**TCIPP**	**TBOEP**	**EHDPP**	**TMTP**	**TPHP**	**TDCIPP**
MEAN	0	0.3	0	24.6	0	0	0	**0**
MIN	0	0	0	2.1	0	0	0	**0**
MAX	0	0.8	0	65.0	0	0	0	**0**
SD	**0**	**0.4**	**0**	**22.1**	**0**	**0**	**0**	**0**

Table 2. 6. Procedural blanks for MP analysis

Sample	**Volume of DI water in SMI Unit (mL)**	**Number of Fibre**	**Number of Pellets**	**Number of Fragments**	**Total MPs**
	700	1	0	0	1
Blank 1	700	0	0	0	0
Blank 2	700	0	0	0	0
Blank 3	700	0	0	0	0
Blank 4	700	1	0	0	1
Blank 5	700	2	0	0	2
Blank 6	700	0	0	0	0
Blank 7	700	0	0	0	0
Blank 8	700	0	0	0	0
Blank 9	700	0	0	0	0
Blank 10	700	1	0	0	1

2.5.9 Determination of Detection limits

The instrument detection limit (IDL) defined as that amount of pollutant that gives a signal to noise ratio of 3:1 was calculated for each of the target compounds and the results are presented in Table 2.6. Sample detection limits (SDLs) were then calculated based on the LOD, final extract volume (FEV), volume of final extract injected (VFEI), sample size (SS) and percentage of internal standard recovery (% IS Rec) using equation 2.3. Calculated method SDL for each analyte is listed in Table 2.7.

$$\mathrm{SDL} = \frac{IDL \times FEV}{VFEI \times SS} \times \frac{100}{\%IS\ Recovery} \ldots\ldots \text{Equation 2.3}$$

Where FEV = final extract volume (µL), VFEI = volume of final extract injected (µL); SS = sample size (g); and %IS recovery = percentage recovery of internal standard used to quantify the target pollutant in a particular sample.

In cases where the concentration in the sample blank exceeded the SDL calculated as above, 10 blanks were prepared and analysed which then allowed us to generate and report the effective SDL (3 times the standard deviation of the blank analyses).

Table 2. 7. Calculated IDL and SDL for OPEs in this study.

ANALYTE	IDL or LOD (ng/injection)	SDL or LOQ (ng/injection)	Non-Detect (ng/g)
TNBP	0.00041	0.05	0.02
TCEP	0.00036	0.03	0.01
TCIPP	0.00055	0.04	0.02
TBOEP	0.0011	0.19	0.09
EHDPP	0.00037	0.07	0.03
TMTP1	0.00097	0.18	0.09
TMTP2	0.00097	0.18	0.09
TPHP	0.00059	0.11	0.05
TDCIPP	0.00052	0.10	0.05

2.5.10 Calculation of concentrations in samples

Concentrations of OPEs in sediment samples were calculated using the equation below:

Concentration = $\frac{A_{NAT}}{A_{IS}} \times \frac{1}{RRF} \times \frac{M_{IS}}{SS}$ .... Equation 2.4

Where A_{IS} = peak area of internal standard in sample; A_{NAT} = peak area of target pollutant in sample; RRF = relative response factor for the target pollutant (calculated using equation 2.1)); M_{IS} = mass of internal standard added to sample (pg) and SS = sample size (g). 0.5 x (SDL) was used as concentration for non-detects or instances where concentrations were below the LOQ.

2.5.11 Statistical Analysis.

Excel (Microsoft Office 365) and IBM SPSS statistics software version 28.0.0. were the main statistical tools used to analysis the data in this study. Quantitative levels and distribution patterns were determined using such parameters as mean, median, minimum, and maximum. The distribution of each data set was evaluated using the Kolmogorov-Smirnov and Shapiro-Wilk tests in SPSS. This showed that the data from the analysis of both sample sets (International samples and UK monthly samples) are not normally distributed (Tables 2.8 and 2.9), so the data were log transformed to ensure normal distribution. To investigate differences among three or more dependent variables, ANOVA was used along with post hoc Tukey test (in some cases), while paired t-tests were used to compare the means between two datasets. Potential correlation between generated data was tested using Pearson correlations with a p value of < 0.05 set as level of statistical significance.

Table 2.8: Tests of Normality for monthly sediment samples from Rivers Sowe, Tame Severn, and Worcester Birmingham Canal

	Kolmogorov-Smirnov			**Shapiro-Wilk**		
	Statistic	df	Sig.	Statistic	df	Sig.
Mean MPs/kg (dw)	.175	48	<.001	.913	48	.002
TnBP	.337	48	<.001	.430	48	<.001
TCEP	.238	48	<.001	.791	48	<.001
TCIPP	.127	48	.052	.907	48	.001
TBOEP	.187	48	<.001	.809	48	<.001
EHDPP	.317	48	<.001	.545	48	<.001
TMTP	.284	48	<.001	.655	48	<.001
TPhP	.316	48	<.001	.452	48	<.001
TDCIPP	.200	48	<.001	.827	48	<.001
Mean 8 OPEs	.193	48	<.001	.712	48	<.001
Σ_8OPE	.193	48	<.001	.712	48	<.001

Table 2.9: Tests of Normality for internal sediment samples

	Kolmogorov-Smirnov[a]			**Shapiro-Wilk**		
	Statistic	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
TnBP	.291	30	<.001	.750	30	<.001
TCEP	.122	30	.200*	.903	30	.010
TCIPP	.182	30	.012	.726	30	<.001
TBOEP	.385	30	<.001	.332	30	<.001
EHDPP	.367	30	<.001	.338	30	<.001
TMTP	.295	30	<.001	.567	30	<.001
TPhP	.373	30	<.001	.321	30	<.001
TDCIPP	.398	30	<.001	.279	30	<.001
Mean 8 OPEs	.243	30	<.001	.767	30	<.001
∑8 OPEs	.243	30	<.001	.767	30	<.001
Mean MPs/kg (dw)	.278	30	<.001	.600	30	<.001

CHAPTER 3

3.1 Occurrence, distribution, and seasonal variation of OPEs in UK freshwater.

3.1.1 Synopsis

In this chapter, sediment samples collected from the Worcester and Birmingham canal, and the Rivers Tame, Severn, and Sowe were analysed for 8 OPEs (TNBP, TCEP, TCIPP, TDCIPP, TBOEP, TPHP, EHDPP and TMTP). The concentration of these OPEs (ng/g) as well as their variation with different parameters at the study locations is investigated and presented. The concentrations of OPEs in this study are then compared to reported concentrations of OPEs in sediments from other parts of the world to identify spatial and seasonal trends of OPEs in UK freshwater sediment for the first time. The ecological risk of the OPEs on aquatic organisms at these study locations is also assessed and presented.

3.2 Occurrence and concentrations of OPEs in sediment samples.

3.2.1 Worcester- Birmingham canal

Concentrations of Σ_8OPEs in sediments from the Worcester-Birmingham canal over the 12 months of sampling ranged from 24 to 295 ng/g dw, while mean concentrations of each individual target OPE ranged from 3 (TDCIPP) to 53 ng/g (TBOEP) dw (Table 3.1) (Figure 3.1). Of the chlorinated OPEs, TCIPP had the highest mean concentration of 9.0 ng/g followed by TCEP (6.0 ng/g). The most abundant non-halogenated OPE was TBOEP and the least TMTP with mean concentrations of 4.1 ng/g (Figure 3.2). Detection frequencies were 100 % for TCEP, TBOEP, TPHP, TDCIPP and TMTP. For TCIPP and TNBP, it was 75% while for EHDPP, detection frequency was 92%.

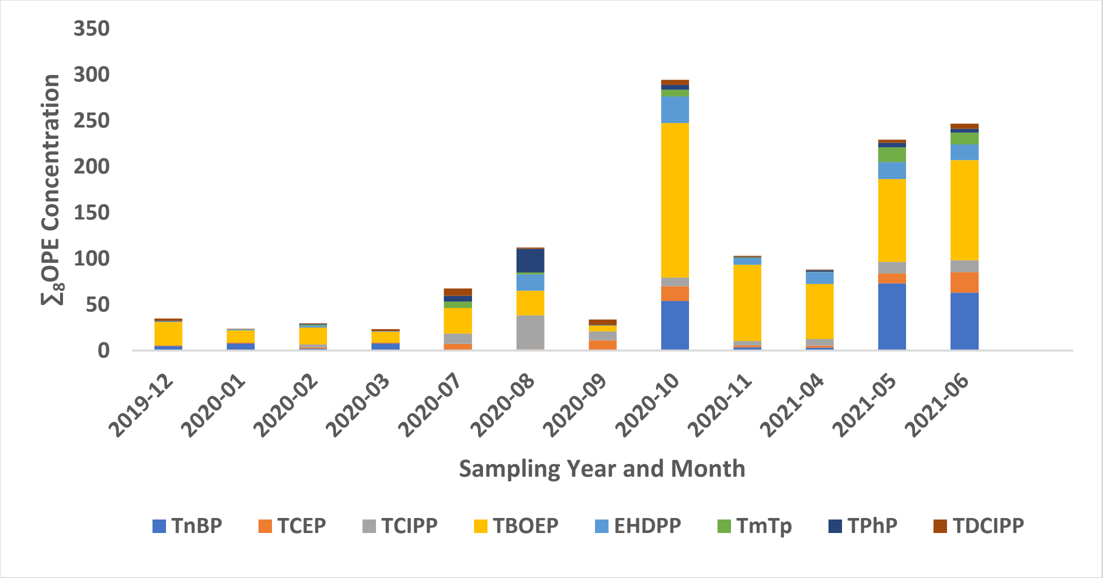


Figure 3. 1. Concentrations of target OPEs in surficial sediments from the Worcester- Birmingham canal.

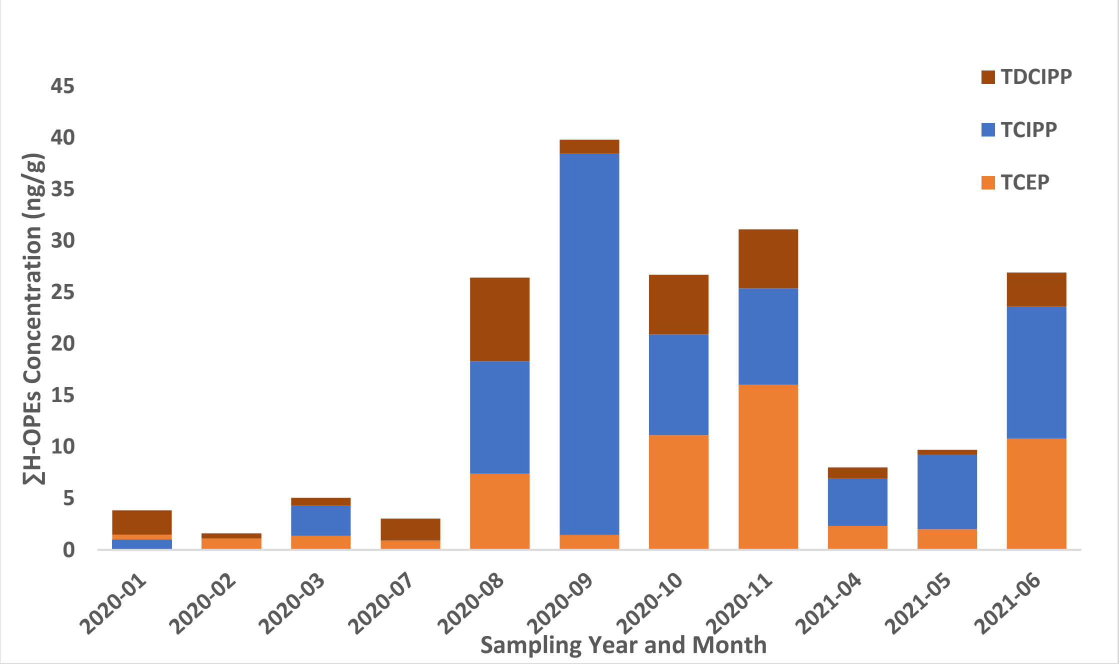


Figure 3. 2. Concentrations of target halogenated OPEs in surficial sediments from the Worcester- Birmingham canal.

Table 3. 1. Descriptive statistical summary of OPE Concentrations (ng/g) in surficial sediments from the Worcester & Birmingham canal.

Year – Month of sample collection	**OPE concentrations for Worcester & Birmingham canal (ng/g)**								
	TNBP	**TCEP**	**TCIPP**	**TBOEP**	**EHDPP**	**TMTP**	**TPHP**	**TDCIPP**	**Σ_8OPEs**
2019-12	5.1	0.5	0.02	25	0.3	0.4	1	2	35
2020-01	7.7	1	0.02	13	1	0.3	0.3	1	24
2020-02	2.3	1	3	19	2	1	1	1	30
2020-03	7.7	1	0.02	12	0.5	0.2	0.1	2	24
2020-07	0.02	7	11	28	0.02	7	6	8	68
2020-08	0.02	1	37	27	18	2	26	1	112
2020-09	0.02	11	10	6	0.1	0.5	0.2	6	34
2020-10	54	16	9	168	29	7	5	6	295
2020-11	3.4	2	5	83	7	1	1	1	103
2021-04	3.2	2	7	60	13	0.2	2	1	88
2021-05	73	11	13	90	18	16	5	3	229
2021-06	63	22	13	109	17	13	4	6	247
Minimum	0.02	0.5	0.02	6	0.02	0.2	0.1	1	24
Mean	18.28	6	9	53	9	4	4	3	107
SD	27.59	7	10	50	10	6	7	3	96
Maximum	73	22	37	168	29	16	26	8	295
Median	4.23	2	8	27	5	1	1	2	78

3.2.2 River Severn in Worcester

For the River Severn at Worcester, concentrations of Σ_8OPEs over the 12 months of sampling ranged from 21 to 98 ng/g dw with mean concentrations of each target OPE ranging from 1.0 (EHDPP, TMTP, and TDCIPP) to 31 (TBOEP) ng/g dw (Table 3.2). Consistent with our observations at the Worcester-Birmingham canal, TBOEP had the highest mean concentration of 31 ng/g (Figure 3.3) and concentrations of Cl-OPEs were: TCIPP>TCEP>TDCIPP (Figure 3.4). However, the least abundant OPE at this location (EHDPP) differed from that detected in the Worcester-Birmingham canal. Detection frequencies for TCEP, TBOEP, TPHP, TCIPP and TDCIPP, were 100%. For TNBP71%, TMTP 92 and EHDPP 83%, detection frequencies were 71%, 92% and 83% respectively.

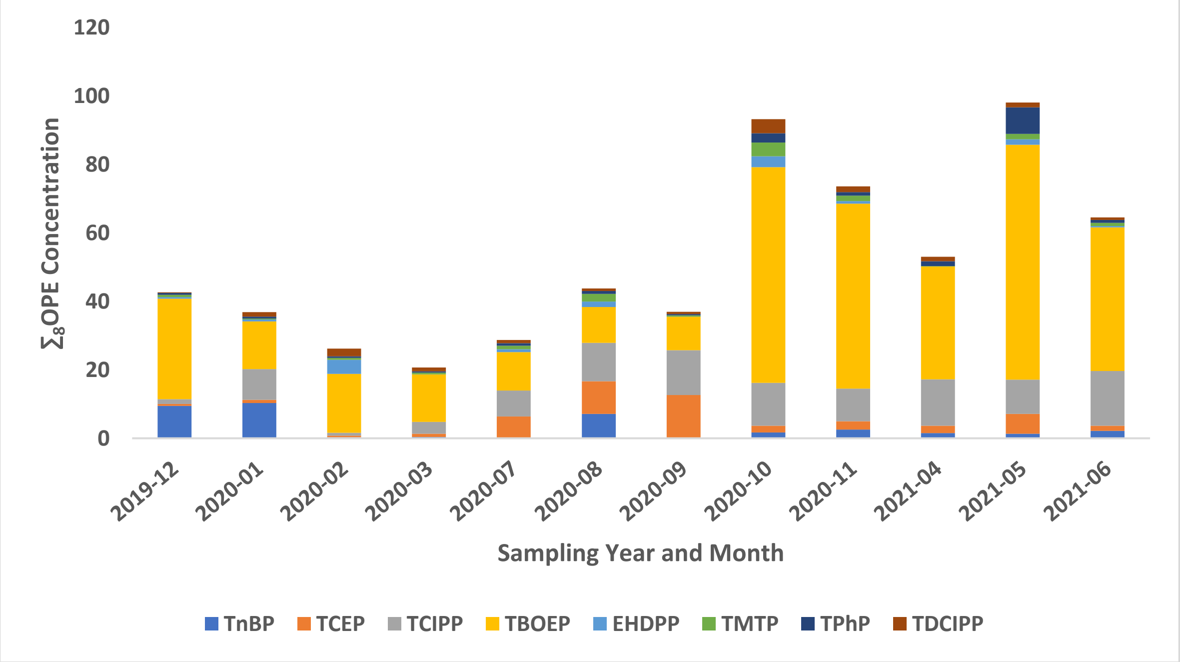


Figure 3. 3. Concentrations (ng/g) and profiles of target OPEs in surficial sediments from the River Severn in Worcester.

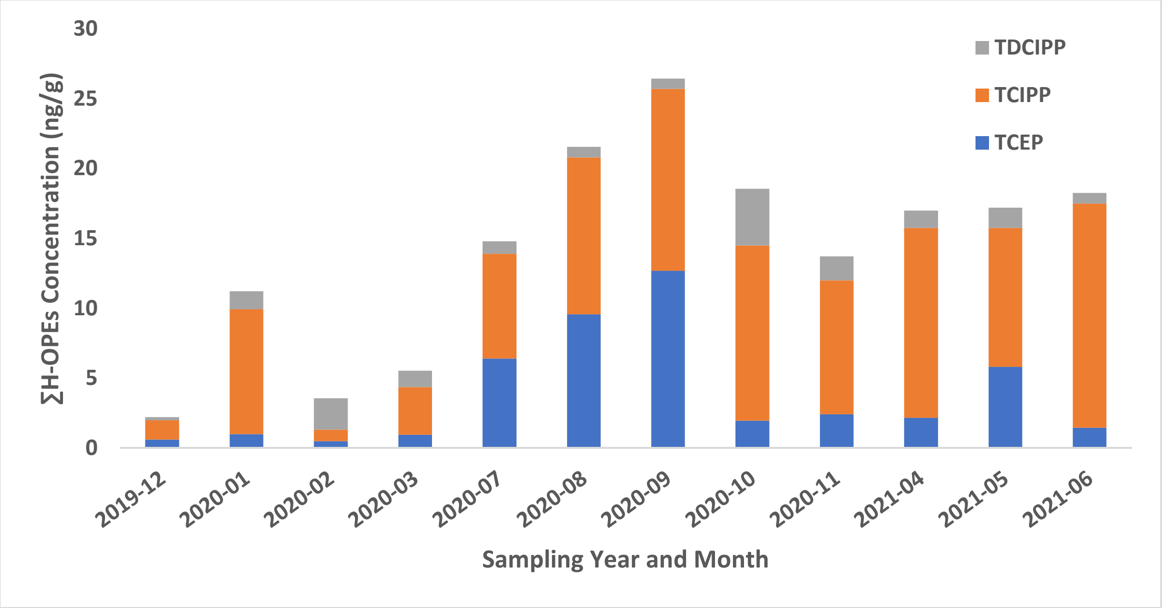


Figure 3. 4. Concentrations of target halogenated OPEs in surficial sediments from the River Severn in Worcester.

Table 3. 2. Concentrations (ng/g) of target OPEs in surficial sediments from the River Severn in Worcester

Year and Month of sample collection	**OPE concentrations for Severn River in Worcester**								
	TNBP	**TCEP**	**TCIPP**	**TBOEP**	**EHDPP**	**TMTP**	**TPHP**	**TDCIPP**	**Σ_8OPES**
2019-12	9.5	0.6	1.39	29	0.5	0.6	1	0.2	43
2020-01	10.3	1	8.95	14	1	0.4	0.5	1	37
2020-02	0.3	0.5	1	17	4	1	0.5	2	26
2020-03	0.4	1	3.41	14	0.02	0.5	0.3	1	21
2020-07	0.02	6	8	11	0.8	1	1	1	29
2020-08	7.1	10	11	10	2	2	1	1	44
2020-09	0.02	13	13	10	0.1	0.3	0.4	1	37
2020-10	1.8	2	13	63	3	4	3	4	93
2020-11	2.6	2	10	54	1	2	1	2	74
2021-04	1.5	2	14	33	0	0.0	1	1	53
2021-05	1.4	6	10	69	2	2	8	1	98
2021-06	2.2	1	16	42	0	1	1	1	65
Minimum	0.02	0.5	0.82	10	0.02	0.04	0.3	0.2	21
Mean	3.1	4	9	31	1	1	1	1	52
SD	3.7	4	5	22	1	1	2	1	26
Maximum	10.3	13	16	69	4	4	8	4	98
Median	1.6	2	10	23	1	1	1	1	43

3.2.3 River Tame in Water Orton

Concentrations of Σ_8OPEs in surficial sediment from the River Tame at Water Orton over the 12 months period of sampling ranged from 19 to 109 ng/g dw, with mean concentrations of individual OPEs ranging from 2.0 (TDCIPP) to 35 ng/g (TBOEP) dw (Table 3.3 and Figure 3.6). As observed for the Worcester-Birmingham canal and the River Severn, the relative abundance of CL-OPEs was TCIPP>TCEP>TDCIPP (Figure 3.5) and detection frequencies were: 100% for TCEP, TBOEP, and TPHP. For TCIPP, TDCIPP and TMTP it was 92 % while for EHDPP and TNBP, it was 83% and 58% respectively.

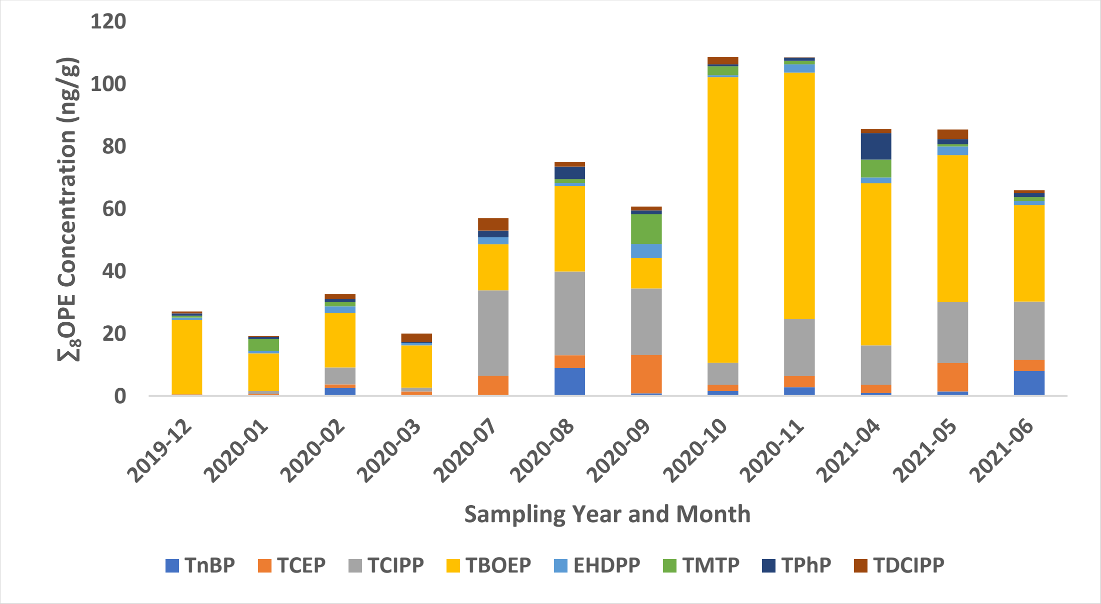


Figure 3. 5. Concentrations of target halogenated OPEs in surficial sediments from River Tame at Water Orton.

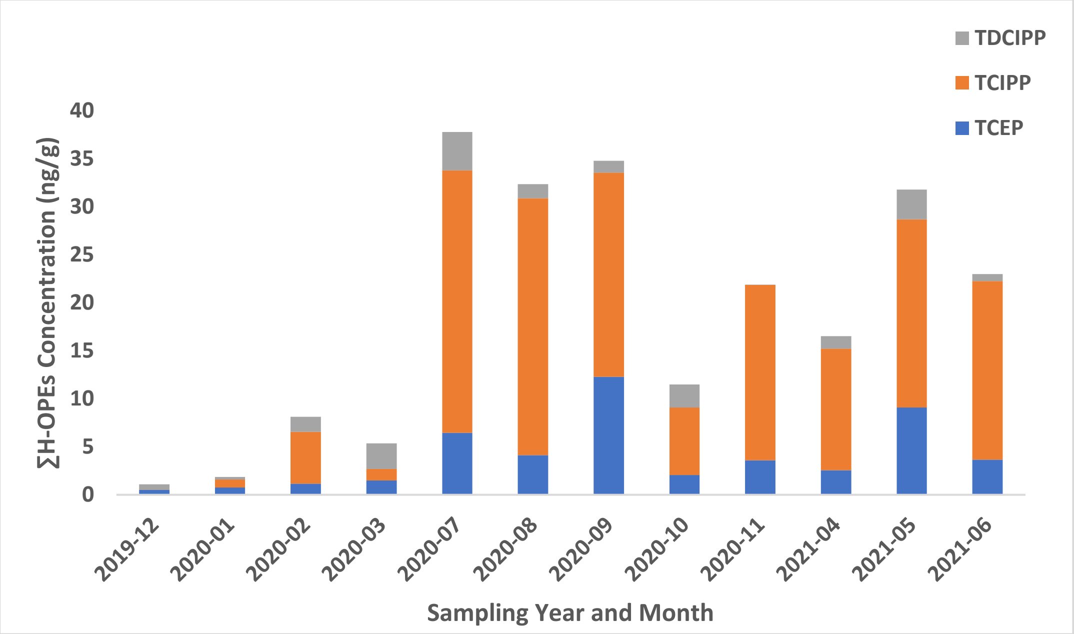


Figure 3. 6. Concentrations of target halogenated OPEs in surficial sediments from River Tame at Water Orton.

Table 3. 3. Concentrations of target OPEs in surficial sediments from the River Tame at Water Orton

	TNBP	**TCEP**	**TCIPP**	**TBOEP**	**EHDPP**	**TMTP**	**TPHP**	**TDCIPP**	**Σ8OPEs**
2019-12	0.02	0.52	0.02	23.77	0.79	0.68	0.75	0.54	27.09
2020-01	0.02	0.77	0.82	12.07	0.69	3.93	0.63	0.27	19.2
2020-02	2.55	1.16	5.41	17.55	2.12	1.42	0.95	1.57	32.73
2020-03	0.02	1.5	1.2	13.57	0.69	0.12	0.27	2.68	20.05
2020-07	0.02	6.45	27.35	14.8	2.26	0.04	2.1	4.02	57.04
2020-08	8.99	4.11	26.78	27.55	0.91	1.22	4.04	1.47	75.07
2020-09	0.87	12.3	21.27	9.9	4.43	9.48	1.21	1.23	60.69
2020-10	1.6	2.05	7.05	91.5	0.7	2.8	0.65	2.4	108.75
2020-11	2.8	3.6	18.25	79	2.75	1.12	1	0.03	108.55
2021-04	1	2.58	12.65	52	1.8	5.8	8.5	1.3	85.63
2021-05	1.5	9.1	19.6	47	2.81	0.67	1.65	3.1	85.43
2021-06	8	3.65	18.6	31	1.3	1.25	1.35	0.76	65.91
Minimum	0.02	0.52	0.02	9.9	0.69	0.04	0.27	0.03	19.2
Mean	2.28	3.98	13.25	34.98	1.77	2.38	1.93	1.61	62.18
SD	3.06	3.62	10.07	27.11	1.16	2.80	2.29	1.21	32.11
Maximum	8.99	12.3	27.35	91.5	4.43	9.48	8.5	4.02	108.75
Median	1.25	3.09	15.45	25.66	1.55	1.235	1.105	1.385	63.3

3.2.4 River Sowe in Coventry

Concentrations of target OPEs in surficial sediment from the River Sowe at Coventry are provided in Table 3.4. Concentrations of Σ_8OPEs over the 12 months of monitoring ranged from 24 to 98 ng/g dw, with mean concentrations of individual OPEs ranging from 0.93 (TPHP) to 32 ng/g (TBOEP) dw (Figure 3.7). In line with our observations at other locations, the relative abundance of Cl-OPEs was TCIPP>TCEP>TDCIPP (Figure 3.8). Detection frequencies for TCEP, TBOEP, TMTP, TPHP, EHDP and TDCIPP were 100% while for TCIPP and TNBP, detection frequencies 83% and 67 % respectively.

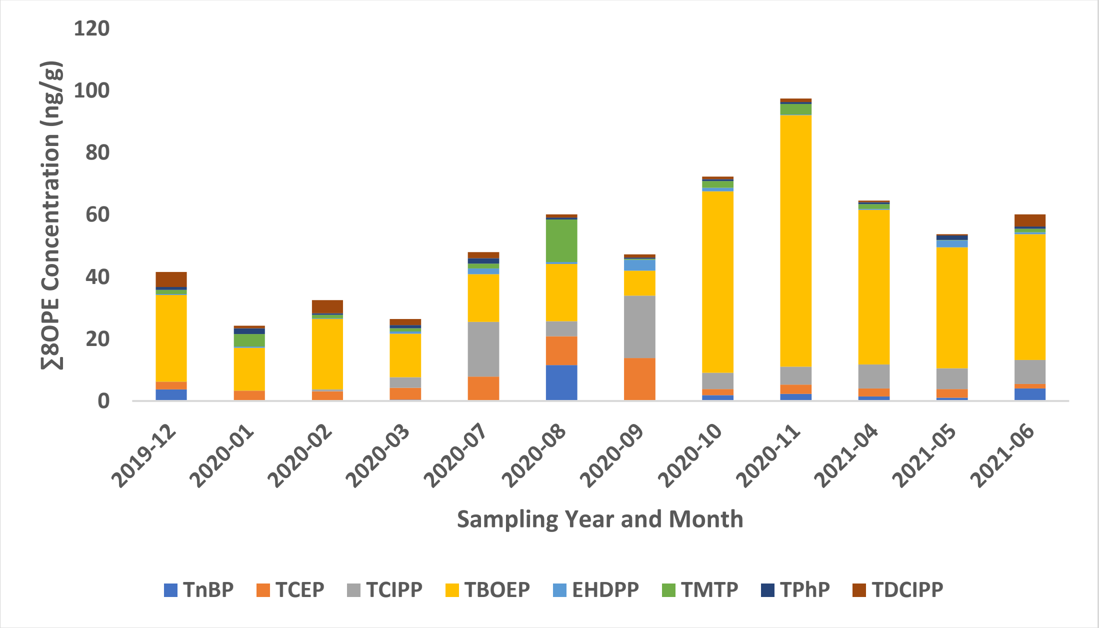


Figure 3. 7. Concentrations (ng/g) of target OPEs in surficial sediments from the River Sowe at Coventry.

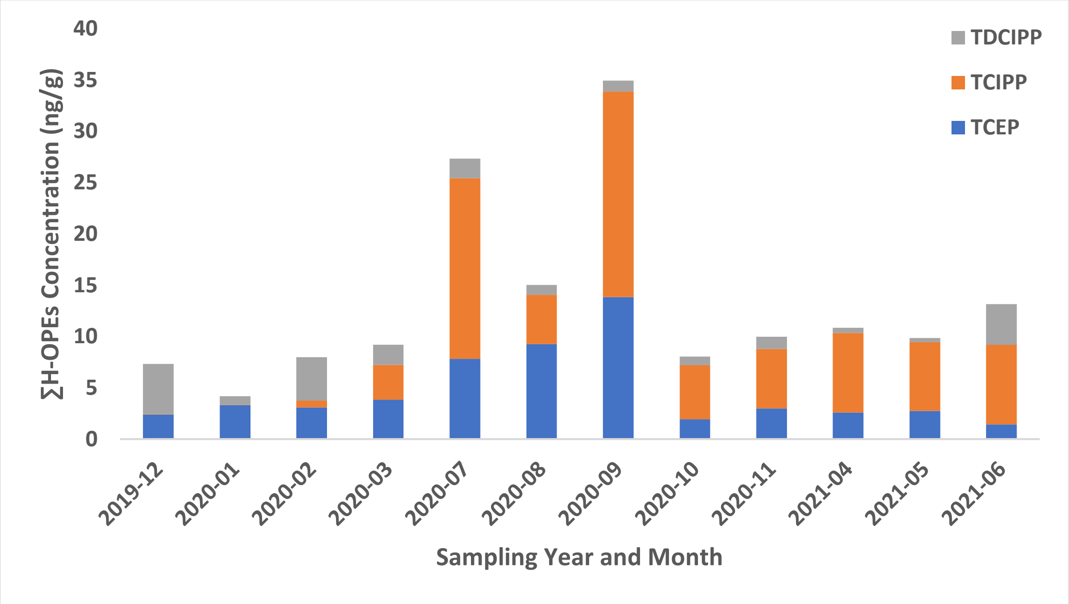


Figure 3. 8. Concentrations (ng/g) of target halogenated OPEs in surficial sediments from the River Sowe at Coventry.

Table 3. 3. Concentrations (ng/g) of target OPEs in surficial sediments from the River Sowe at Coventry

Year and Month of sample collection	**OPE Concentrations for Sowe River in Coventry**								
	TNBP	**TCEP**	**TCIPP**	**TBOEP**	**EHDPP**	**TMTP**	**TPHP**	**TDCIPP**	**Σ_8OPEs**
2019-12	3.75	2.41	0.02	27.99	0.31	1.33	0.88	4.91	41.6
2020-01	0.02	3.31	0.02	13.82	0.35	4.06	1.86	0.87	24.31
2020-02	0.02	3.09	0.66	22.65	0.26	1.1	0.5	4.25	32.53
2020-03	0.46	3.83	3.41	14	0.71	1	1.03	1.95	26.39
2020-07	0.02	7.84	17.6	15.45	1.79	1.58	1.75	1.91	47.94
2020-08	11.6	9.25	4.81	18.5	0.59	13.75	0.66	0.96	60.12
2020-09	0.02	13.85	20.04	8.11	3.52	0.29	0.39	1.05	47.27
2020-10	1.9	1.95	5.25	58.5	1.1	2.15	0.65	0.85	72.35
2020-11	2.3	3	5.8	81	0.25	3.4	0.6	1.16	97.51
2021-04	1.45	2.6	7.75	49.8	0.26	1.55	0.65	0.5	64.56
2021-05	1.1	2.75	6.7	39	2.2	0.13	1.45	0.41	53.74
2021-06	4.05	1.45	7.75	40.5	0.65	1.08	0.75	3.95	60.18
Minimum	0.02	1.45	0.02	8.11	0.25	0.13	0.39	0.41	24.31
Mean	2.22	4.61	6.65	32.44	1.00	2.62	0.93	1.90	52.38
SD	3.28	3.74	6.34	22.00	1.02	3.69	0.49	1.57	20.73
Maximum	11.6	13.85	20.04	81	3.52	13.75	1.86	4.91	97.51
Median	1.275	3.045	5.525	25.32	0.62	1.44	0.705	1.105	50.84

As shown in Figure 3.9 for all four sampled UK freshwater systems (Worcester and Birmingham canal, River Severn, River Sowe, and River Tame), the highest average $\sum_8$ OPEs concentration over the 12 months period was recorded in the Worcester and Birmingham canal. This study location was the only location (of all four sampled UK freshwater systems) not receiving direct input from a wastewater treatment plant. It is however, the most urban location as samples were collected from a point adjacent to the University of Birmingham train station. Also, there were reports of pollution (a rainbow sheen with fuel like smell) at a point along the canal around March 2021 (WorcesterNews, 2022). The second highest average $\sum_8$ OPEs concentration was for the River Tame where the sampling points were before and after the wastewater treatment plant servicing the highest population of all three sampling locations (approximately 2.5 million people within the Birmingham area). There was only a minor difference in $\sum_8$ OPEs concentrations measured at the Rivers Severn and Sowe, which is suggestive of a similar relationship between population serviced by the respective wastewater treatment plants and the abundance of OPEs. Sediments samples collected from river Sowe (close to WWTP 2 which serves a population of 420,470) shows a slightly higher concentration of OPEs than samples from River Severn (close to WWTP3 which services a total population of 50,000 to 200, 000 people).

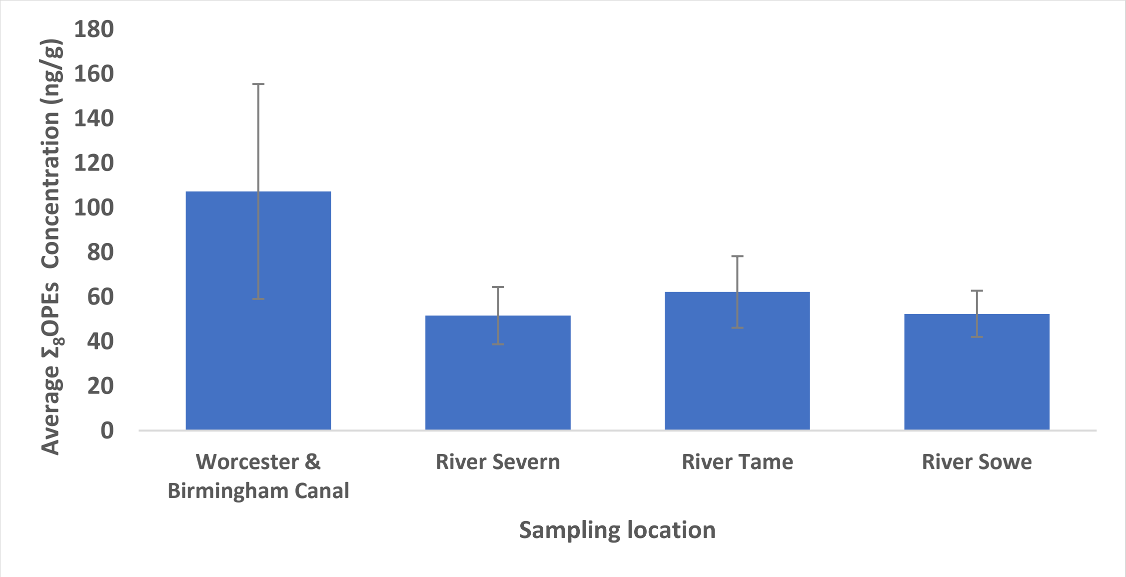


Figure 3. 9. Average $\sum_8$ OPEs concentration (y-error bar denotes standard deviation) over the 12 months period for Worcester and Birmingham canal, River Severn River Sowe, and River Tame.

Among the individual target OPEs assessed across the four sampling locations, TBOEP emerged as the most abundant non-halogenated OPE, while TCIPP stood out as the most prevalent halogenated OPE. Conversely, TDCIPP exhibited the lowest abundance among the halogenated OPEs across all four sampling locations. Notably, the least abundant OPE in Rivers Sowe, Tame, and Worcester Birmingham canals was TDCIPP; however, in the case of River Sowe, TPHP displayed the lowest abundance. As depicted in Figure 3.10, it is evident that the Worcester and Birmingham canal exhibited the highest concentrations for EHDPP, TBOEP, TCEP, TDCIPP, TMTP, TnBP, and TPHP. Conversely, River Tame recorded the highest concentration solely for TCIPP.

To assess the statistical significance of the observed variations in the concentrations of the sum of eight OPEs across the study locations, an analysis of variance (ANOVA) test was employed. The results of the test indicated that there is no significant difference in the $\sum_8$ OPEs concentration across the four study locations. This conclusion is supported by the calculated p-value (0.531), which exceeded the predetermined significance level of 0.05, indicating that the observed differences are not statistically significant.

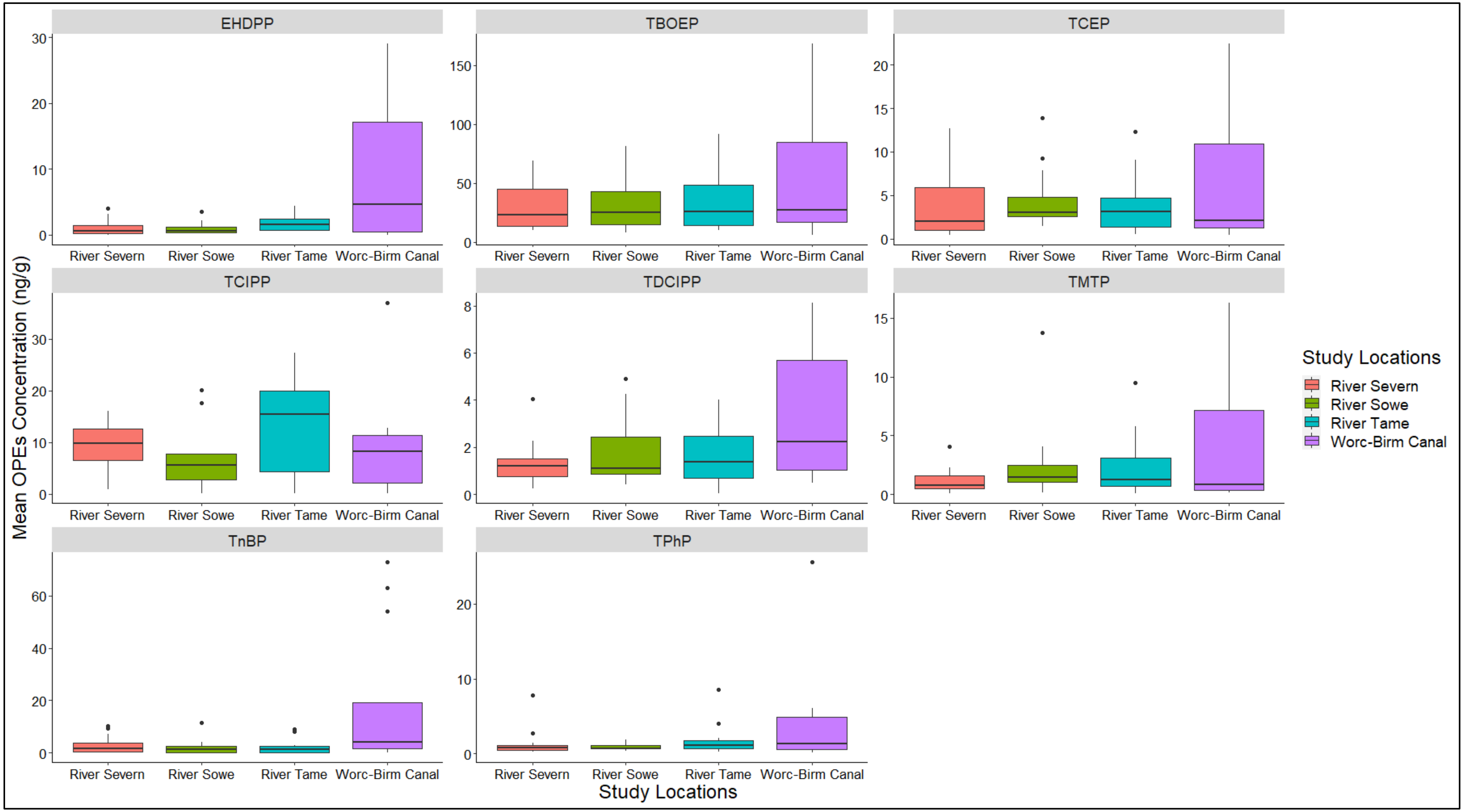


Figure 3. 10. Comparison of the mean concentration of each of the 8 target OPEs in all four sampling locations (Worcester and Birmingham canal, River Severn River Sowe, and River Tame).

3.3 Seasonal variations

The United Kingdom experiences four distinct seasons throughout the year, and Table 3.7 provides a comprehensive overview of these seasons, their corresponding months, and noteworthy characteristics associated with each season (MetOffice, 2022). The discussion of seasonal variation in this section will be based on these four seasons as the notable features associated with these seasons may influence the level of OPEs detected. As shown on Table 3.7 and in Figure 3.11, the highest $\sum_8$ OPEs concentration for all study locations fall within the Autumn months (September, October, and November) while the lowest concentrations all fall within the winter months (December, January, and February). The highest $\sum_8$ OPEs concentration for the Worcester and Birmingham Canal, River Tame, River Sowe, and River Severn are 431 ng/g, 278 ng/g, 217 ng/g, and 204 ng/g respectively. While the lowest (in winter) are 88 ng/g, 79 ng/g, 98 ng/g, and 106 ng/g respectively.

Table 3. 4. UK seasons, months, notable features, and average concentrations of $\sum_8$ OPEs across study locations.

Season	**Months**	**Notable features**	**$\sum_8$ OPEs at River Severn (ng/g)**	**$\sum_8$ OPEs at River Sowe (ng/g)**	**$\sum_8$ OPEs at River Tame (ng/g)**	**$\sum_8$ OPEs at Worcester & Birmingham Canal (ng/g)**
Summer	June to end of August	Usually has the hottest temperatures. sunniest days Sometimes driest season Varying rainfall as with all seasons in the UK	137	168	198	426
Autumn	September to November	Cooler temperature Stormier weather Shorter days.	204	217	278	431
Winter	December to February	coldest months shortest days often wet and windy frost and even snow often	106	98	79	88
Spring	March to May	longer and warmer days often calm and dry	172	145	191	342

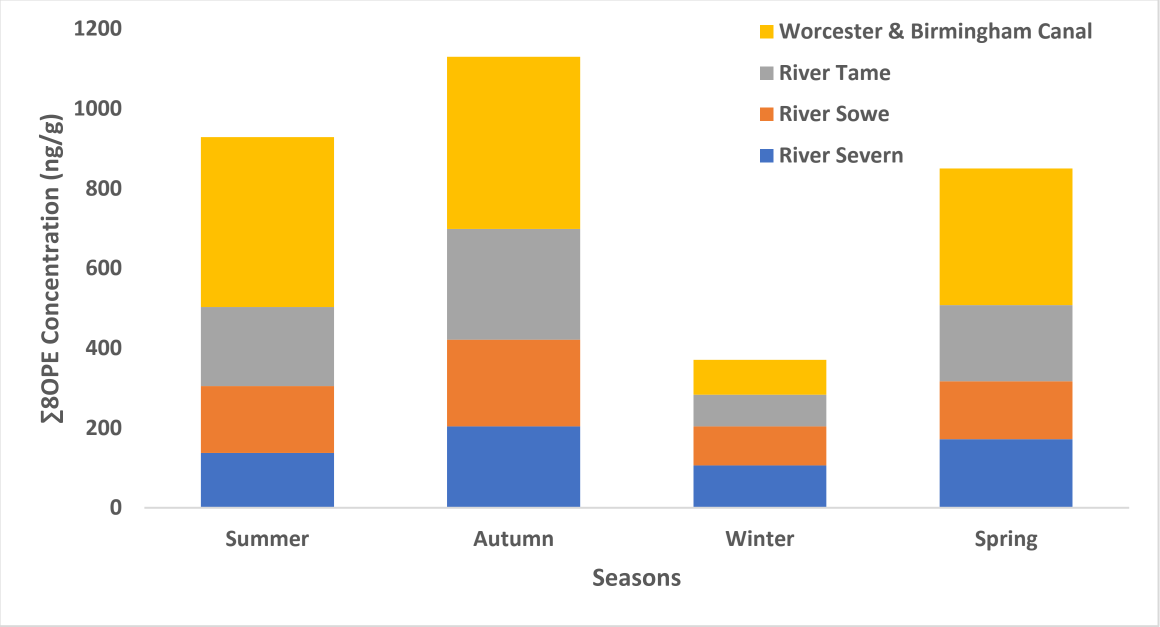
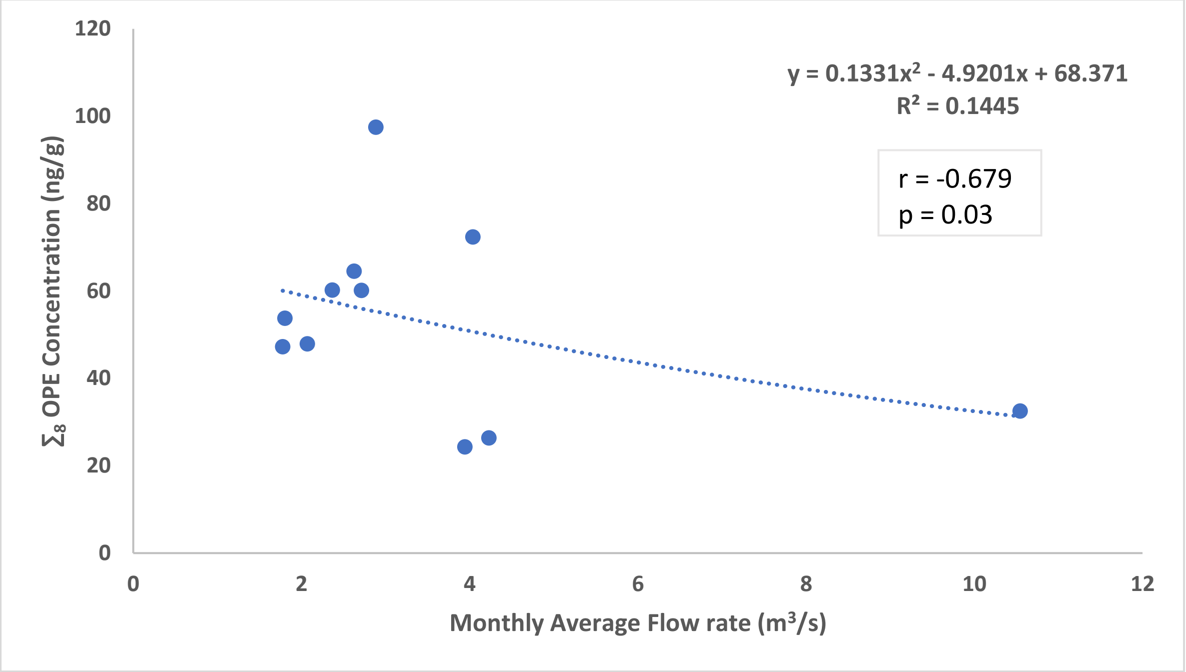


Figure 3. 11. Seasonal Variation in average concentrations (ng/g) of $\sum_8$ OPEs in surficial sediments across study locations.

Furthermore, to further understand the seasonal fluctuations in the concentrations of OPEs, we examined the possible correlation between OPE concentration and two vital hydrological parameters, namely river flow rates(NRFA, 2022) and water levels (River_Levels_uk, 2022) across the four seasons. This was informed by preceding studies, which have reported significant variations in sediment pollutant concentration as a function of hydro-dynamic conditions (Zheng et al., 2011, Ma et al., 2022, Luo et al., 2021). Such variations underscore the potential influence of these factors in determining the distribution and concentration of pollutants like OPEs in the aqueous environment.

The $\sum_8$ OPEs concentrations in sediment samples at River Severn, River Tame and River Sowe all varied inversely to the river level and flow rate across the four seasons as shown on Figures 3.12, 3.13 and 3.14. The highest flow rate and river level were observed between winter and spring, and this coincided with the seasons with the lowest $\sum_8$ OPEs concentrations.



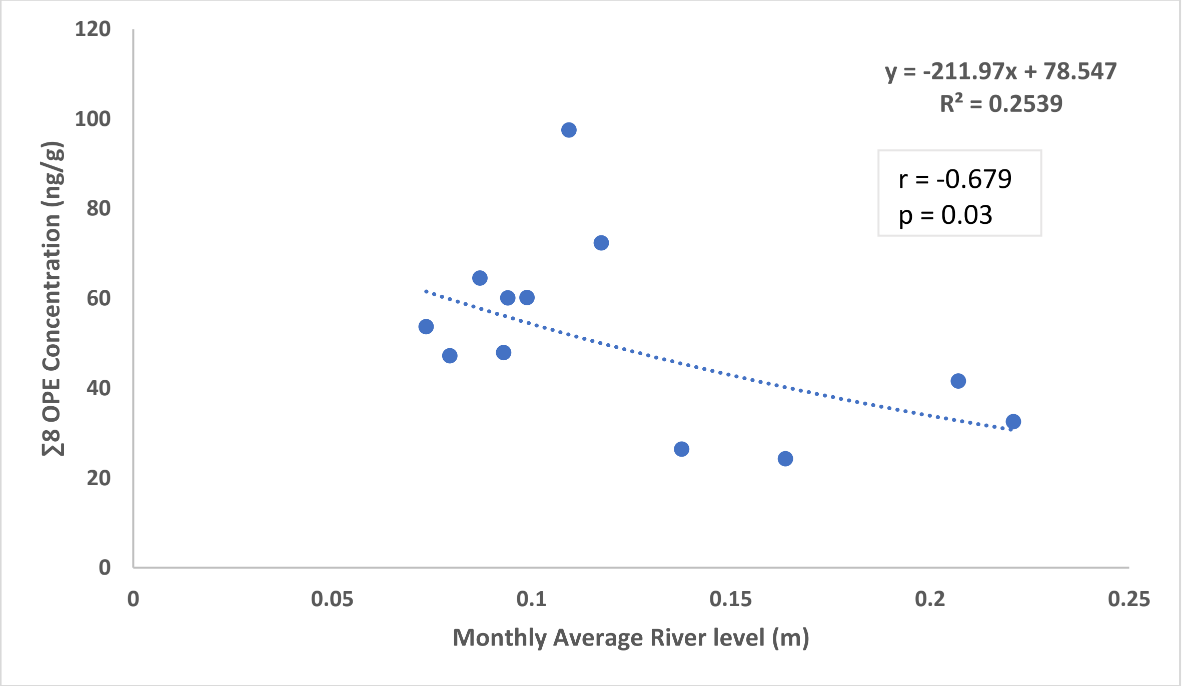
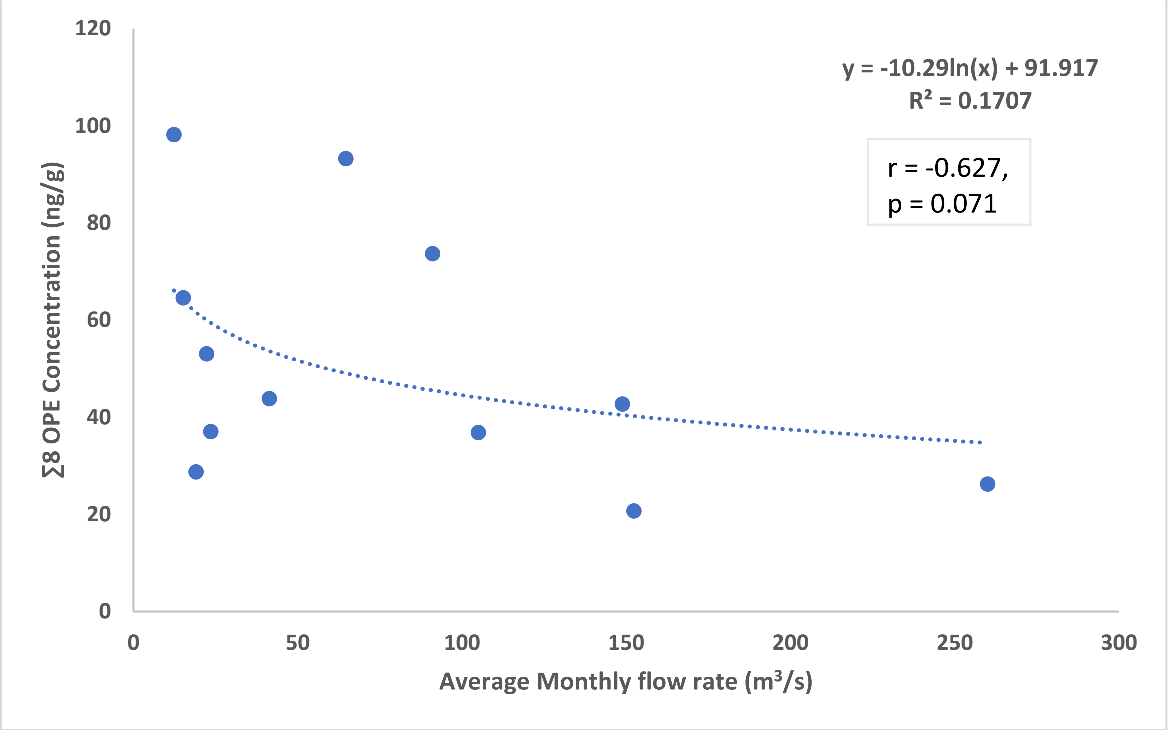


Figure 3. 12. Comparison between $\sum_8$ OPEs concentrations in sediment samples with flow rate and river level at River Sowe.



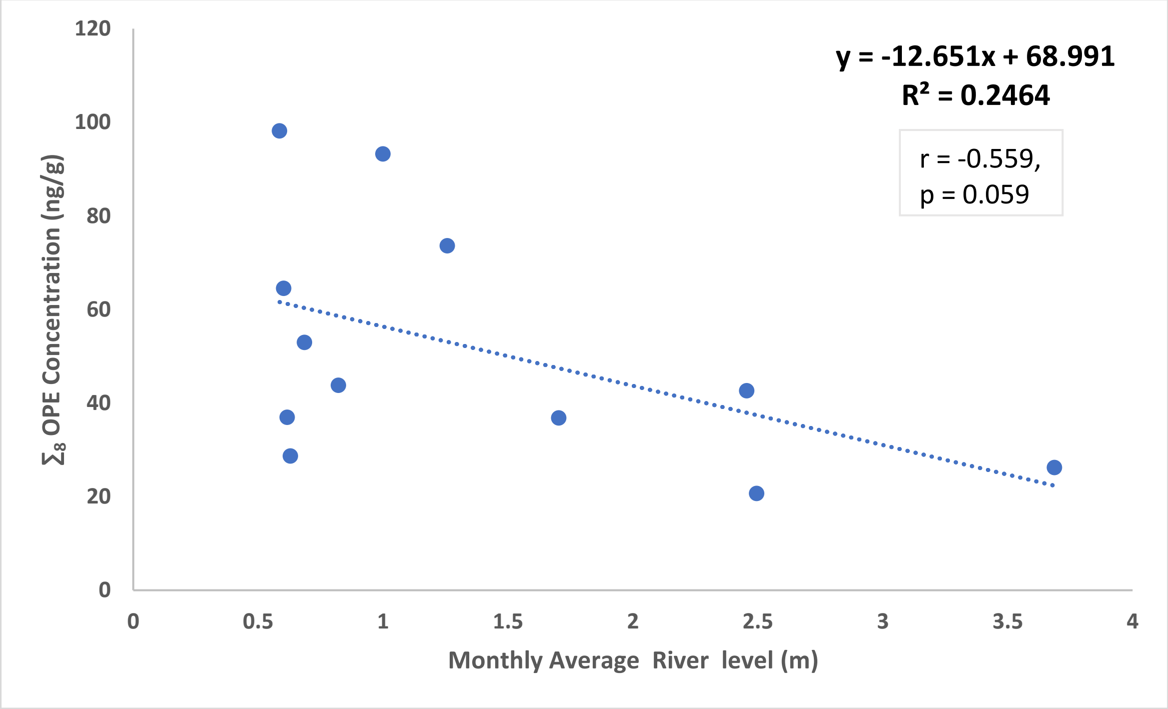
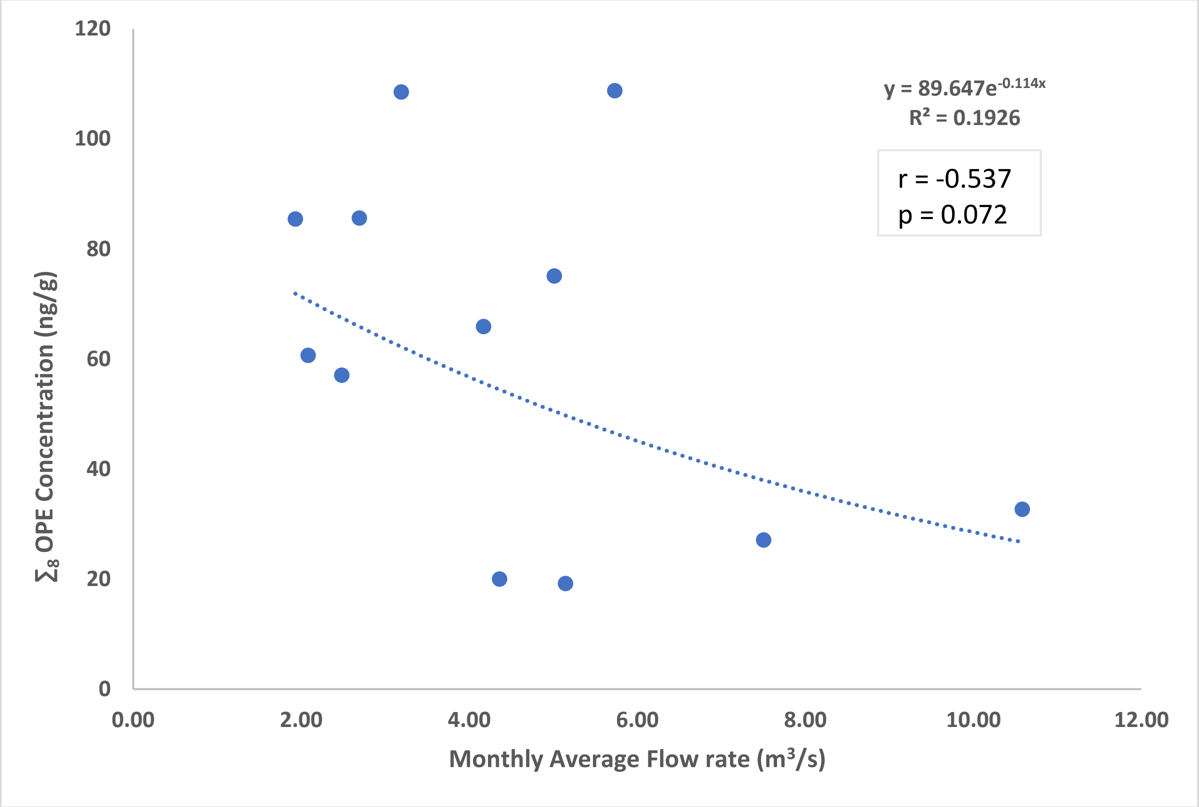


Figure 3. 13. Comparison between $\sum_8$ OPEs concentrations in sediment samples with flow rate and river level at River Severn.



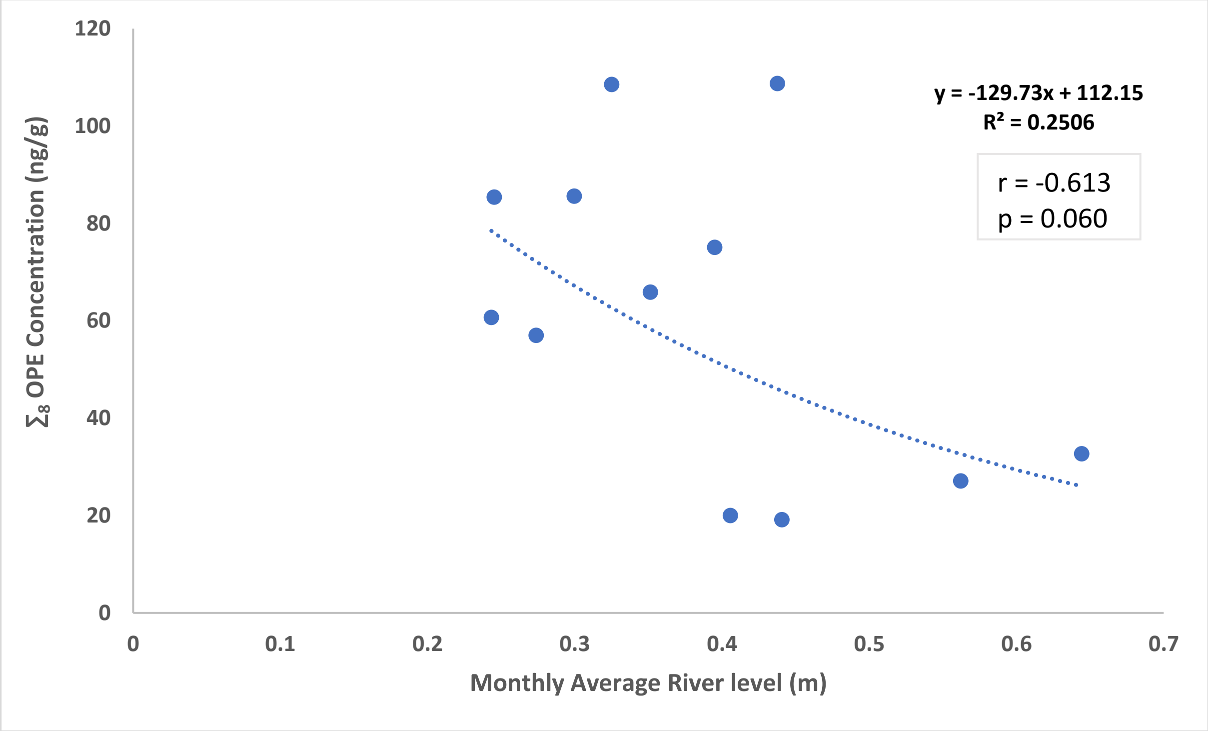


Figure 3. 14. Comparison between $\sum_8$ OPEs concentrations in sediment samples with flow rate and river level at River Tame.

For the fourth sampling location (Worcester and Birmingham Canal), flow rate and water level data were not available, but the pattern is not expected to differ much from the patterns in the other sampling locations as they are all located within the West Midlands. Furthermore, seeing that this is a canal with many locks, the flowrate and water level are expected to have minimal variation as they are controlled by the locks. This observed relationship between river level, flow rate and OPE concentration can be attributed to the diluting effect of the higher river level as a result of higher rainfall and the fact that slow flowrate enables more time for contaminant partitioning/adsorption to sediment particles (Luo et al., 2021)

A Pearson correlation test was used to assess the statistical significance of the observed trends. The results of the correlation tests reveal negative correlations between the cumulative concentration of the eight OPEs ($\sum_8$ OPEs) and two hydrodynamic factors, namely flow rate and river level, across all study locations.

In the case of the River Sowe site (Figure 3.12), the correlation analysis between $\sum_8$ OPEs concentration and river level demonstrated a substantial negative correlation; however, statistical significance was not attained ($r = -0.559$, $p = 0.059$). Similarly, when examining the relationship between $\sum_8$OPEs concentration and flow rate, a strong negative correlation was observed, but it fell just short of statistical significance, with a p-value of 0.071 ($r = -0.627$).

For the River Severn (Figure 3.13), a significant correlation was observed between $\sum_8$OPEs and flow rate ($r = -0.679$, $p = 0.03$), indicating a strong negative correlation between these variables with a statistically significant result. Conversely, when examining the relationship between $\sum_8$OPEs concentration and river level, a negative correlation was observed; however, statistical significance was not achieved ($r = -0.513$, $p = 0.09$).

At the River Tame site (Figure 3.14), both the flow rate and river level exhibited a substantial correlation with $\sum_8$OPEs; however, the statistical significance of these correlations could not be established. The p-values associated with both variables were 0.072 and 0.060, respectively, indicating a tendency towards significance but falling short of the threshold. The Pearson correlation coefficients calculated for these relationships were -0.537 and -0.613, respectively, indicating a moderately strong negative association between the variables.

3.4 Variation Upstream and Downstream of WWTPs

To investigate the effect of wastewater treatment plants on OPE pollution in freshwater systems in the UK, sediment samples were collected upstream and downstream of three WWTPs along River Severn, River Sowe, and River Tame. The following is a discussion of the variation of the 8 target OPEs at the respective sampling points.

3.4.1 River Severn

Figure 3.15 shows the total OPE concentration upstream and downstream of WWTPs at River Severn for each month over the 12 months of sampling. In this period, $\sum_8$ OPEs concentrations downstream of the WWTPs effluent discharge exceeded that measured upstream for 7 out of the 12 months, also for each individual target OPE, mean concentrations downstream of WWTPs exceeded those upstream for 6 out of 8 as shown in Figure 3.16.

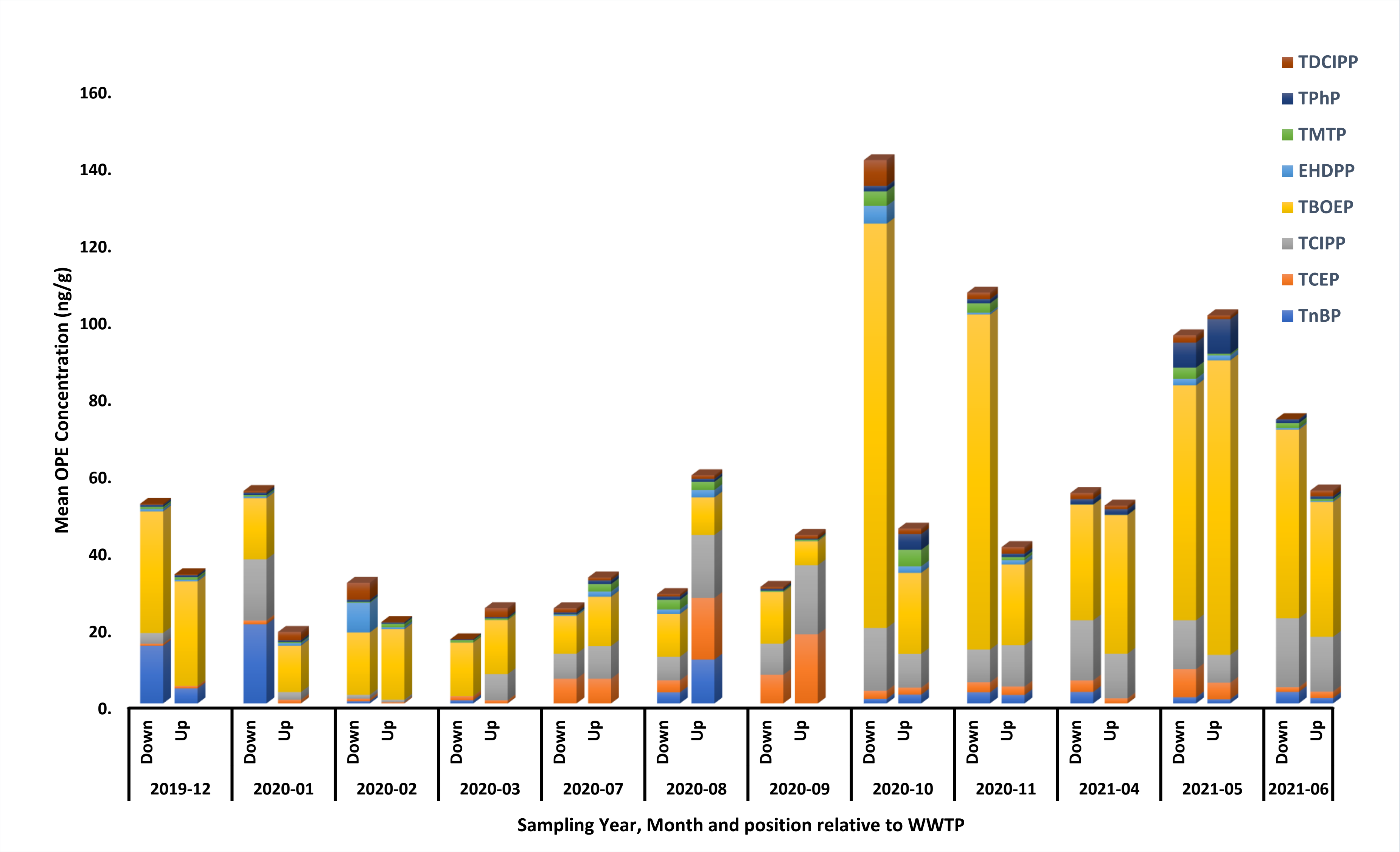


Figure 3. 15. Mean concentrations in sediments upstream and downstream of WWTPs at River Severn.

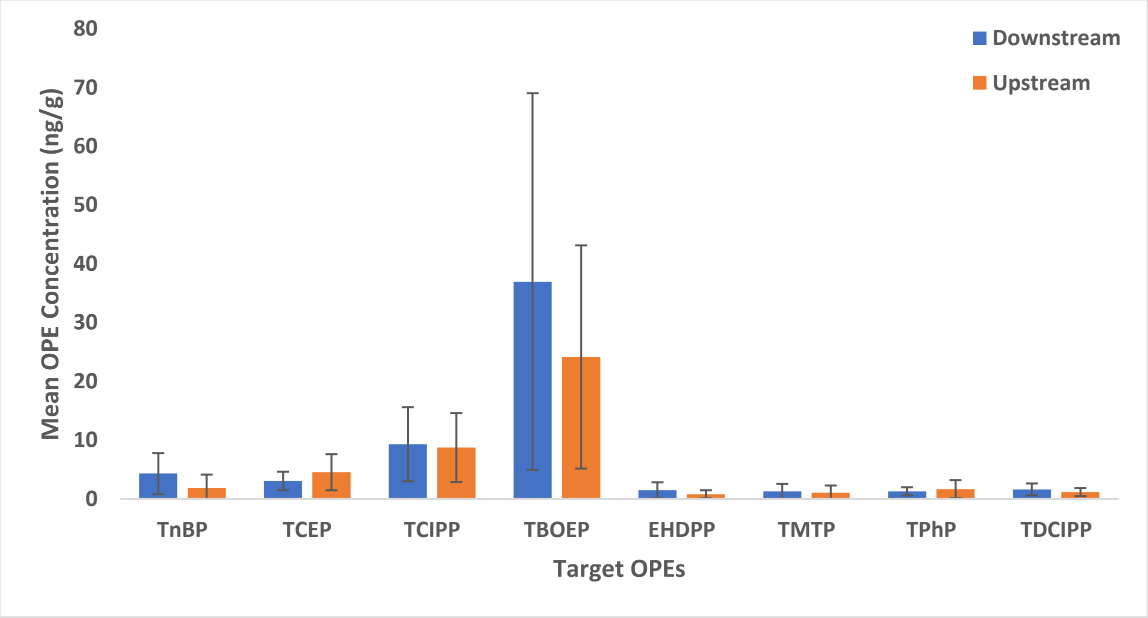


Figure 3. 16. Mean OPE concentration upstream and downstream of WWTP for each target OPE in surficial sediments from the River Severn at Worcester (y-error bar denotes standard deviation).

In order to test the statistical significance of this relationship, a paired sample t-test was performed with the null hypothesis (H_0) that mean OPE concentration downstream of the WWTP is equal to mean OPE concentration upstream (TOPE $_{DS}$ = TOPE $_{US}$). The alternative hypothesis (H_A) therefore will be that the mean OPE concentration downstream is not equal to that upstream of the WWTP (TOPE $_{DS}$ ≠ TOPE $_{US}$) across the 12 months as well as for each target OPE. Paired t-tests were also conducted for individual OPEs upstream and downstream of the WWTPs to eliminate the possibility that using mean OPEs for the paired t-test might mask some differences for individual OPEs (Figure 3.16).

The paired sample t-test for the mean OPEs revealed no statistical significance upstream and downstream of the WWTPs. This is because the p-value was greater than 0.05; hence the null hypotheses could not be rejected. For individual OPEs (TNBP, TCEP, TCIPP, TBOEP, EHDPP, TPHP, TMTP and TDCIPP), there was no significant relationship for 7 out of the 8 OPEs. There was a statistically significant difference between the concentration of TNBP upstream of the WWTP and downstream with a p value of 0.04.

3.4.2 River Sowe

With the River Sowe as shown in Figure 3.17, mean of the OPE concentrations upstream and downstream of a WWTP for each month over the 12 months of sampling shows a similar trend to that observed for the River Severn. The concentrations downstream of WWTP effluent discharge exceeded those upstream for 10 out of the 12 months and were only lower for August 2020 and May 2021 (both of which are among the months with similar results for the Severn River). For each target OPE as shown in Figure 3.18, mean concentrations downstream of the WWTP were more than upstream for all 8 target OPEs. A paired sample t-test was then performed to examine the statistical significance of this observation and the p-value was 0.005. This therefore means that the null hypothesis can be rejected, and the total OPE concentration downstream of the WWTP over the 12 months period are significantly higher than upstream of the WWTP.

For individual OPEs, there was a significant difference in the concentrations of TPHP and TCEP between upstream and downstream of the WWTPs as indicated by p values of 0.02 and 0.05 respectively. For the other target OPEs, the p values were all greater than 0.05 so the null hypotheses could not be rejected.

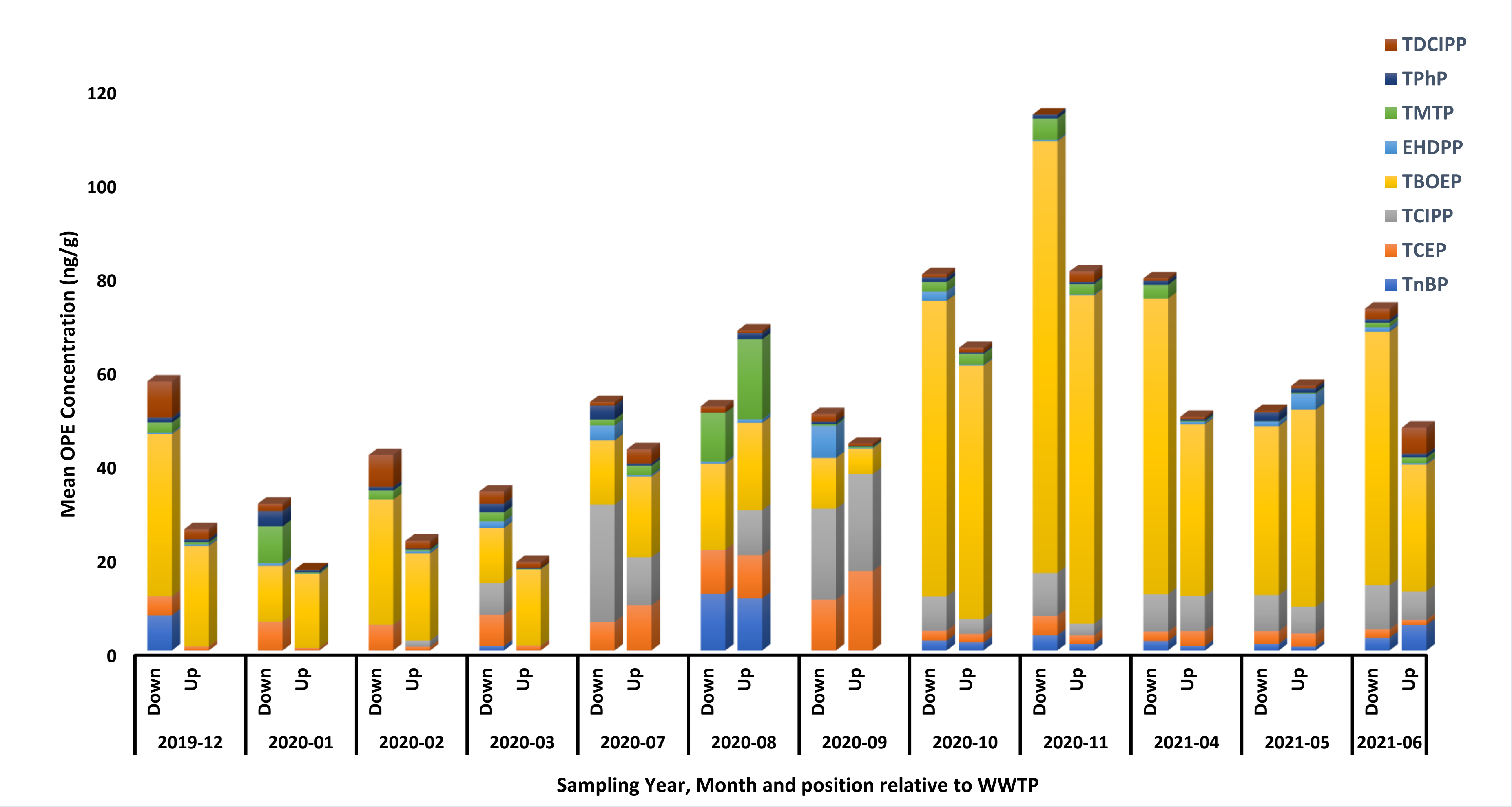


Figure 3. 17. Mean OPE concentrations before and after a WWTP in surficial sediments from the River Sowe.

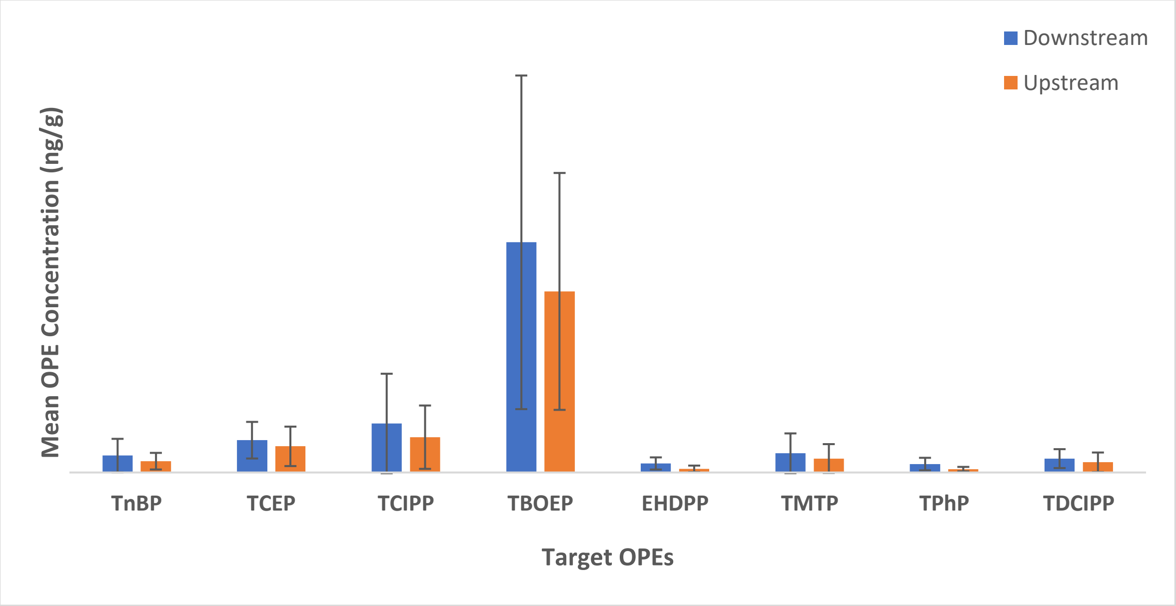


Figure 3. 18. Mean OPE concentration before and after WWTP for each target OPE in surficial sediments from the River Sowe (y-error bar denotes standard deviation).

3.4.3 River Tame

For the River Tame, mean OPE concentrations downstream of WWTP were only higher than those upstream for 5 out of 12 months (Figure 3.19). Also, for each target OPE as shown in Figure 3.20, 4 out of 8 showed higher mean concentrations downstream of WWTP than upstream. A paired sample t-test was performed, and the p-value exceeded 0.05 for total OPEs upstream and downstream of WWTP. A paired sample t test to compare the concentration individual target OPEs upstream and downstream of WWTPs revealed no significant difference for all the target OPEs (Figure 3.20). The null hypothesis could not be rejected as the p values ranged from 0.07 for TDCIPP to 0.7 for TCEP.

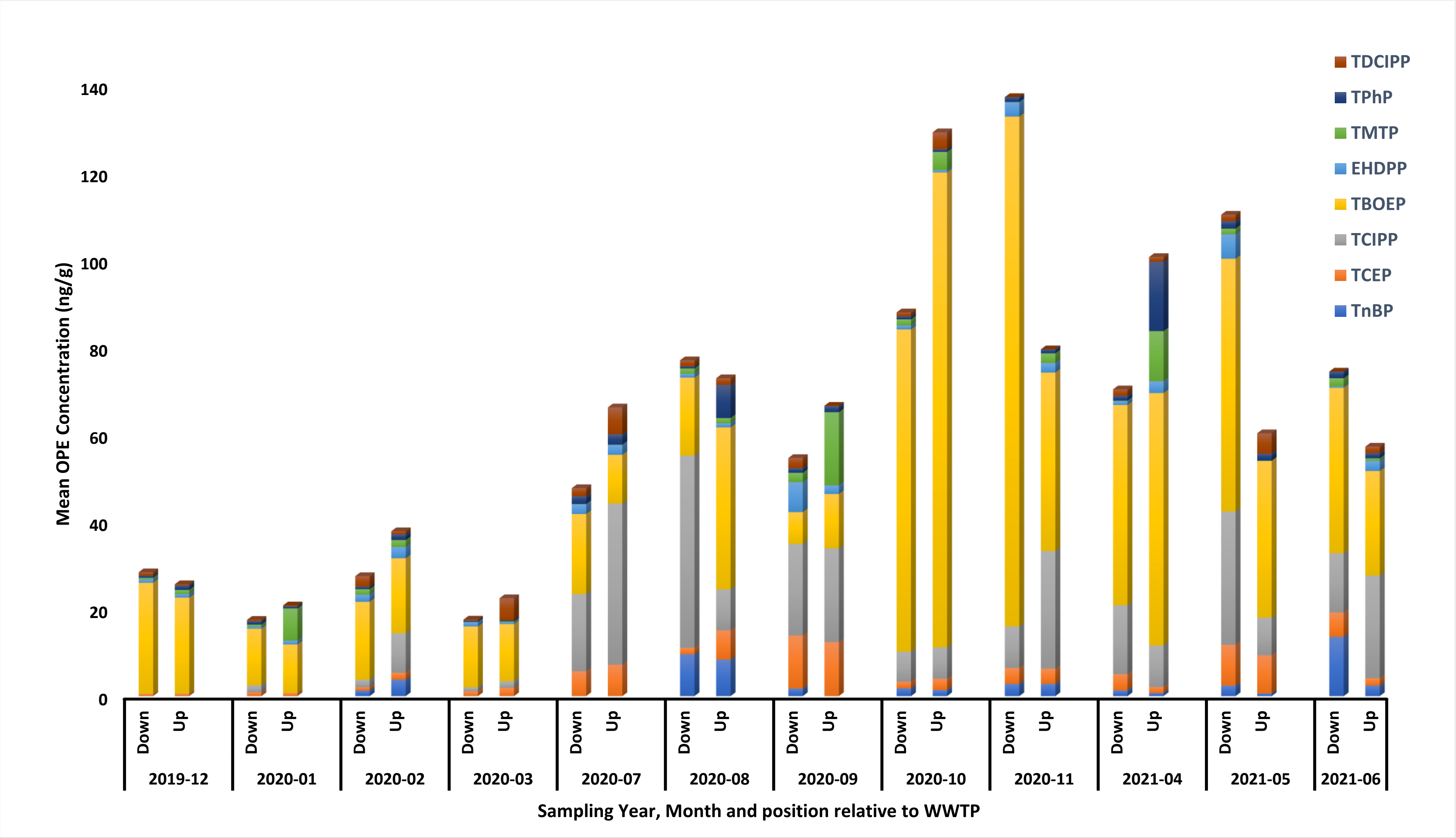


Figure 3. 19. $\sum_8$OPE concentrations in surficial sediment before and after a WWTP on the River Tame.

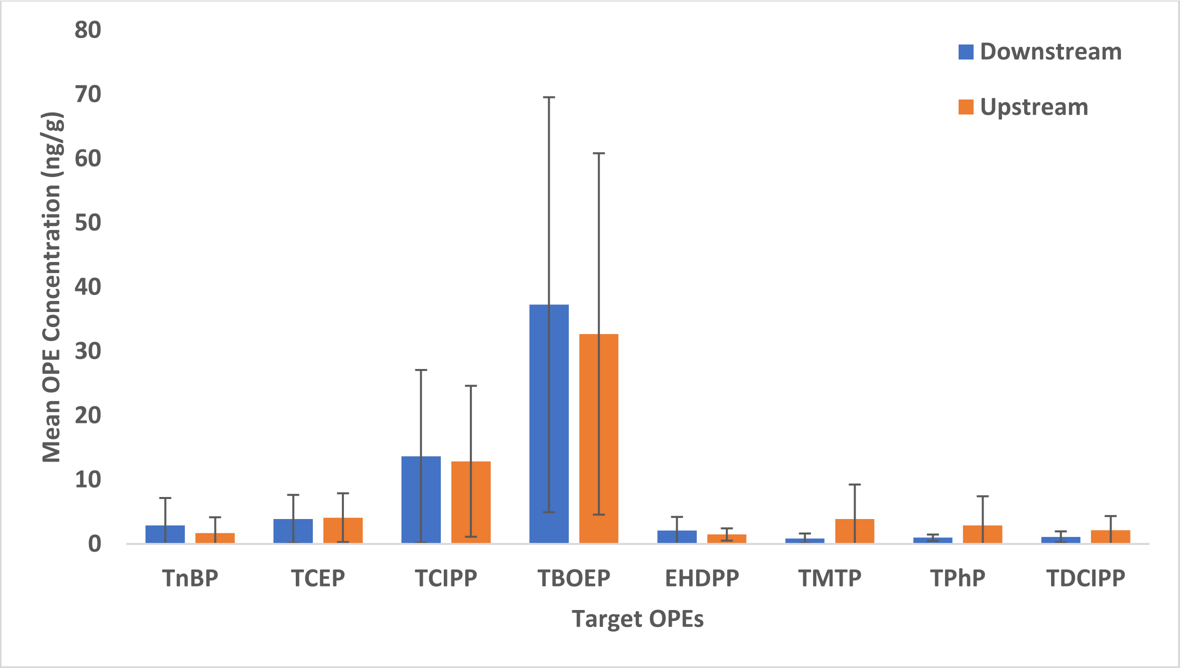


Figure 3. 20. Mean OPE concentrations upstream and downstream of WWTP for each target OPE in surficial sediments from the River Tame (y-error bar denotes standard deviation).

Table 3. 5. Paired sample t-test for total OPEs before and after WWTP over the 12 months sampling period at the Tame River

Similar to some previous studies (Fries and Püttmann, 2003, Li et al., 2020), the results presented here (especially for river Severn and river Sowe) suggests that WWTPs might contribute to OPEs in the sampled rivers however, available research has not provided conclusive evidence on the role of WWTPs as sources of OPEs to the environment (Meyer and Bester, 2004). This is due to a combination of factors including lack of definitive information about removal efficiency by treatment operations, potential degradation of parent OPEs (since they are not highly persistent to aerobic and anaerobic degradation) and their excretion as metabolites (di- and mono-esters) in humans and other animals (Meyer and Bester, 2004, Pantelaki and Voutsa, 2022, Petropoulou et al., 2016).

3.5 Ecological risk assessment

To assess the risk of the target OPEs on aquatic organisms, the risk quotient (RQ) method which has been used by several researchers was used (Shi et al., 2016, Gao et al., 2020, Yadav and Devi, 2020). The RQ is calculated as a quotient of the measured environmental concentration (MEC) and the predicted no effect concentration (PNEC) as shown in equation 3.1.

$$\boldsymbol{RQ = \frac{MEC}{PNEC}} \quad \text{………………………………………………………………. …………}3.1$$

$$\boldsymbol{PNEC = \frac{EC_{50}}{f}} \quad \text{………………………………………………………………………..……}3.2$$

PNEC is calculated by dividing the toxicological relevant concentration (EC_{50}) by an assessment factor (f) sometimes referred to as an uncertainty factor and/or a safety factor, or a combination of both (which is a numeric factor applied to the PNEC in order to derive a safe environmental concentration for a particular substance) (Kwon and Lee, 2017, Karrer, 2019). The PNEC values used in this study were obtained from previous studies (Wang et al., 2019b, Hu et al., 2021, Verbruggen et al., 2006) and the RQ for all study locations are shown in Table 3.6.

The potential risk from these OPEs to aquatic organisms were determined from the RQ values calculated such that RQ values ranging from 0.01 to 0.1 were classed as low risk. RQ values between 0.1 and 1 as moderate risk while RQ values above 1 were classed as high risk (Liu et al., 2017, Yan et al., 2017, Xing et al., 2018). All target compounds in all study locations in this study fall within the low-risk category except for EHDPP in the Worcester and Birmingham canal which can be considered to pose moderate risk since it has a RQ value of 0.29. Also, the RQs for EHDPP in Rivers Sowe and river Tame are quite close to the moderate level. This result is very similar to the work of Xing et al., 2018 where EHDPP was also reported as a predominant contributor to ecological risk in surface water (Xing et al., 2018). The PNEC value used for any ecological risk assessment plays a key role in the evaluation of the risk and the fact that this value can vary significantly depending on the PNEC derivation methodology used has been a concern (Jung et al., 2021). It has been reported that the two approaches that have been proposed for deriving the PNEC value of chemicals (the assessment factor (AF) approach and the species sensitivity distribution (SSD) approach) can lead to overestimation or underestimation of risk (Jung et al., 2021). Furthermore, the lack of knowledge on the toxicological impact of exposure to chemical mixtures must be acknowledged. The toxic effect of chemicals can change as a result of the influence of other chemicals on their biological action as well as other possible interactions between chemicals (synergistic or antagonistic effects)(Binderup et al., 2003, Bopp et al., 2019) however, most current risk assessments are based on single chemicals (Bopp et al., 2019).

Table 3. 6. RQ for Target OPEs at River Sowe, River Tame, River Severn and Worcester Birmingham Canal.

River Tame			
OPEs	**MEC (ng/g)**	**PNEC (ng/g)**	**RQ**
TnBP	2.3	900	0.003
TCEP	4.0	386	0.010
TCIPP	13.2	1700	0.008
TBOEP	35.0	2480	0.014
EHDPP	1.8	30	0.059
TMTP	2.4	/	/
TPhP	1.9	130	0.015
TDCIPP	1.6	320	0.005

Worcester & Birm Canal			
OPEs	**MEC (ng/g)**	**PNEC (ng/g)**	**RQ**
TnBP	18.3	900	0.020
TCEP	6.4	386	0.017
TCIPP	9.0	1700	0.005
TBOEP	53.3	2480	0.022
EHDPP	8.8	30	0.292
TMTP	4.1	/	/
TPhP	4.2	130	0.032
TDCIPP	3.1	320	0.010

River Sowe			
OPEs	**MEC (ng/g)**	**PNEC (ng/g)**	**RQ**
TnBP	2.2	900	0.002
TCEP	4.6	386	0.012
TCIPP	6.7	1700	0.004
TBOEP	32.4	2480	0.013
EHDPP	1.0	30	0.033
TMTP	2.6	/	/
TPhP	0.9	130	0.007
TDCIPP	1.9	320	0.006

River Severn			
OPEs	**MEC (ng/g)**	**PNEC (ng/g)**	**RQ**
TnBP	3.1	900	0.003
TCEP	3.8	386	0.010
TCIPP	9.0	1700	0.005
TBOEP	30.6	2480	0.012
EHDPP	1.1	30	0.037
TMTP	1.2	/	/
TPhP	1.5	130	0.011
TDCIPP	1.4	320	0.004

3.6 Comparison with Results from other parts of the world

Although there are several studies on organophosphate esters in environmental media such as water (Vasseghian et al., 2022, Zhu et al., 2022), air (Hartmann et al., 2004, Zhou et al., 2017), indoor dust (Brommer et al., 2012, Brommer and Harrad, 2015), food (He et al., 2018a, Zhou et al., 2022). The current body of literature pertaining to OPEs within freshwater sediments is notably scarce. This observation is substantiated by a systematic review conducted via the Web of Knowledge database, utilizing diverse combinations of the terms "Organophosphate," "Freshwater," and "Sediments." This search only came up with thirty pertinent studies in the five-year span from 2017 to 2022. Importantly, upon further scrutiny, only fifteen of these studies presented a research focus and findings relevant to the ones in the current investigation. One result of our study is the observation of higher OPE concentrations downstream of WWTPs compared to upstream (with a statistically significant difference observed for the River Sowe). This result agrees with the findings of several previous studies where WWTPs were identified as potential sources of OPEs to freshwater systems (Zhang et al., 2022b, Cristale and Lacorte, 2013, Xing et al., 2018, Chokwe and Okonkwo, 2019). In terms of seasonal variation, the lowest concentration of OPEs in this study was recorded in winter as was the case with Wu et al., 2020 and the highest concentration of OPEs in this study was also recorded in summer and autumn just like Wu et al., 2020 who reported the highest concentrations of OPEs between June and September (Wu et al., 2020b). Table 3.7 shows that the concentrations of OPEs detected in the current investigation exhibit similarities with certain preceding studies, while simultaneously demonstrating discrepancies with others.

Table 3. 7. Comparison between OPE concentrations in surficial freshwater sediments in this study and results from other parts of the world.

Study	**Location**	**TNBP (ng/g)**	**TCEP (ng/g)**	**TCIPP (ng/g)**	**TBOEP (ng/g)**	**EHDP (ng/g)**	**TMTP (ng/g)**	**TPHP (ng/g)**	**TDCIPP (ng/g)**
This study	River Tame	2.28	3.98	13.25	34.97	1.77	2.38	1.92	1.61
This study	River Sowe	2.22	4.61	6.65	32.44	1.00	2.62	0.93	1.90
This study	River Severn	3.08	3.78	9.01	30.57	1.12	1.16	1.47	1.38
This study	Worcester and Birmingham Canal	18.28	6.44	8.96	53.34	8.82	4.08	4.21	3.11
(Cristale et al., 2013a)	River Besòs (River Sediment)	8.40	7.12	164.71	< LOD	37.71	-	13.26	8.74
(Xing et al., 2018)	Luoma Lake, China (Lake Sediment)	0.02	0.38	0.06	0.001	0.06	0.06	0.01	-
(Wang et al., 2018b, Liu et al., 2018b)	Lake Taihu, China (Lake sediment)	0.00	3.00	1.20	0	0.38	0.38	1.70	0.10
(Giulivo et al., 2017)	Evrotas River Sediment (Greece)	2.39	1.78	4.59	1.47	4.82	4.82	0.36	1.63
(Giulivo et al., 2017)	Adige River (Italy)	5.52	2.51	14.93	2.36	37.31	37.31	0.86	2.31
(Giulivo et al., 2017)	Sava River (Slovenia, Croatia, Bosnia and Herzegovina and Serbia)	7.65	0.79	6.6	3.16	4.76	4.76	-	0.36
(Cao et al., 2017)	Lakes Superior (Sediment)	0.34	0.15	0.15	0.03	0.01	0.01	0.04	-
(Cao et al., 2017)	Lake Michigan. (Sediment)	0.52	ND	0.39	1.52	0.03	0.03	0.17	-
(Cao et al., 2017)	Lake Ontario (Sediment)	1.43	ND	0.71	7.29	0.31	0.31	2.62	-
(Chokwe and Okonkwo, 2019)	Vaal River, South Africa.	37.51	2.56	1.14	26.21	-	-	2.88	0.66
(Yadav and Devi, 2020)	Kathmandu valley, Nepal. (River Sediment)	76.41	18.46	112.59	-	135.60	-	39.60	5.66
(Gao et al., 2020)	The Arctic	-	1.30	1.15	2.64	0.24	-	0.37	0.39

Table 3.7 presents a comparison between this study and 12 other studies from different parts of the world. The average concentrations of each target OPE in each study location (for this study) is lower than the concentration reported in some previous studies (Cristale et al., 2013a, Xing et al., 2018) but fall broadly in the middle of the range previously reported (Table 3.7). The most abundant OPE in all locations in this study was TBOEP and it shows a higher average concentration than was reported in all 12 previous studies considered here. TBOEP was also reported as one of the most abundant OPEs in sediments of the Vaal River in south Africa (Chokwe and Okonkwo, 2019) and this can be attributed to the fact that it is one of the predominant OPEs found in the environment (Giraudo et al., 2017). For TCEP and TCIPP, the average concentration in all the locations in this study (River Tame, River Sowe, River Severn, and the Worcester Birmingham canal) was higher than the concentration reported in most of the studies considered. Also, the inverse relationship between river flow rate and OPE concentration reported in this study is similar to the findings of Cristale et al, 2013 following a study targeting 10 OPEs at River Besòs in Spain (A River impacted by industrial effluent and high urban population) (Cristale et al., 2013a).

This study is the first report of OPEs in UK freshwater sediments and the concentrations fall broadly within the range reported in previous studies. The concentrations of OPEs varied by season and is influenced by flowrate and water level. The impact of WWTPs on OPE concentrations in sediment was assessed and the result suggests that urbanisation and proximity to potential anthropogenic sources of contamination might have a greater influence on freshwater sediment contamination with OPEs than WWTPs. An assessment of the risk posed by the OPEs to aquatic organism appears to be low although the PNEC are based on historical studies calculated with the highest uncertainty factor of 1000.

CHAPTER 4

4.1 Occurrence, distribution, and seasonal variation of MPs in freshwater sediment and relationship to OPEs.

4.1.1 Synopsis

Results of the identification and characterisation of MPs in sediment samples collected from Worcester- Birmingham canal, River Tame, River Severn and River Sowe are analyzed and presented in this chapter. The abundance, distribution, and seasonal variation of MPs in these sediment samples are investigated and presented. The concentrations of OPEs in the sediments as presented in Chapter 3 are then compared to the abundance and distribution of the MPs to establish any possible relationships. MPs were found in all sampling locations all year round. MPs abundance across all study locations are presented in MPs per kg of dry weight sediment sample (MPs/kg dw).

4.2 Occurrence and concentrations of MPs in UK freshwater sediments

As outlined in Chapter 2, the evaluation of microplastics (MPs) in the sediment samples involved the utilization of 30 grams of sediment. The enumeration of the MPs was consequently extrapolated to ascertain the count of MPs per kilogram as illustrated in Table 4.1. Figure 4.1 also shows the range, median and quartiles of the total number of MPs/kg for each month across all four study locations. The mean number of MPs/kg dw for all 4 study locations ranged from 67 – 267 (Worcester and Birmingham canal), 133 – 283 (River Tame), 66.67 – 317 (River Severn) and 67 – 233 (River Sowe) per month.

The highest mean number of MPs/kg was recorded in the River Tame (Figure 4.2) which as mentioned in chapter 3, is the study location with effluent from the wastewater treatment plant servicing the highest population of all three sampling locations (approximately 2.5 million people within the Birmingham area). The lowest mean number of MPs/kg was at the Worcester and Birmingham canal which although is the only location (of all four sampled UK freshwater systems) not receiving direct input from a wastewater treatment plant, is at the most urban location.

Table 4. 1: Mean and Median number of MPs/ 30gs of sediment samples analysed and Mean number of MPs/kgs of sediment.

Sampling Date	**Location**	**Mean MPs/30 g (dw)**	**Median MPs/30 g (dw)**	**Mean MPs/kg (dw)**
2019-12	River Sowe	3.5	2.3	117
2019-12	River Tame	6.5	3.8	217
2019-12	Worcester & Birmingham Canal	4.0	2.0	133
2019-12	River Severn	9.5	5.0	317
2020-01	River Sowe	4.0	2.5	133
2020-01	River Tame	8.5	4.3	283
2020-01	Worcester & Birmingham Canal	8.0	4.0	267
2020-01	River Severn	9.5	5.5	317
2020-02	River Sowe	5.0	2.4	167
2020-02	River Tame	6.0	4.0	200
2020-02	Worcester & Birmingham Canal	8.0	4.0	267
2020-02	River Severn	5.5	3.0	183
2020-03	River Sowe	5.5	3.5	183
2020-03	River Tame	8.5	4.5	283
2020-03	Worcester & Birmingham Canal	2.0	1.0	67
2020-03	River Severn	7.5	5.0	250
2020-07	River Sowe	2.0	1.0	67
2020-07	River Tame	6.5	3.5	217
2020-07	Worcester & Birmingham Canal	2.0	1.0	67
2020-07	River Severn	4.0	2.0	133
2020-08	River Sowe	7.0	4.5	233
2020-08	River Tame	8.0	3.8	267
2020-08	Worcester & Birmingham Canal	2.0	2.0	67
2020-08	River Severn	6.0	3.5	200
2020-09	River Sowe	3.5	2.0	117
2020-09	River Tame	5.0	3.0	167
2020-09	Worcester & Birmingham Canal	4.0	3.0	133
2020-09	River Severn	6.5	3.5	217
2020-10	River Sowe	2.0	1.0	67
2020-10	River Tame	4.0	2.5	133
2020-10	Worcester & Birmingham Canal	3.0	2.0	100
2020-10	River Severn	6.0	3.0	200
2020-11	River Sowe	3.0	2.0	100

2020-11	River Tame	6.0	3.5	200
2020-11	Worcester & Birmingham Canal	2.0	1.0	67
2020-11	River Severn	3.5	2.0	117
2021-04	River Sowe	2.5	2.0	83
2021-04	River Tame	4.5	2.8	150
2021-04	Worcester & Birmingham Canal	3.0	2.0	100
2021-04	River Severn	2.5	1.5	83
2021-05	River Sowe	3.0	1.5	100
2021-05	River Tame	4.0	2.5	133
2021-05	Worcester & Birmingham Canal	2.0	1.0	67
2021-05	River Severn	4.0	2.3	133
2021-06	River Sowe	2.0	1.3	67
2021-06	River Tame	4.0	2.5	133
2021-06	Worcester & Birmingham Canal	2.0	1.5	67
2021-06	River Severn	2.0	1.0	67

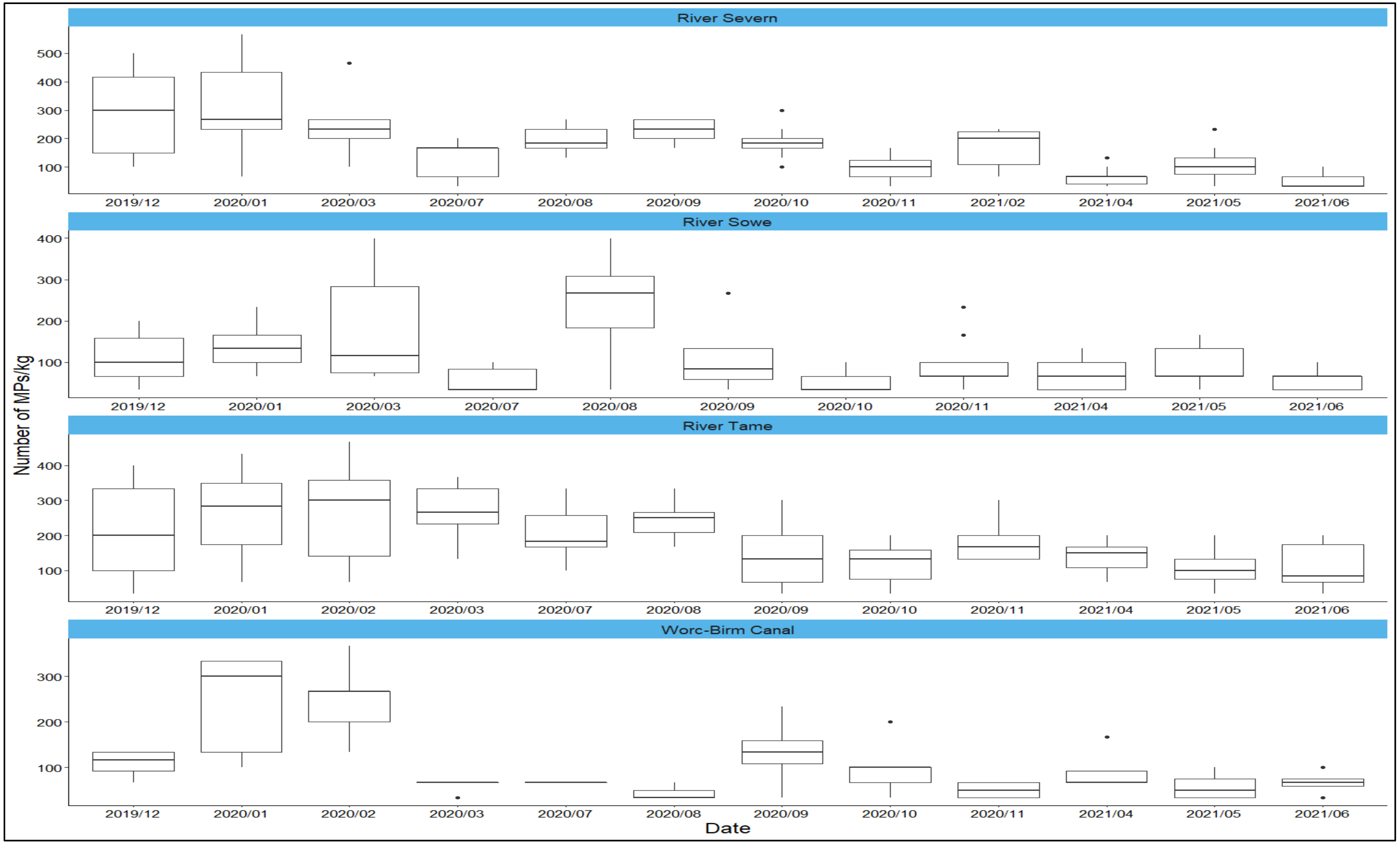


Figure 4. 1. Boxplots of the total number of MPs/kg for each month across all four study locations (River Severn, River Sowe, River Tame and Worcester & Birmingham Canal

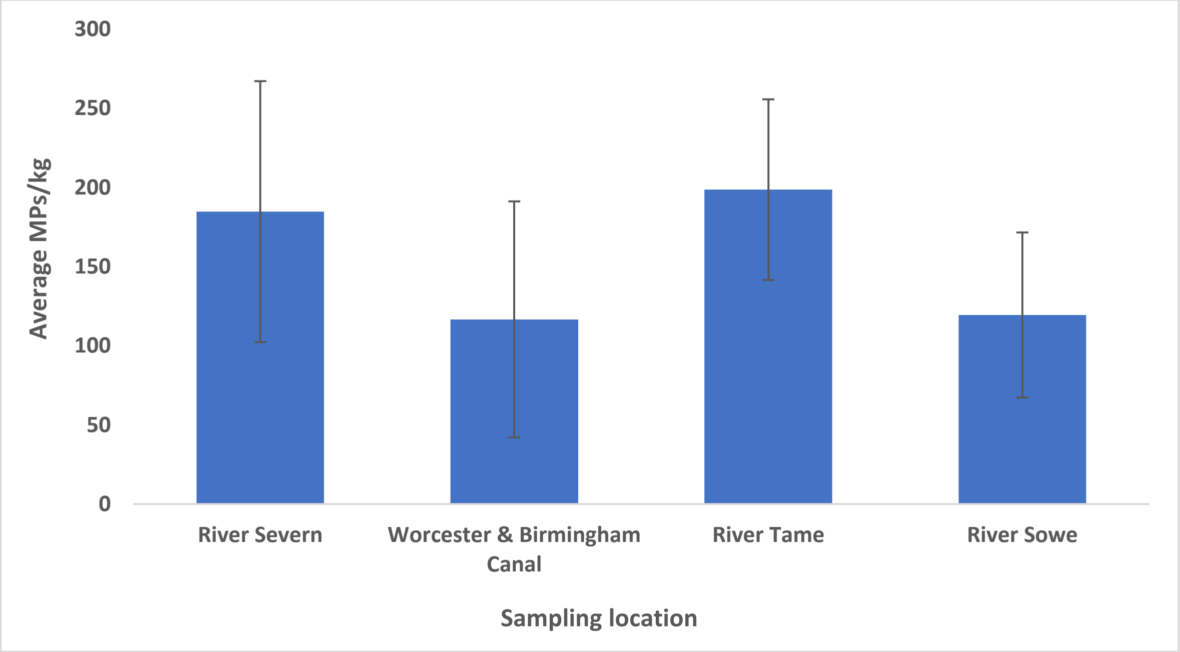


Figure 4. 2. Average MP/kg in River Severn, River Sowe, River Tame and Worcester & Birmingham Canal. (Y error bars = 1 standard deviation).

To ascertain the significance of the observed disparities in the abundance of MPs across the study sites, a one-way Analysis of Variance (ANOVA) coupled with Tukey's post hoc test were employed. The MPs' abundance in the Worcester Birmingham Canal was significantly higher than that in the River Severn ($P = 0.039$) and the River Tame ($P = 0.005$). Similarly, the quantity of MPs in the River Tame significantly surpassed that in the River Sowe ($P = 0.02$). The prevalence of MPs as found in this study aligns with the quantities documented by Wu et al., 2020 in China (25 to 560 µm) and Sekudewicz et al., 2021 in Poland (190 to 580).

4.3 Morphological characteristics of MPs

Following the identification of MPs using the stereo microscope, the various MPs were grouped into fibres, fragments, and pellets as shown in Figure 4.3 (Zhao et al., 2014).

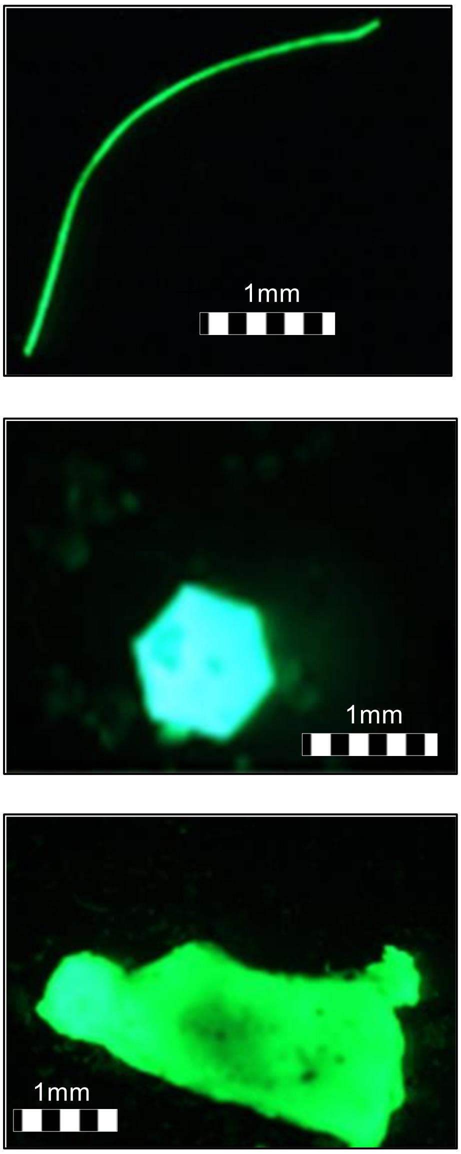


Figure 4. 3. Fibres, fragments, and pellets as identified by Nikon SMZ-1000 stereo microscope set up.

The most dominant morphological group in all the isolated MPs across all 4 study locations over the 12 months period are fragments (71%) and the least abundant are fibres (9%) (Figure 4.4). This low percentage of fibres can be attributed to a number of factors one of which is their shape and low density which can reduce their settlement (Hoellein et al., 2019). Also, previous studies have reported that the Nile Red method is not very effective with fibres and microfibres- especially polyamide or polyester fibres (Tamminga et al., 2017, Shim et al., 2016, Nel et al., 2021). The abundance of fragments suggest the possibility of the MPs in these locations originating from secondary sources (Karim, 2021).

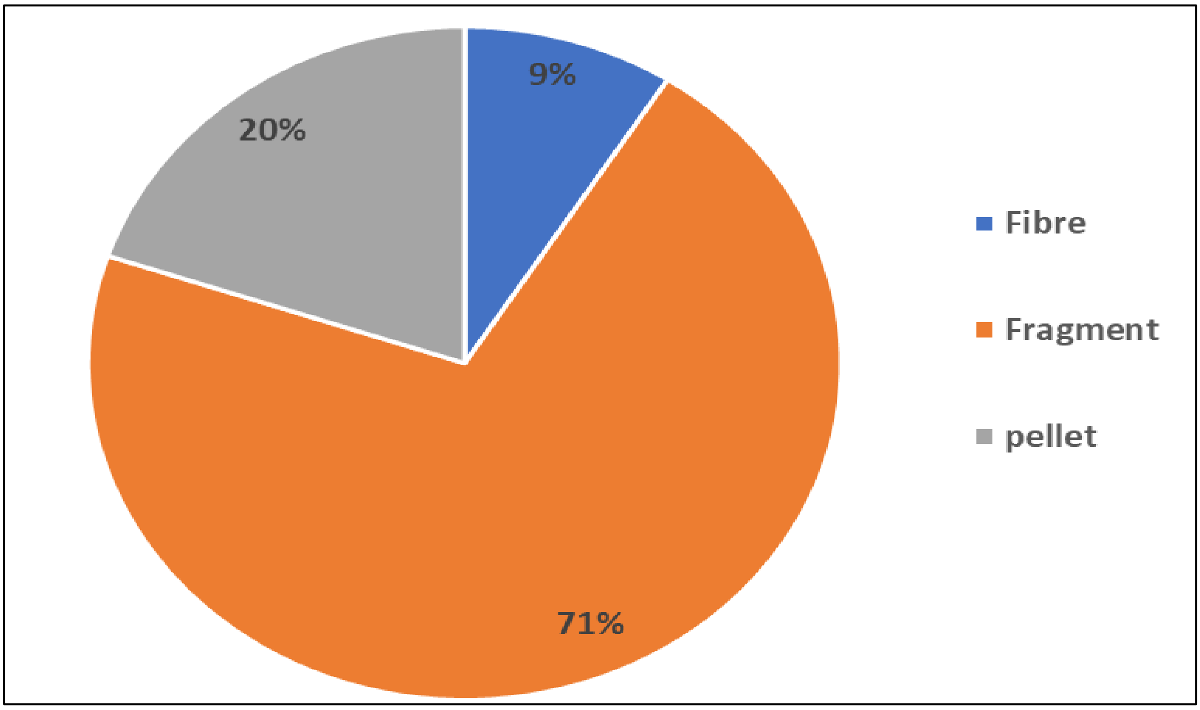


Figure 4. 4. Morphology of isolated MPs from all sampling locations over the entire period of sampling.

The ratio at individual study locations were similar to Figure 4.4 with fragments being the most abundant shape (Figure 4.5). At the Birmingham and Worcester Canal, the most dominant MP type is fragments (84%) and the least dominant fibres (5%). Fragments were also dominant at River Tame (85%), River Severn (85%) and River Sowe (80%). Like the present study, the most dominant MP shape reported in most of the reviewed studies (N = 10) as shown on Table 4.3 are fragments and the second most common shapes were fibres. A study by Margenat et al., 2021 reported 95% of identified MPs as fragments (Margenat et al., 2021). Similarly, in a study by Ramírez-Álvarez et al.,(2020b) fragments and fibres accounted for 70% and 28% respectively of the MPs identified (Ramírez-Álvarez et al., 2020b). This abundance of fragments in sediments as also reported by some previous studies can be attributed to the fact that freshwater environments receive a continuous influx of plastic waste from various sources, such as urban runoff, industrial discharges, and improper waste management fragments which often sink to riverbeds (Wang et al., 2017).

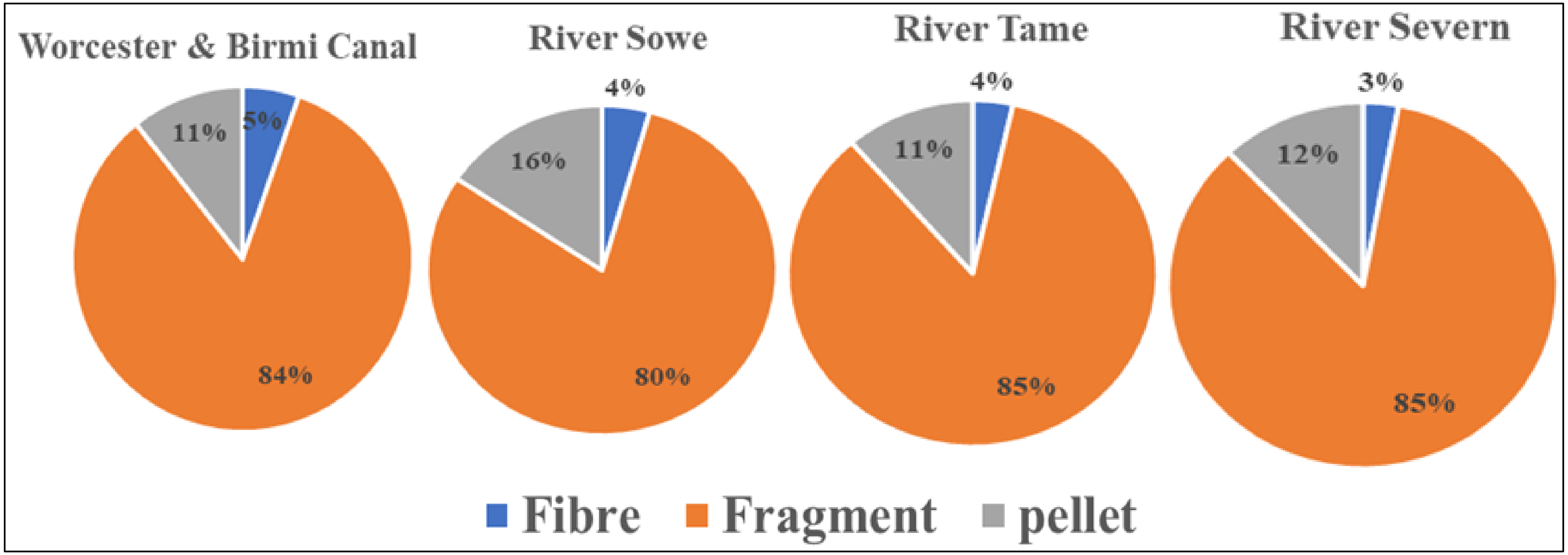


Figure 4. 5. Morphology of isolated MPs from the Worcester- Birmingham canal, River Tame, River Severn, and River Sowe.

In terms of size variation, there was an increasing trend from larger (5000 µm) to smaller size (63 µm) across all study locations. The cut off point for lower size range in this study is 63 µm because a 63 µm mesh size sieve was used prior to sample analysis as outlined in section 2.2.1. At the Worcester and Birmingham Canal, the most abundant size range (52%) is 63 – 199 µm. This is followed by the 200 – 399 µm size range which accounts for 24% of the MPs isolated in this location (Figure 4.6).

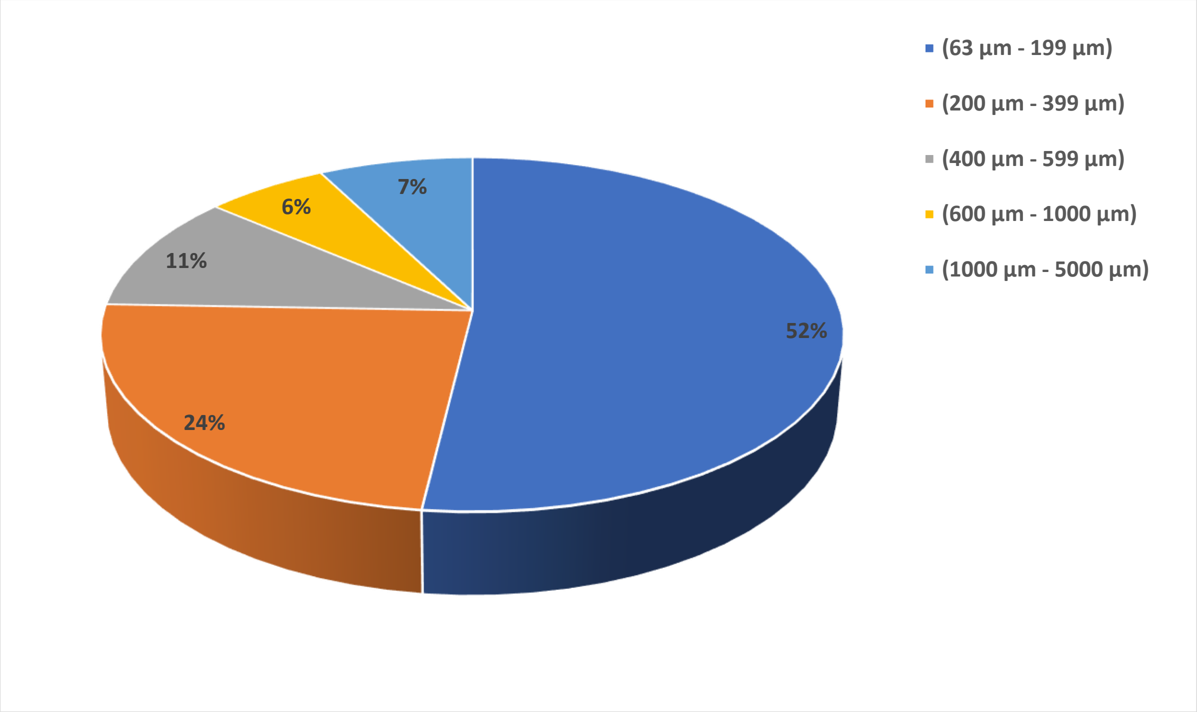


Figure 4. 6. Size range of MPs at the Worcester and Birmingham Canal.

As shown on Figure 4.7 the most abundant size range at the River Severn (29%) is 200 – 399 µm and the second most abundant size range is the 63 – 199 µm (25%) size range which then places MPs with sizes lower than 400 µm at 54%. The larger size ranges in this study (1000 – 5000 µm) account for only 7% of the isolated MPs in this location which agrees with most previous studies reviewed as part of this work. (Table 4.3).

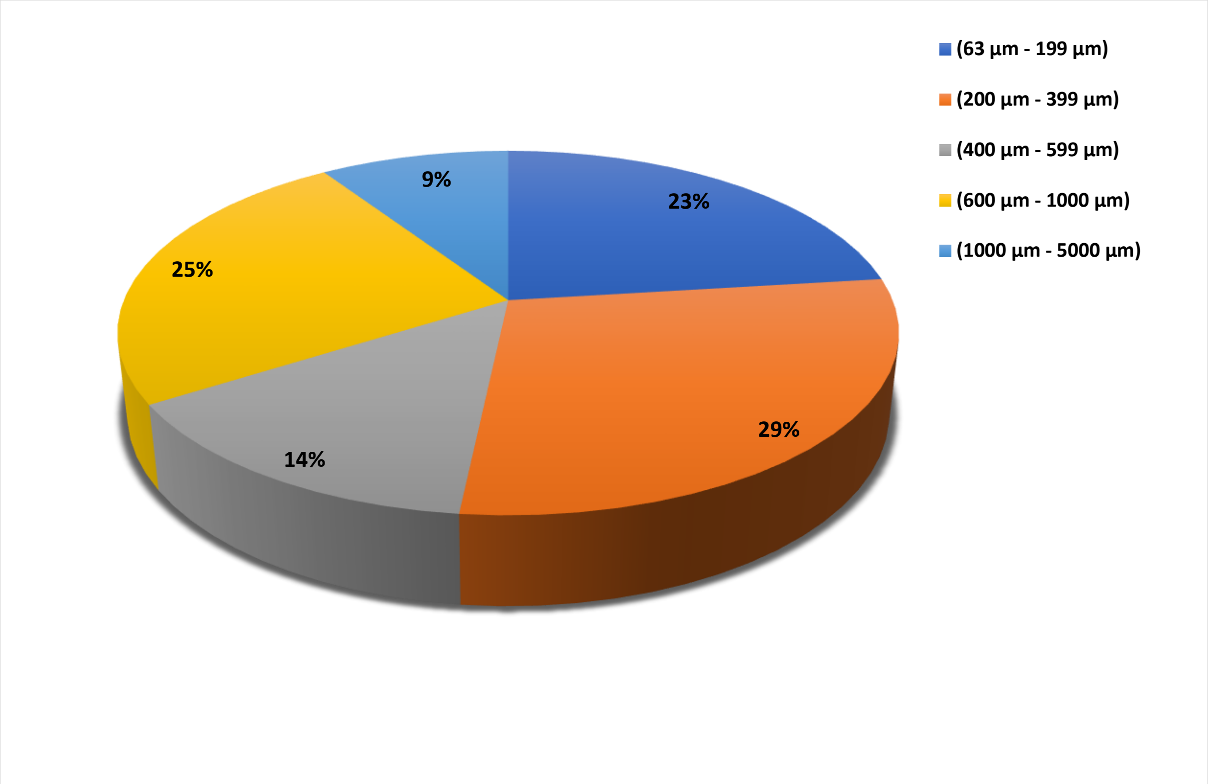


Figure 4. 7. Size range of MPs at the River Severn.

At the River Sowe, 63 – 199 µm was the most abundant size range (41%) (Figure 4.8), as well as River Tame (39%) (Figure 4.9). Unlike in the Worcester and Birmingham Canal (the only location without WWTP input), where more than 50 % of isolated MPs fall within the lower size range (Figure 4.6), the MPs are relatively more evenly distributed across the five size ranges in the locations with WWTPs (River Severn, River Sowe, and River Tame) compared to the location without WWTPs (Worcester and Birmingham Canal).

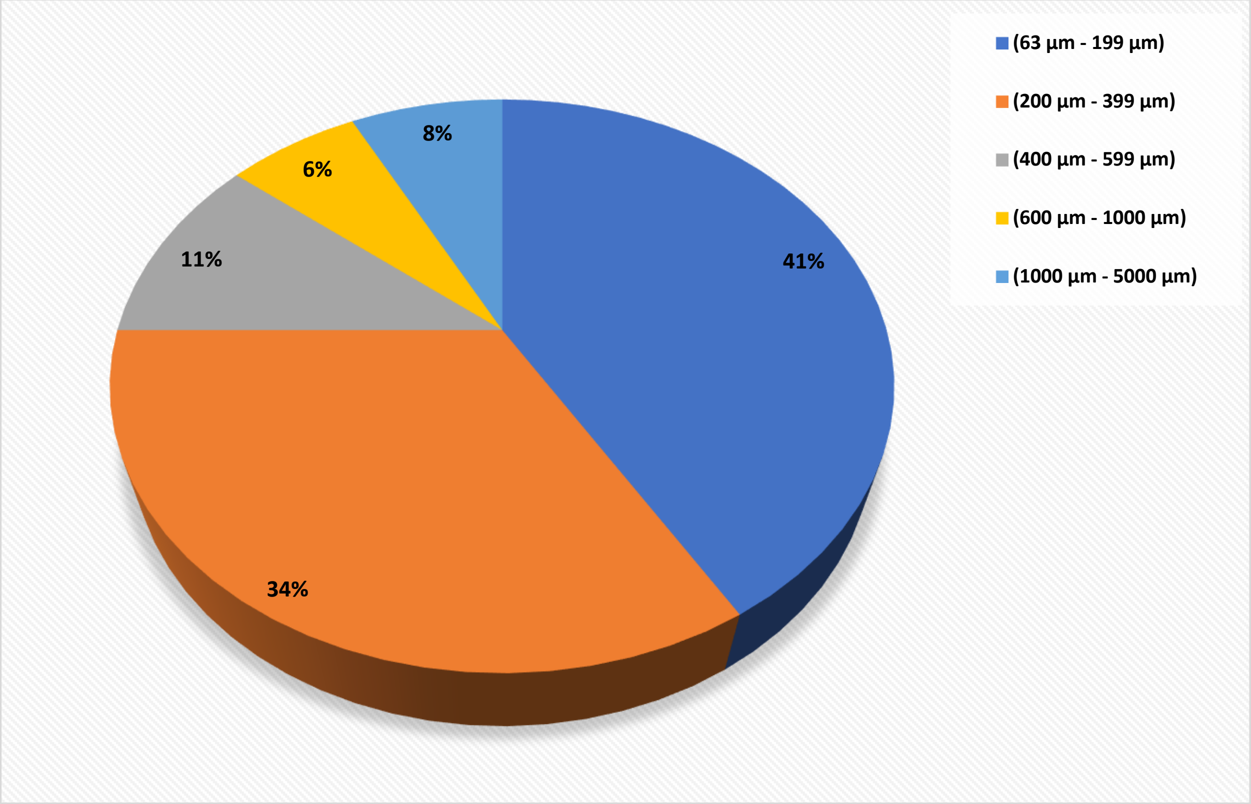


Figure 4. 8. Size range of MPs at the River Sowe.

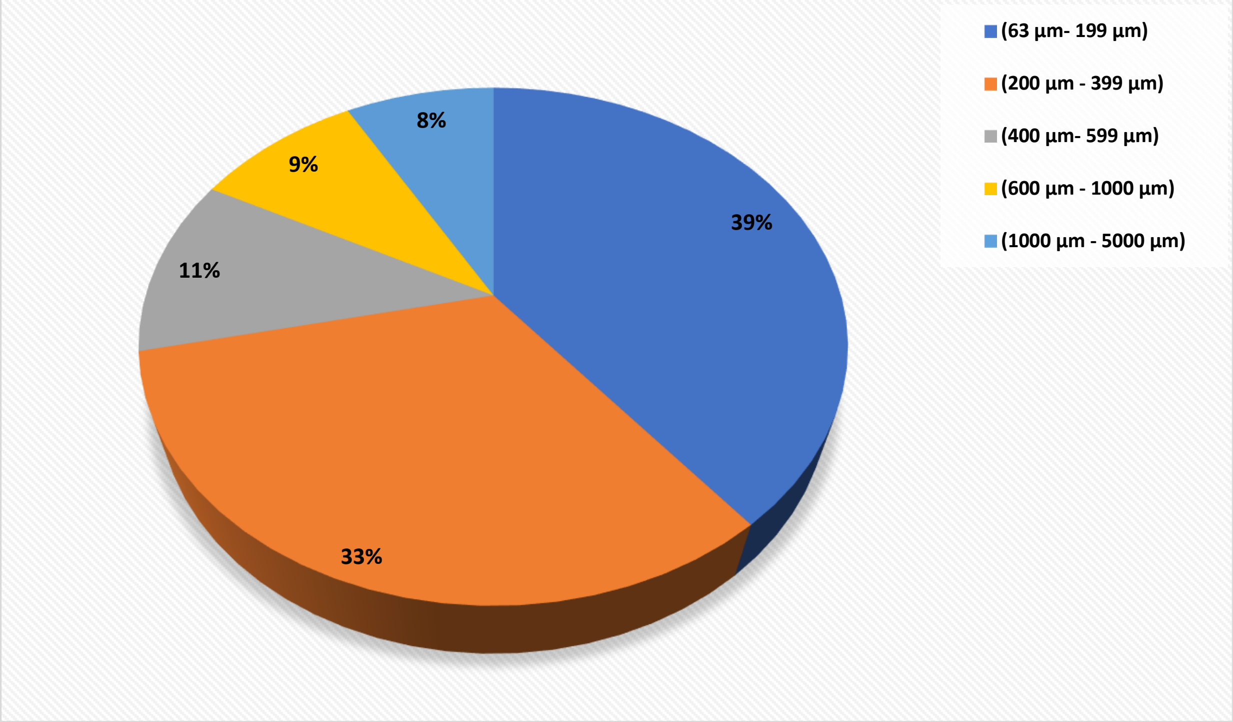


Figure 4. 9. Size range of MPs at the River Tame.

The variation of size range in the present study is also similar to results from previous studies in other parts of the world. The smaller size range (< 1 mm) was the most dominant as reported in studies from other parts of the world such as China (Wu et al., 2020a), and Korea (Park et al., 2020).

4.4 Variation upstream and downstream of WWTPs

Variations were observed between the abundance, as well as distribution of MPs upstream and downstream of River Tame, River Sowe and River Severn. Overall, 57% of the mean number of MPs identified across all three locations over the 12 months period were detected downstream of the WWTPs while 43% was upstream (Figure 4.10).

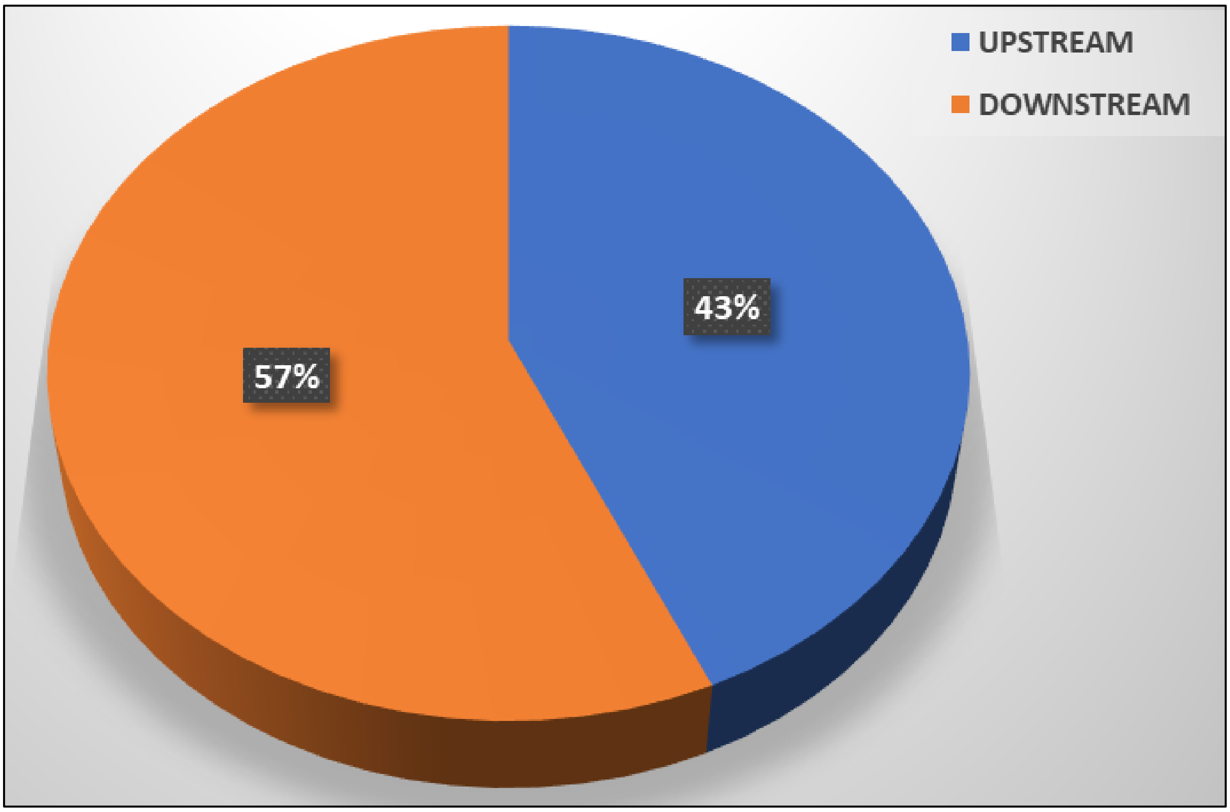


Figure 4. 10. Distribution of Mean MPs upstream and downstream of WWTPs across all three river locations over the 12-month sampling period.

Figures 4.10 and 4.11 show that there were more MPs downstream of WWTPs than upstream for all three locations (River Tame, River Sowe, and River Severn). For the River Severn

(WWTP3), the mean number of MPs upstream and downstream of WWTPs ranged from 67 to 267 and 67 to 433 MPs/kg dw respectively. River Sowe (WWTP2) which has the least MP abundance had a monthly mean number of MPs upstream and downstream of WWTP2 ranging from 67 to 167 and 33 to 300 MPs/kg dw respectively.

The monthly mean number of MPs upstream and downstream of the WWTP (1) on the River Tame ranged from 100 to 267 and 100 to 367 MPs/kg dw respectively.

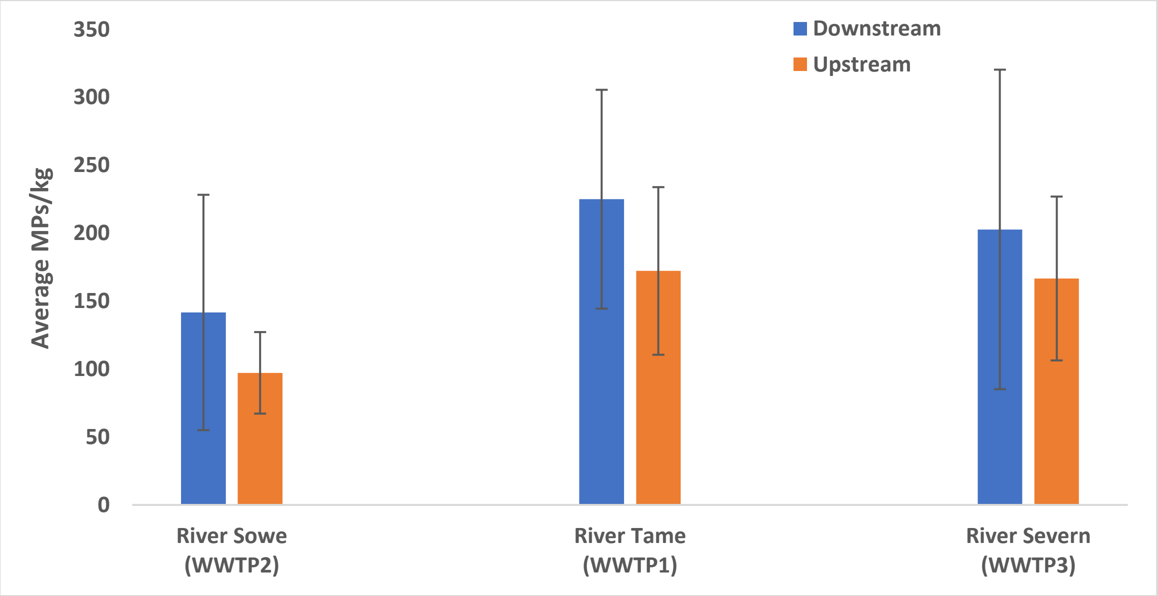


Figure 4. 11. Mean MPs/kg Upstream and Downstream of WWTPs. (Y error bars are standard deviation error bars showing variation around the mean).

The observed difference between the abundance of MPs upstream and downstream of WWTPs across all study locations were however not statistically significant as the p-values obtained after a paired sample t-test were all greater than 0.05. This however does not negate the observed relationship between MP abundance upstream and downstream of WWTPs in Figure 4.11 as previous studies identified WWTPs as potential contributors to MP pollution in the freshwater environment (Margenat et al., 2021, Sá et al., 2022, Sekudewicz et al., 2021). In 5 out of 10 reviewed studies from other parts of the world (Table 4.3), WWTPs were identified as a possible contributor of MP (mostly Fibres) pollution in freshwater systems.

Regarding morphological distribution, the analysis revealed a greater abundance of fragments downstream compared to upstream across all four study locations, as depicted in Figure 4.12. However, the distribution of fibres and pellets exhibited less distinct patterns. Specifically, while the number of fibres downstream was higher for both Rivers Severn and Tame, it remained constant upstream and downstream for River Sowe. Likewise, a higher number of pellets were observed downstream for Rivers Sowe and Tame, yet for River Severn, the higher number of pellets was recorded upstream as opposed to downstream.

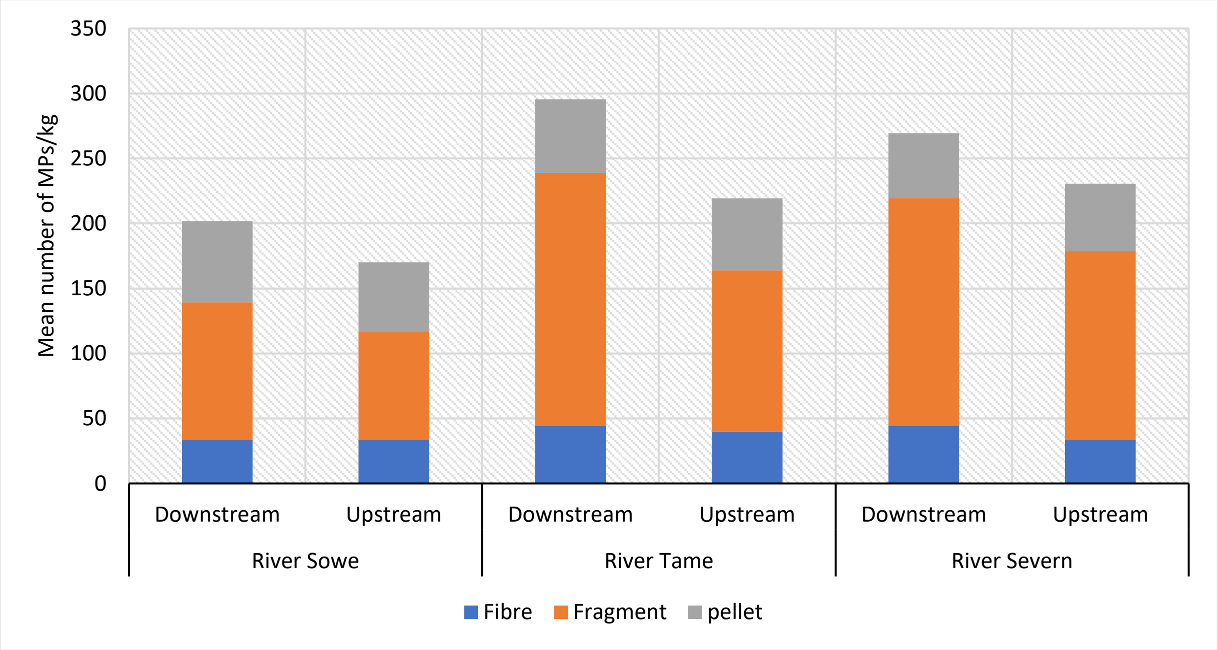


Figure 4. 12: Morphological distribution of MPs upstream and Downstream

The distribution of sizes for fragments, fibres, and pellets at all four study sites, both upstream and downstream, is illustrated in Figure 4.13. It is noteworthy that, on average, both fragments and fibres exhibited marginally longer lengths in the downstream regions compared to their upstream counterparts.

With respect to fragments, the average length upstream varied between 40 and 935 µm, having a median value of 376 µm. Conversely, in the downstream regions, the length spanned from 40 to 639 µm, with a median of 345 µm. The interquartile range, that is, the 25th and 75th percentiles for upstream regions were 194 and 543, respectively, whereas for the downstream regions, they were 242 and 441, respectively.

In the case of fibres, the average length upstream ranged from 290 to 6189 µm with a median of 2495 µm, while downstream, the lengths ranged from 285 to 6140 µm with a median value of 1560 µm. The respective lower and upper quartiles for the upstream regions were found to be 663 and 4854, and for the downstream regions, they were 693 and 3351.

In contrast, for pellets, the box plots did not exhibit a notable difference between the upstream and downstream data. The average length upstream fluctuated between 35 and 1110 µm with a median value of 268 µm, whereas for downstream regions, it ranged between 33 and 817 µm with a median of 351 µm. The lower and upper quartiles for upstream regions were 100 and 573, respectively, and for downstream, they were 143 and 632, respectively.

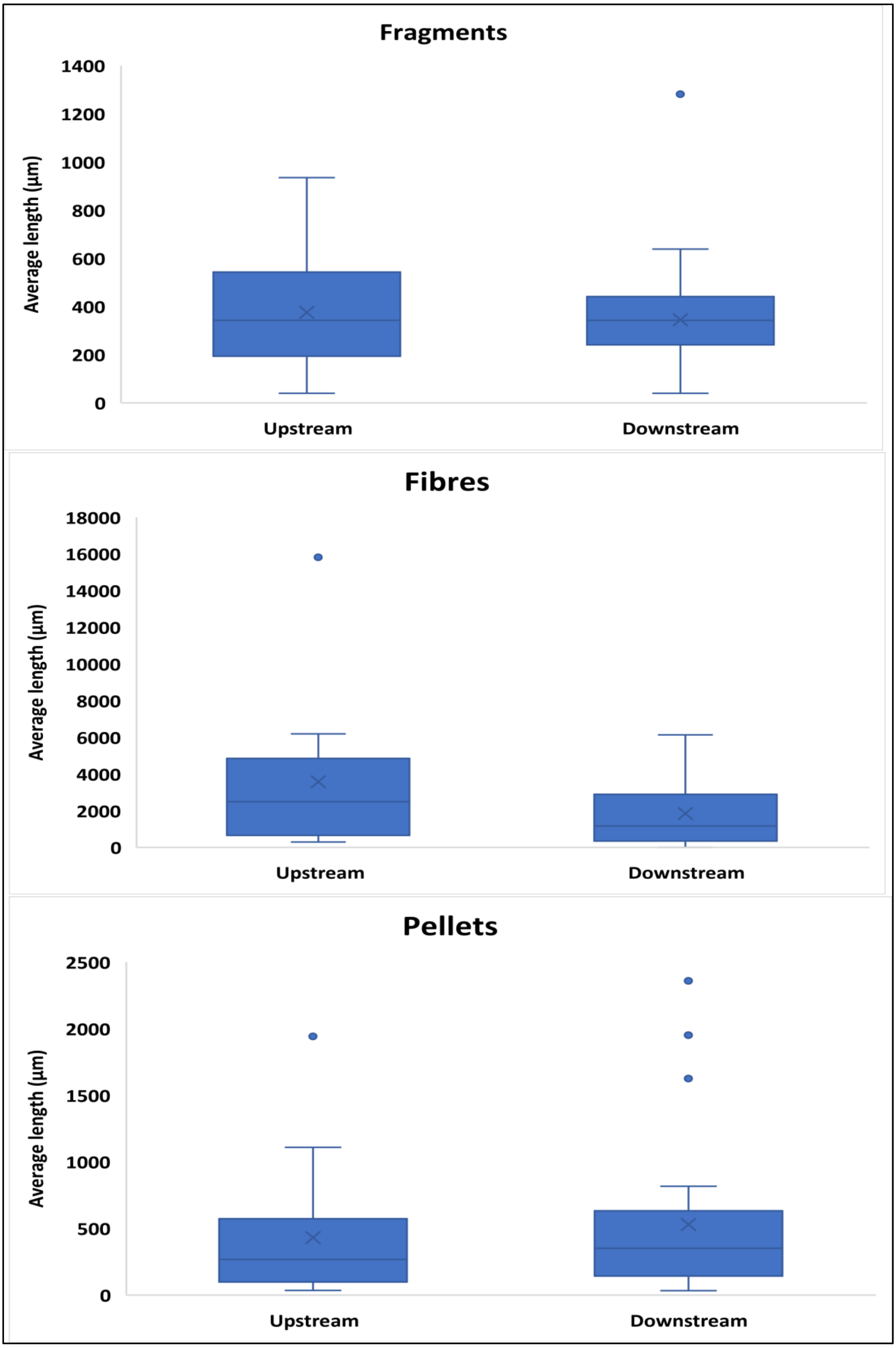


Figure 4. 13: Box plots showing the distribution of fragments, fibres and pellets upstream and Downstream.

4.5 Seasonal variations

This section presents the impact of the four seasons discussed in the previous chapter on the abundance and distribution of MPs in the target freshwater systems. The relationship between MP abundance/distribution and seasonal variation has been reported with varying results by different researchers. Some previous studies have reported increased abundance of MPs in dry seasons than in rainy seasons (Xia et al., 2021, Wicaksono et al., 2021, Wu et al., 2020a) with the explanation that increased flow rate can mobilize previously settled MPs in sediment (Ballent et al., 2016, Zhang et al., 2017). However, other studies have reported contrary results (Rodrigues et al., 2018, Park et al., 2020, Gupta et al., 2021) with the possible explanation that increased washing of terrestrial MPs into the water system because of heavy rainfalls can increase MP abundance in the receiving waterways during rainy seasons.

In this study, the highest number of MPs per kg was recorded in the winter months (December, January, and February) for all four study locations (Worcester Birmingham canal, River Tame, River Sowe, and the River Severn) as shown in Table 4.2 and Figure 4.14. This result is interesting as it gives an inverse of what was observed for seasonal variation of OPE concentration in the study locations (Table 3.7 and Figure 3.9). A possible explanation for this observation would be more terrestrial MPs being washed off into the rivers as a result of the higher rainfall and corresponding higher flow rate in winter in all 4 studied locations.

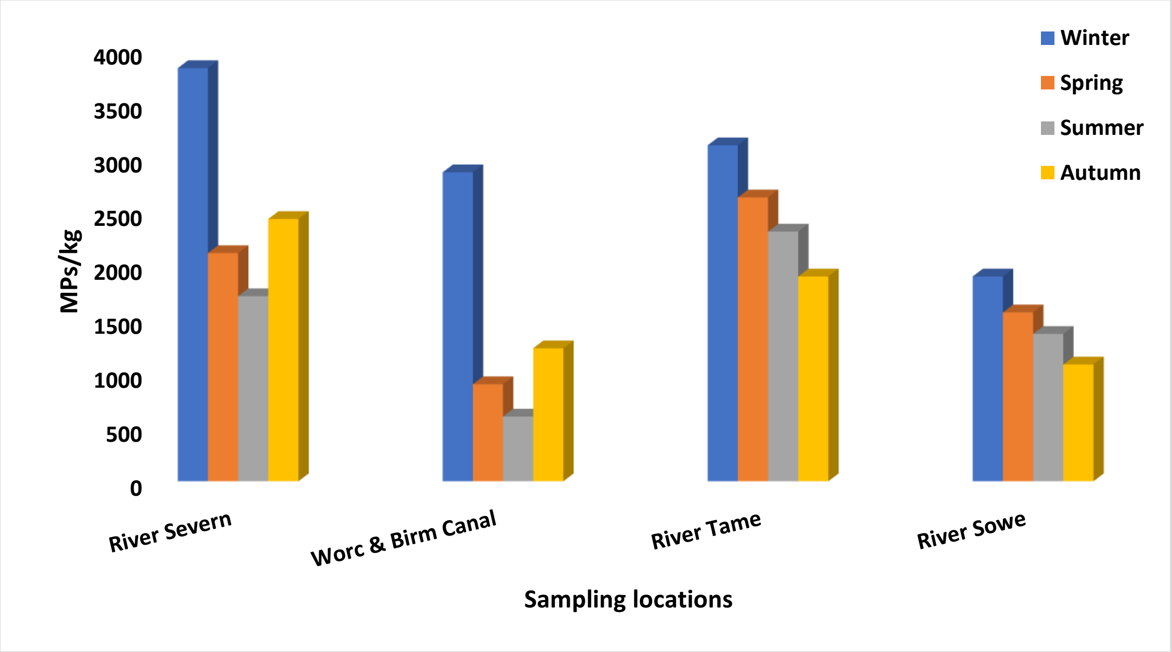


Figure 4. 14. Seasonal variation of MPs abundance across study locations.

In comparison with results from other parts of the world (Table 4.3), only two out of the 10 studies considered the seasonal variation of MPs over a similar seasonal classification as that used in this study (summer, winter, spring and autumn) and both reported the highest concentration of MPs in winter. Other studies reviewed here reported MP variation in rainy and dry seasons with one study, reporting highest concentration of MPs in the rainy season and three reporting highest concentration in the dry season. The higher concentrations reported in rainy season is similar to the results of the present study as higher rainfall was recorded in the winter months.

Table 4. 2. UK seasons, months, notable features, and MP abundance

Season	Months	Notable features	MPs/kg (dw) at River Severn	MPs/kg (dw) at River Sowe	MPs/kg (dw) at River Tame	MPs/kg (dw) at W&B Canal
Summer	June to end of August	Usually has the hottest temperatures. Sunniest days Sometimes driest season Varying rainfall as with all seasons in the UK	1717	1367	2317	600
Autumn	September to November	Cooler temperature Stormier weather Shorter days.	2433	1083	1900	1233
Winter	December to February	Coldest months Shortest days Often wet and windy Frost and even snow often	3833	1900	3117	2867
Spring	March to May	longer and warmer days Often calm and dry	2117	1567	2633	900

Table 4. 3. Comparison between this study and studies from other parts of the world.

Study	Location	Mean MP Abundance (MPs/kg)	Dominant shape	Dominant Size range (µm)	Effect of WWTP	Seasonal Variation	Most abundant type
This study	WWTP1	133 to 283	Fragments	200 to 399	Upstream < Downstream	Highest in spring	
This study	WWTP2	67 to 233	Fragments	63 to 199	Upstream < Downstream	Highest in winter	
This study	WWTP3	67 to 317	Fragments	200 to 399	Upstream < Downstream	Highest in winter	
This study	W&B Can	67 to 267	Fragments	63 to 199	Upstream < Downstream	Highest in winter	
(Viitala et al., 2022)	Finland	100 ± 410	Fibres	100 to 500	Possible contributor as MPs concentrations were highest in sediment samples closest to the discharge site of WWTP effluents.	NA	PE, PP, PES, and PS
(Wu et al., 2020a)	China	25 to 560	Fragments	100 to 1000	Possible contributor of MP	More abundant in dry season	PE, PP, PS and PVC
(Wicaksono et al., 2021)	Indonesia	16.67 ± 20.82 to 150 ± 36.06	Fragments	1000–5000	NA	More abundant in dry season	PE and PP
(Margenat et al., 2021)	Spain	NA	Fragments	Higher number of small MPs (10–64)	Contribute to MP pollution	NA	PVC
(Sekudewicz et al., 2021)	Poland	190 to 580	Fibre	300 to 750	Possible contributor of MP	NA	PE, PP,
(Park et al., 2020)	Korea	340 to 500	Fragments	100 to 300	unclear	More abundant in the rainy season	PE and PP
(Karim, 2021)	Finland	9 to 65 items/g	Fragment	28.2 to 828.6	NA	Highest in winter	PP
(Xia et al., 2021)	China	(6.95–149.35) × 10^3	Fibres	50–500	NA	Higher in dry season	PE
(Hossain et al., 2022)	Bangladesh	143.33 ± 3.33 to 1240 ± 5.77	Fibres	<1000	NA	NA	PE, PET, PA/Nylon, Rayon, and PP
(Sá et al., 2022)	Portugal	565	Fibres	<2000	Possible contributor of MP	Highest in winter and Autumn.	polyethylene terephthalate and polyacrylate

In order to understand the impact of weather conditions (some of which depend on these seasonal variations), the MP abundance was compared to the flow rate and river level data for all study locations as shown in Figures 4.15, 4.16 and 4.17.

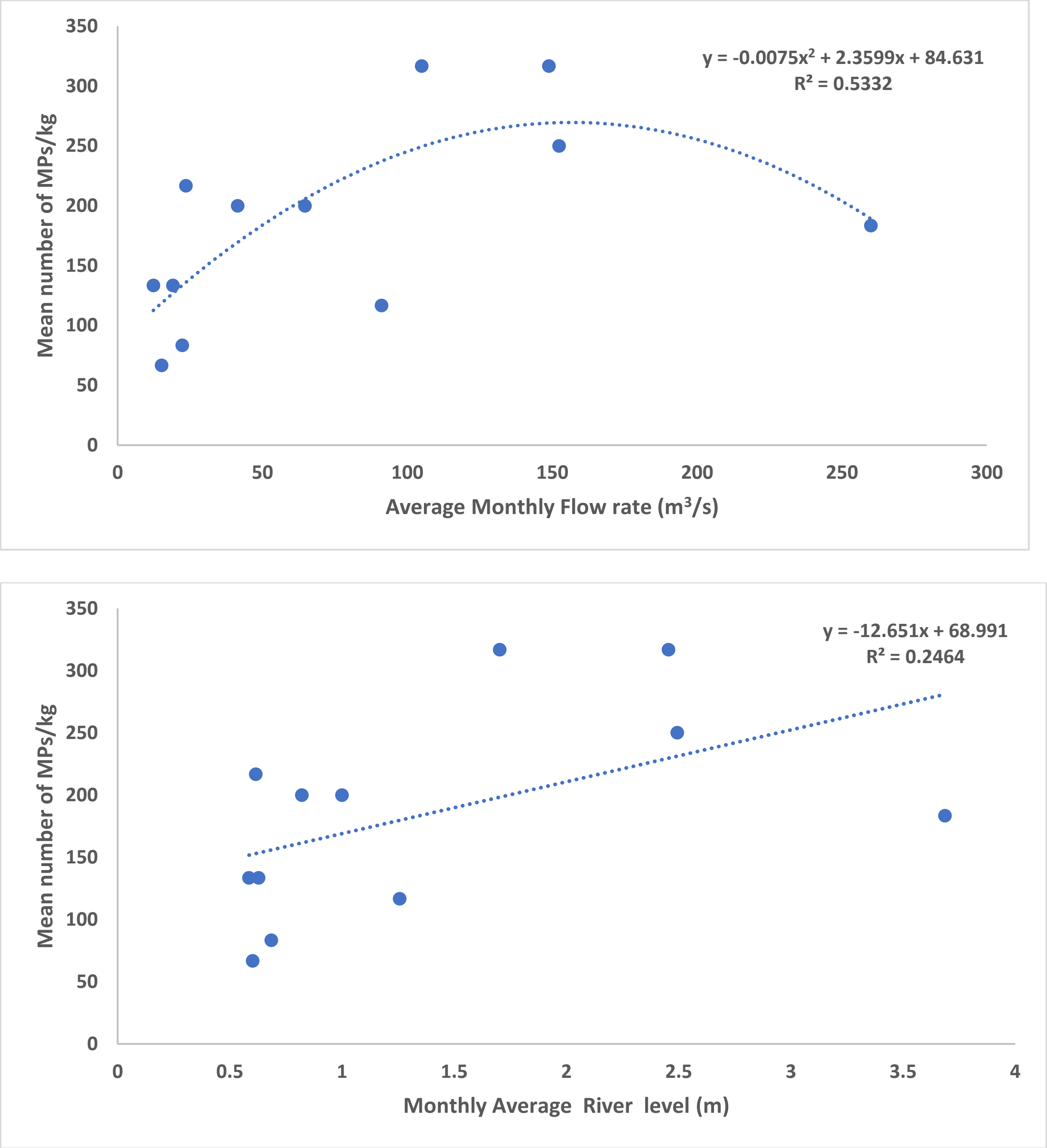
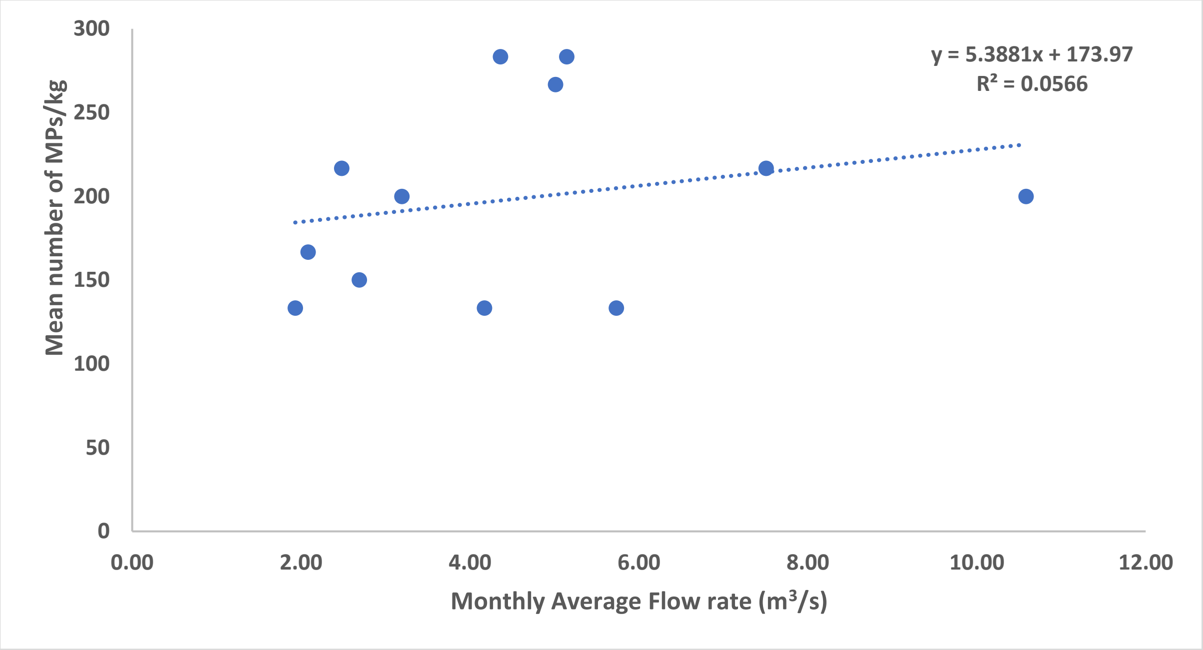


Figure 4. 15. Relationship between MP abundance, River flow rate and River level in River Severn with line of best fit.



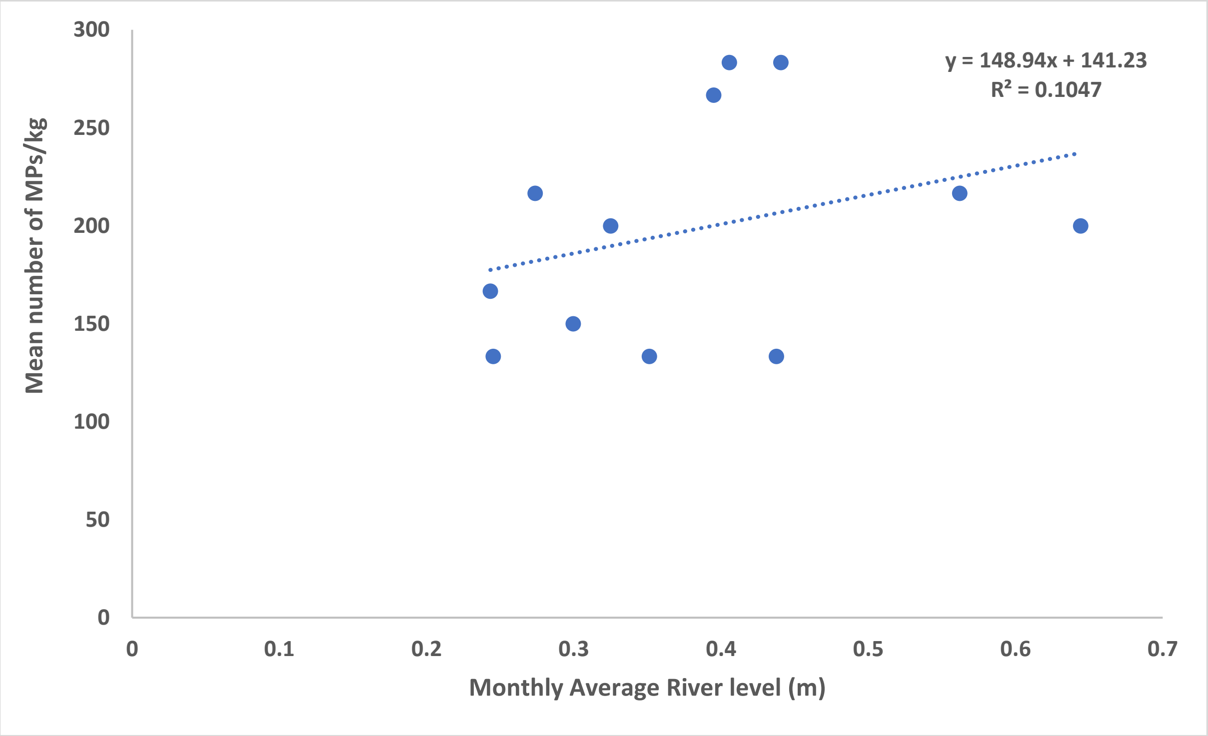
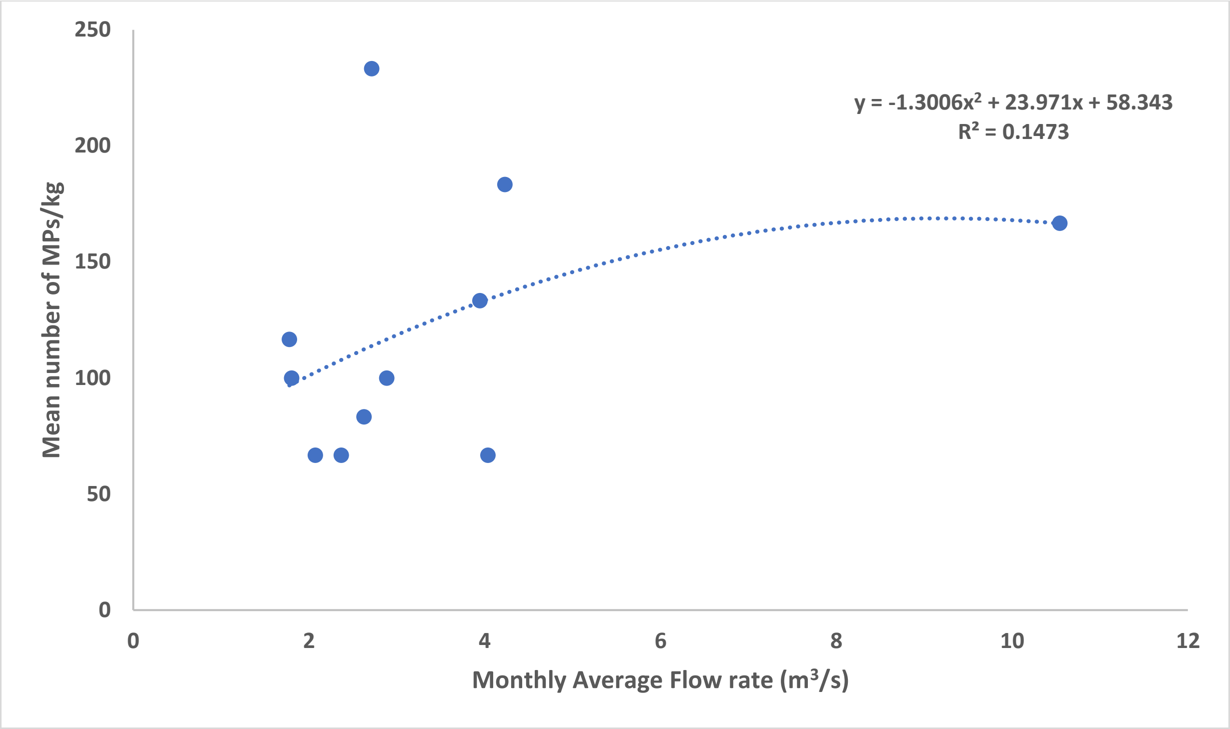


Figure 4. 16. Relationship between MP abundance, River flow rate and River level in the River Tame with line of best fit.



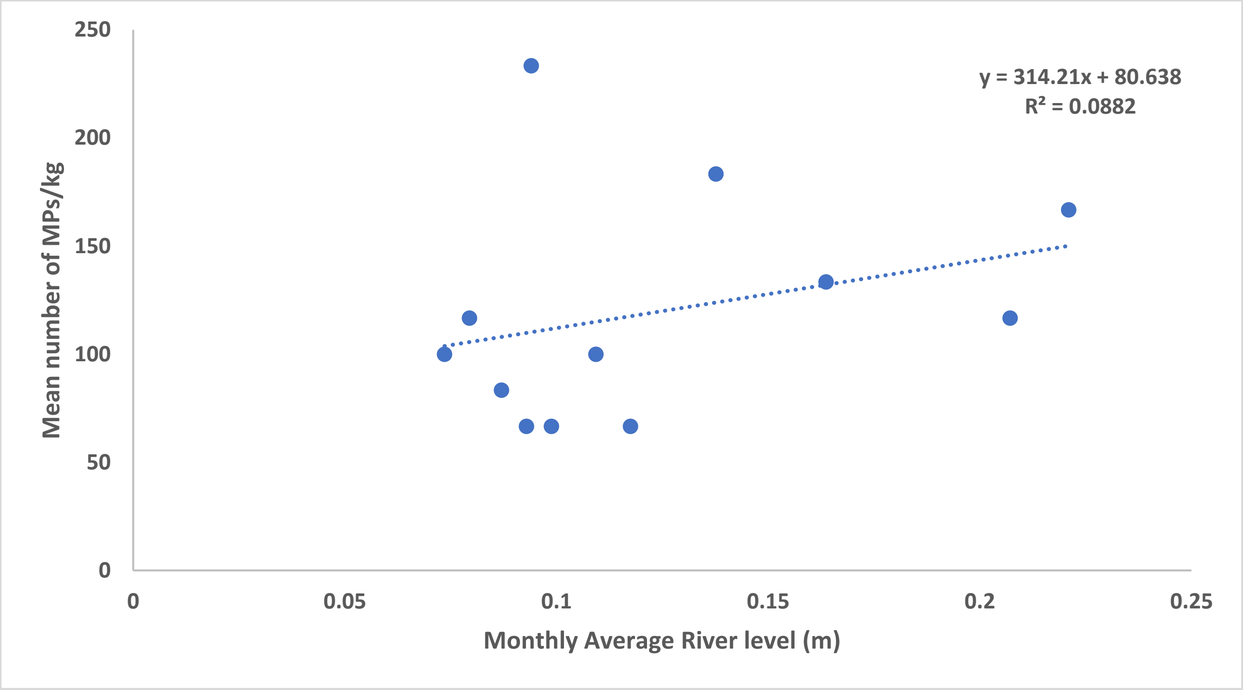


Figure 4. 17. Relationship between MP abundance, River flow rate and River level in the River Sowe with line of best fit.

For the River Sowe (WWTP2), the highest flow rate and river level data were recorded in the winter months of January and February as was the case with MP abundance where the highest concentration was recorded in winter. The highest MP abundance at River Severn (WWTP3) and River Tame were also recorded in winter and this falls within the same period of highest flow rate and river level data at River Severn (January and February). This period also falls withing the flood of mid – late February 2020 at River Severn. Although we couldn't access flow rate and water level data for the Worcester and Birmingham Canal, the flow rate and water level are not expected to vary much as discussed in the previous chapter.

A Pearson correlation analysis was conducted to assess the statistical significance of the observed trends, with findings delineated in Tables 4.4, 4.5, and 4.6 as well as Figures 4.15, 4.16, and 4.17. The analysis revealed positive correlations at the River Severn between MPs/kg and two key metrics: the river level (r = 0.584, p = 0.046) and the river flow rate (r = -0.694, p = 0.026). This suggests a significant relationship between these variables at the 0.05 level. Conversely, in the cases of the River Tame and River Sowe, the correlation analysis did not yield any statistically significant associations, as evidenced by all p-values exceeding the 0.05 threshold. This indicates a lack of significant correlation between the total number of MPs/kg and both the river level and flow rate in these water bodies.

Table 4. 4. Correlation test between total MPs/kg, River level and Flow rate at River Severn

River Severn				
		Mean MPs//kg	River Level (m)	Flow Rate (m^3/s)
Mean MPs//kg	Pearson Correlation	1	.584*	.694*
	Sig.		.046	.026
River Level (m)	Pearson Correlation		1	.982**
	Sig.			<.001
River Level (m)	Pearson Correlation			1
*. Correlation is significant at the 0.05 level.				
**. Correlation is significant at the 0.01 level.				

Table 4. 5. Correlation test between total MPs/kg, River level and Flow rate at River Tame

River Tame				
		Mean MPs//kg	River Level (m)	Flow Rate (m^3/s)
Mean MPs//kg	Pearson Correlation	1	.370	.353
	Sig.		.236	.317
River Level (m)	Pearson Correlation		1	.993**
	Sig.			<.001
River Level (m)	Pearson Correlation			1
**. Correlation is significant at the 0.01 level.				

Table 4. 6. Correlation test between total MPs/kg, River level and Flow rate at River Sowe

River Sowe				
		Mean MPs//kg	River Level (m)	Flow Rate (m^3/s)
Total MPs//kg	Pearson Correlation	1	.463	.575
	Sig.		.130	.105
River Level (m)	Pearson Correlation		1	.953**
	Sig.			<.001
Mean MPs//kg	Pearson Correlation			1
**. Correlation is significant at the 0.01 level.				

4.6 Comparison of MPs abundance and OPE concentrations in the four sampled locations.

As shown in Figure 4.19, the results obtained from various locations were similar in some respects. The most abundant OPE in all 4 study locations was TBOEP and the most abundant halogenated OPE across all four sampling locations was TCIPP. Furthermore, in terms of MPs morphology, fragments accounted for the most dominant shape across all four locations. The highest concentration of OPEs was recorded at the Worcester and Birmingham Canal while the lowest OPE concentration was recorded at the River Severn and the River Sowe with both locations having almost the same concentration (Figure 3.9). Similar comparison between microplastic abundance across all four study locations reveals a different result with the highest concentration of MPs recorded at the River Tame (location with the WWTP servicing the highest population of people of all 4 locations) (Figure 4.19).

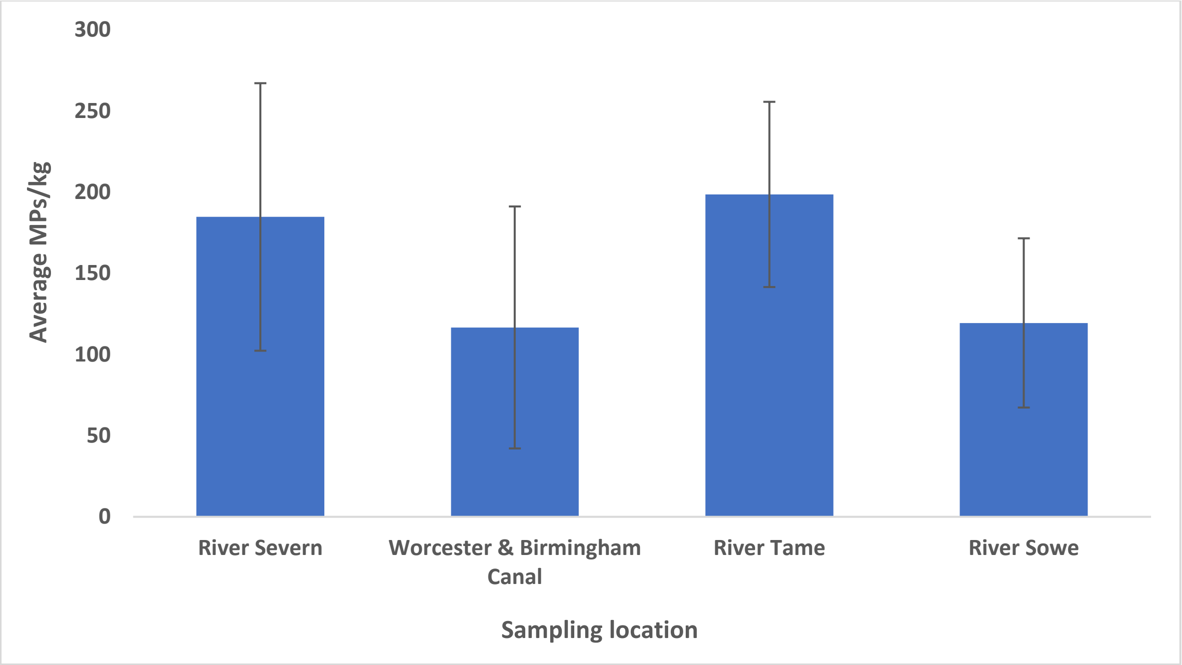
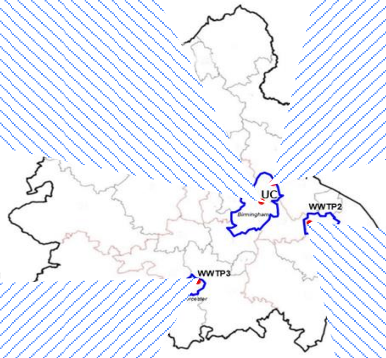


Figure 4. 18. Average total MP concentration over the 12 months period for Worcester and Birmingham canal, River Severn, River Sowe, and River Tame (Y error bars = 1 standard deviation).

Worcester and Birmingham Canal	
Mean OPE concentration range	3.11 to 53.34 ng g^{-1}(dw)
Dominant OPE	TBOEP
Dominant Halogenated OPE	TCIPP
Mean MP number/kg range	66.67 to 266.67 MPs/kg
Dominant Morphology	Fragments
Dominant Size range	63 to 199 µm
MP, OPE correlation	r = -0.588, p = 0.044

River Tame	
Mean OPE concentration range	1.61 to 34.97 ng g^{-1} dry (dw)
Dominant OPE	TBOEP
Dominant Halogenated OPE	TCIPP
OPE Before WWTP vs OPE after WWTP	Not statistically significant (p = 0.889)
Mean MP number/kg range	133.33 to 283.33 MPs/kg
Dominant Morphology	Fragments
Dominant Size range	200 to 399 µm
MP, OPE correlation	r = -0.444, p = 0 .030



River Severn	
Mean OPE concentration range	1.12 to 51.69 ng g^{-1} (dw)
Dominant OPE	TBOEP
Dominant Halogenated OPE	TCIPP
OPE Before WWTP vs OPE after WWTP	Not statistically significant (p = 0.169)
Mean MP number/kg range	66.67 to 316.67 MPs/kg
Dominant Morphology	Fragments
Dominant Size range	200 to 399 µm
MP, OPE correlation.	r = -0.230, p = 0.280

River Sowe	
Mean OPE concentration range	0.02 to 34.97 ng g^{-1} (dw)
Dominant OPE	TBOEB
Dominant Halogenated OPE	TCIPP
OPE Before WWTP vs OPE after WWTP	Statistically significant (p = 0.005)
Mean MP number/kg range	66.67 to 233.33 MPs/kg
Dominant Morphology	Fragments
Dominant Size range	63 to 199 µm
MP, OPE correlation	r = -0.588, p = 0.044

Figure 4. 19. Comparison between parameters of interest at the four sampling locations

Seasonal variation of OPEs across all four study locations show a similar result as the highest concentrations of OPEs were recorded in the autumn months (Figure 3.9). Also, the lowest concentrations of OPE at all 4 sampling locations were recorded in the winter months.

Seasonal variation of MPs showed an inverse result with the highest concentration of MPs recorded in the winter months at all four sampling locations (River Severn, River Sowe, River Tame, and Worcester- Birmingham canal) as shown in Figure 4.17 and 4.18. This can be attributed to the higher river flow rates in winter as a result of the higher rainfall which has the tendency of washing terrestrial MPs into the target water body (Qin et al., 2023, Zhang et al., 2023). Such high flow rate would also lead to higher suspended sediment concentrations in general, which may cause the total OPEs to be diluted into a larger mass of sediment particles; thereby resulting in lower OPE concentrations in sediments.

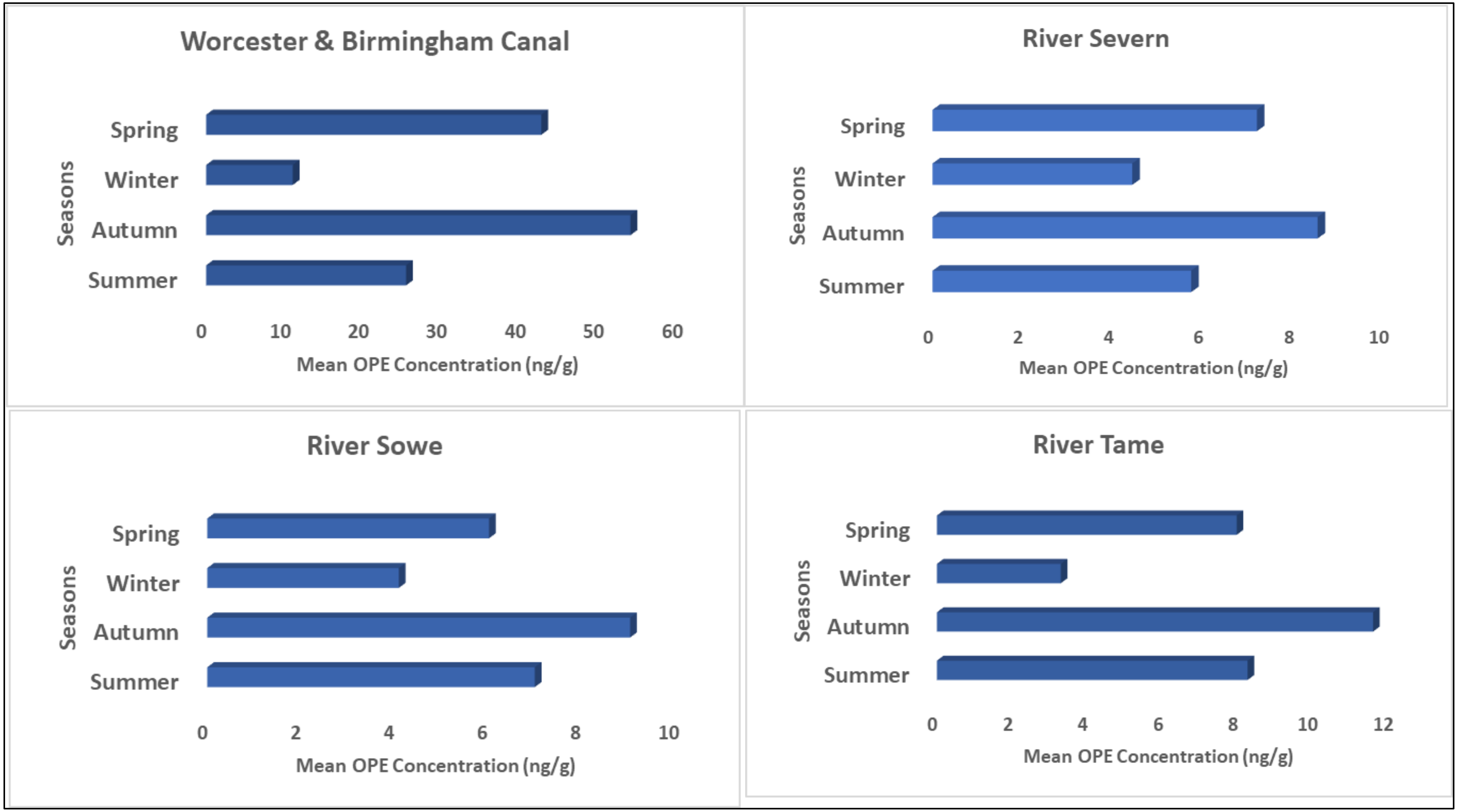


Figure 4. 20. Seasonal variation of OPE concentration by study location.

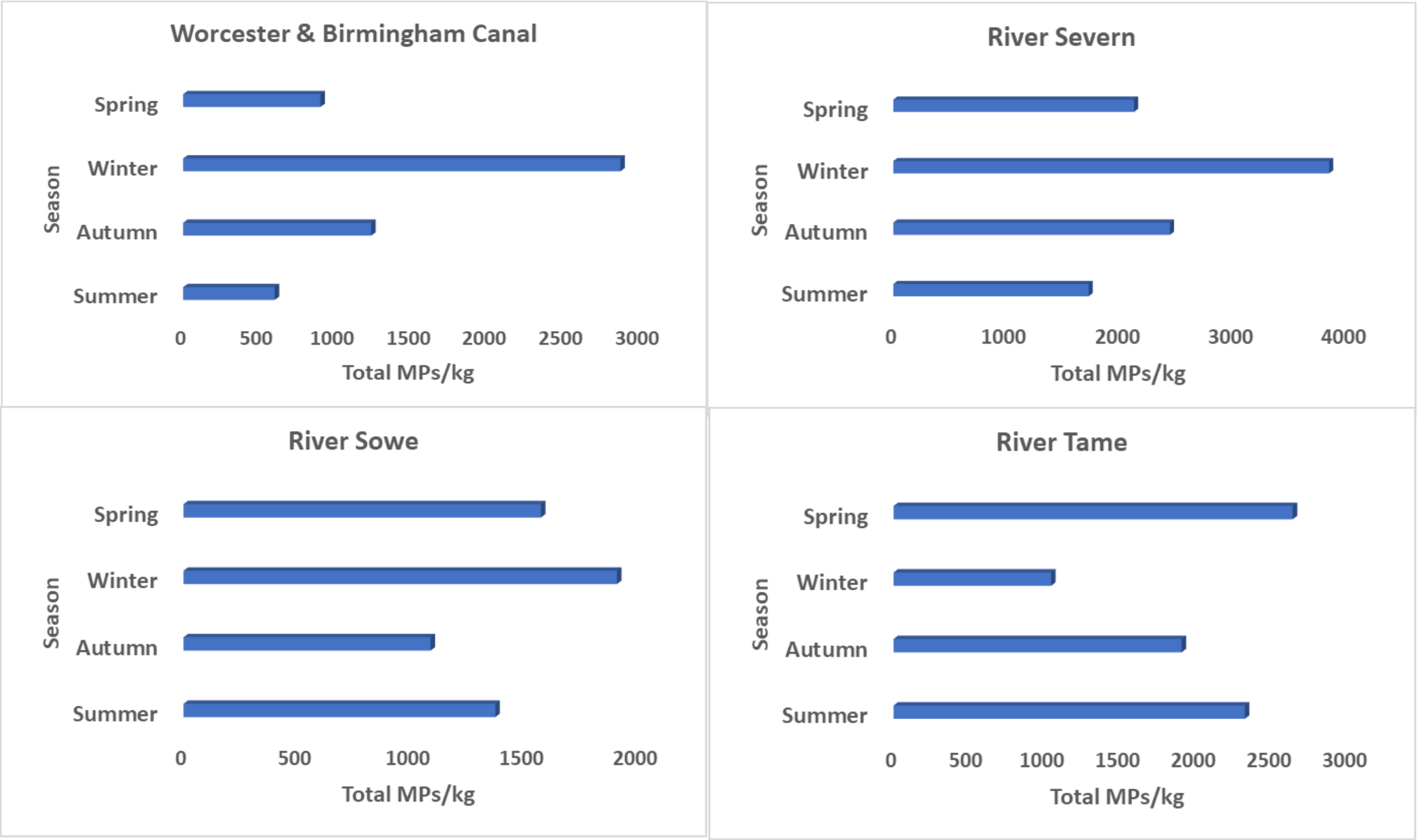


Figure 4. 21. Seasonal variation of MP concentration by study location.

Across all four study locations, MPs measuring 63 to 399 µm were the most abundant size range while larger size ranges (measuring between 600 to 5000 µm) were the least abundant in all four study locations, but this varied in terms of percentage between locations. The three locations with WWTPs had lower percentage of the smaller size range of 63 to 199 µm compared to the Worcester and Birmingham canal which is the most urban location and the only site without a WWTP near the sampling point.

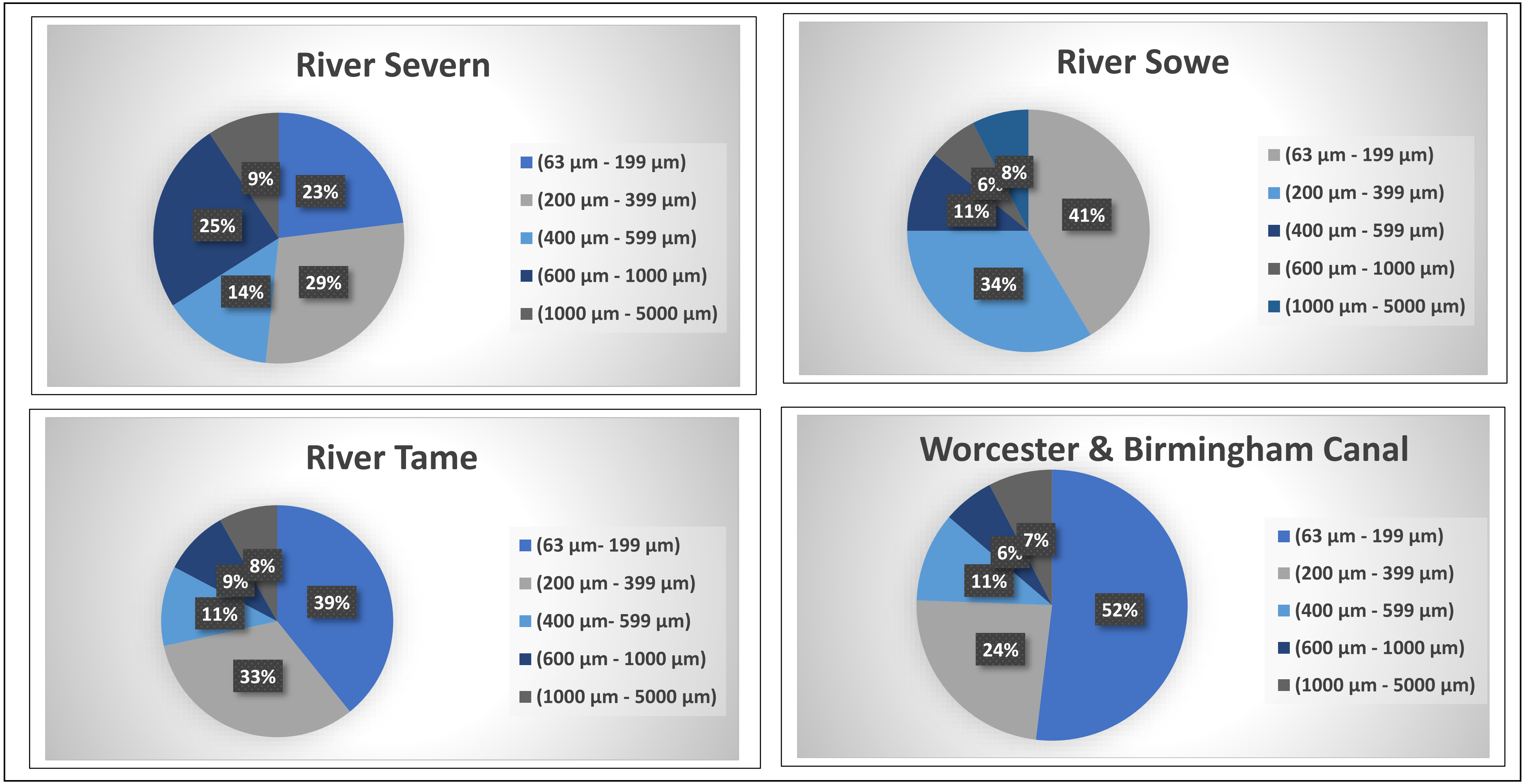


Figure 4. 22. MP size variation across sampling locations.

4.7 Correlation of MPs abundance with OPEs in monthly sediment samples collected from each location.

Studies on the role of MPs as vectors of hazardous chemicals in the environment have looked at the role of MPs in transporting organic pollutants to and from the environment (Fred-Ahmadu et al., 2020, Wang et al., 2018a). However, questions still abound on the interaction between MPs and these pollutants in natural environmental conditions. This is partly due to the difficulty in reproducing the real life environmental conditions such as variations in climatic conditions under which these interactions take place (Mosca Angelucci and Tomei, 2022).

To further understand this interaction between chemical plastic additives in sediments between sediment particles and the MPs themselves, the possibility of correlation between MPs concentrations and the concentration of OPEs in the same sediment samples was investigated. Visual observation as shown in the scattered plot (Figure 4.20) suggests a possible positive correlation at some points and possible negative correlation at others as the trend of both variables over the 12-month period of sampling appear to align in varying ways. However, the patterns revealed in Figure 4.20 are more suggestive of a negative correlation than a positive correlation.

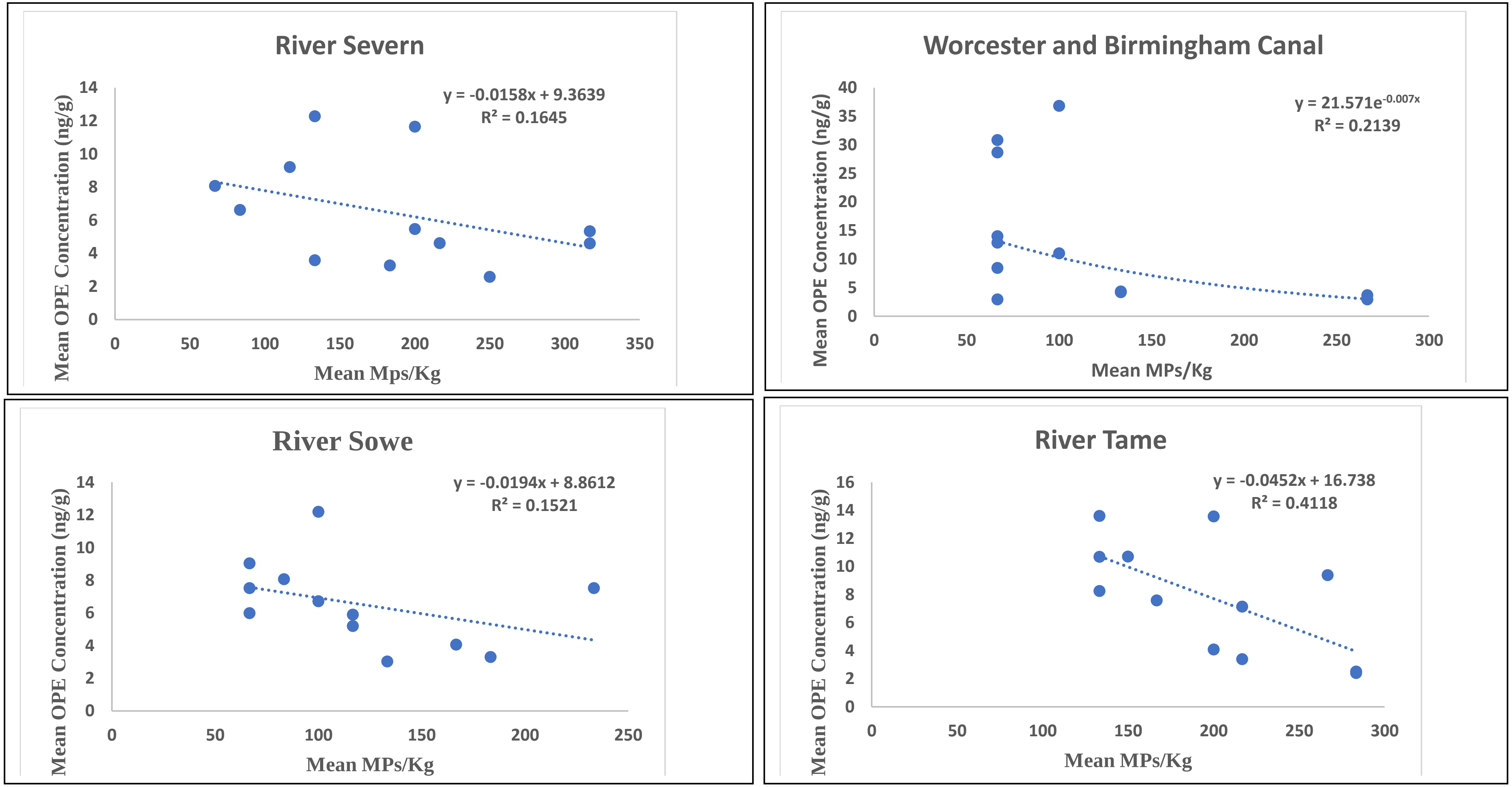


Figure 4. 20. Scattergram plot between mean MPs/kg and Mean OPE concentration over 12 months across all 4 study locations

To assess the statistical significance of the observed correlation, a Pearson correlation test was conducted, and the resulting findings are presented in Table 4.7. The results indicate the presence of negative correlations between the cumulative concentration of $\sum_8$ OPEs concentration, and various parameters related to MP particles. These parameters include the mean number of MPs particles, median number of MPs particles, mean area of MPs particles, and median area of MPs particles across all locations included in the study.

The strength of these correlations varied across the different parameters, ranging from a relatively strong negative correlation between the mean particle number and $\sum_8$ OPEs concentration ($r = -0.326$) to a weaker negative correlation between the mean particle area and $\sum_8$ OPEs concentration ($r = -0.165$). The statistical significance of these observed correlations also exhibited variability, with a strongly significant correlation observed between the mean particle number and $\sum_8$ OPEs concentration ($p = 0.002$), whereas an insignificant correlation was found between the mean particle area and $\sum_8$ OPEs concentration ($p = 0.134$).

Similar results were obtained when examining the correlations between individual target OPEs and the mean number of MPs particles, median number of MPs particles, mean area of MPs particles, and median area of MPs particles. Specifically, a strong and statistically significant negative correlation was observed between the concentration of the most abundant OPE, TPOEP (found across all sampling points), and the mean particle number ($r = -0.309$, $p = 0.004$).

Table 4. 7. Correlation test between total OPE concentration in all locations and the mean particle number, median particle number, mean particle area and median particle area in all study locations.

		TNBP	**TCEP**	**TCIPP**	**TBOEP**	**EHDPP**	**TMTP**	**TPHP**	**TDCIPP**	**∑8 OPEs**
Mean particles number	Pearson Correlation	-.045	-.144	-.214	-.309**	-.012	-.016	-.217*	-.119	-.326**
	Sig.	.685	.192	.051	.004	.911	.888	.047	.282	.002
Median Particle number	Pearson Correlation	-.053	-.143	-.188	-.295**	.030	.002	-.166	-.135	-.299**
	Sig.	.635	.194	.086	.007	.787	.985	.131	.220	.006
Mean particle Area_µm2	Pearson Correlation	-.154	-.193	-.114	-.139	-.024	-.019	-.207	-.188	-.165
	Sig.	.161	.079	.302	.207	.830	.865	.059	.086	.134
Median Particle Area_µm2	Pearson Correlation	-.209	-.196	-.137	-.262*	-.104	-.114	-.216*	-.113	-.283**
	Sig.	.056	.074	.213	.016	.348	.301	.049	.306	.009

**. Correlation is significant at the 0.01 level.
*. Correlation is significant at the 0.05 level.

The Pearson correlation test was repeated separately for each of the four study locations, Worcester and Birmingham Canal (Table 4.8), River Tame (Table 4.9), River Sowe (Table 4.10) and River Severn (Table 4.11) and the results were similar to those obtained in Table 4.7.

At the Worcester & Birmingham canal, a strong correlation between mean particle number and $\sum_8$OPEs concentration was observed (Table 4.8). The r and p values are -588 and 0.04 respectively.

Table 4. 8. Correlation test between $\sum_8$ OPEs concentration and the mean particle number at the Worcester & Birmingham Canal.

Worcester & Birmingham Canal			
		$\sum_8$ OPEs	Mean particle number
$\sum_8$ OPEs	Pearson Correlation	1	-.588*
	Sig.		.044
Mean particles number	Pearson Correlation	-.588*	1
	Sig.	.044	
*. Correlation is significant at the 0.05 level.			

The negative correlation between $\sum_8$OPEs concentration and the mean particle number at River Sowe was insignificant with r and p values are -0.156 and 0.466 respectively (Table 4.9). The r and p values of the negative correlation between $\sum_8$OPEs concentration and median particle number are -0.144 and 0.502 respectively while for $\sum_8$ OPEs concentration and mean particle area; r and p values are -0.053 and 0.806.

Table 4. 9. Correlation test between $\sum_8$ OPEs concentration, and the mean particle number, median particle number and mean particle area at the River Sowe.

River Sowe		$\sum_8$ OPEs
Mean particles number	Pearson Correlation	-.156
	Sig.	.466
Median Particle number	Pearson Correlation	-.144
	Sig.	.502
Area (µm2)	Pearson Correlation	-.053
	Sig.	.806
**. Correlation is significant at the 0.01 level. *. Correlation is significant at the 0.05 level.		

For the River Tame (Table 4.10), the negative correlation between ∑8 OPEs concentration, and the mean particle number was strong and significant (r = -0.444, p = 0.030). A strong and significant negative correlation was also observed between ∑8 OPEs concentration and median particle number (r = -0.477, p = 0.019).

Table 4. 10. Correlation test between ∑8 OPEs concentration, and the mean particle number, median particle number, median particle area and mean particle area at River Tame.

River Tame		∑8 OPEs
Mean particles number	Pearson Correlation	-.444*
	Sig.	.030
Median Particle number	Pearson Correlation	-.477*
	Sig.	.019
Mean Particle Area_µm2	Pearson Correlation	.049
	Sig.	.821
Median Particle Area_µm2	Pearson Correlation	-.002
	Sig.	.991
**. Correlation is significant at the 0.01 level.		
*. Correlation is significant at the 0.05 level.		

At the River Severn (Table 4.11), strong and significant negative correlations were observed for the comparison between ∑8OPEs concentration and mean particle area (r = -0.411, p = 0.046) as well as particle median area (r = -0.447, p = 0.029). However, the correlation between ∑8 OPEs concentration and mean particle number at this location was weak and insignificant (r = -0.230, p = 0.280).

Table 4. 11. Correlation test between ∑8 OPEs concentration, and the mean particle number, median particle number, and mean particle area at River Severn.

River Severn		∑8 OPEs
Mean particles number	Pearson Correlation	-.230
	Sig.	.280
Mean particle Area_µm2	Pearson Correlation	-.411*
	Sig.	.046
Median particle Area_µm2	Pearson Correlation	-.447*
	Sig.	.029
**. Correlation is significant at the 0.01 level. *. Correlation is significant at the 0.05 level.		

The overwhelming trend from both the Scattergram plot and the Pearson correlation tests in all four study locations was a negative correlation between mean MPs number and OPEs concentrations. The observed negative correlation between concentrations of MPs and OPEs is hard to explain. While this study lacks sufficient data to firmly support any particular conclusion, one plausible explanation is the adsorption of organic pollutants onto microplastic particles. Although no previous study has specifically investigated OPEs to the best of our knowledge, extensive research has been conducted on the adsorption of other pollutants, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorobenzenes (CBs), and pesticides, onto microplastics (Fu et al., 2021, Wang et al., 2018c). The adsorption process typically involves a combination of mechanisms, including hydrophobic interactions, partition effects, electrostatic attraction/repulsion, halogen bonding, hydrogen bonding, π-π interactions, and Van der Waals forces (Fu et al., 2021). Physical interactions between OPEs and MPs is facilitated through factors such as crystallinity and surface area of MPs making them a suitable surface for organic pollutants to adhere to thereby reducing the concentration of free pollutants in the sediment. (Mei et al., 2020). The source of the pollution could also play a role in this observed relationship between OPEs and MPs. This might simply be an indication that MPs and OPEs are from different sources.

However, it is important to emphasize that further research is necessary to validate this hypothesis regarding OPEs and microplastic adsorption. The present study lacks comprehensive data on this specific interaction, and thus caution must be exercised in drawing definitive conclusions. Future investigations could focus on examining the adsorption behaviour of OPEs onto microplastic particles, employing appropriate experimental methodologies and comprehensive data collection to elucidate the underlying mechanisms and quantify the extent of the adsorption process.

CHAPTER 5

5.1 Global variation in concentrations of MPs and OPEs in freshwater sediment.

5.1.1 Synopsis

In this chapter, surface sediment samples from 64 locations (Table 2.1) in 14 countries (Nepal, Greece, Poland, Swaziland, Spain, Mozambique, Switzerland, Canada, Malaysia, Republic of Korea, United Kingdom, Chile, Nigeria, and India) and 5 continents were analysed for OPEs and MPs. All sediment samples were kindly provided by the Leverhulme Trust-funded 100 Plastic Rivers project at the University of Birmingham, UK. (https://www.birmingham.ac.uk/research/water-sciences/projects/plastic-rivers.aspx). The 100 Plastic Rivers project is the first systematic and global analysis of microplastics in freshwater ecosystems, working in collaboration with partners in different parts of the world who provide samples collected from a range of rivers following a strict standard protocol. Surficial sediment samples collected at an approximate depth of 6 cm between 2019 and 2021 were transported in glass jars to the University of Birmingham where they were analysed for MPs and OPEs using the methods described in Chapter 2.

This chapter investigates the occurrence and concentrations of MPs and OPEs, evaluates geographical variations in concentrations of these contaminants using comparable sampling and analytical methods for the first time, identifies "hot spots" of pollution and compares detected concentrations of MPs and OPEs in this study to previously reported concentrations in the same region. It is important to bear in mind, that the relatively small sample numbers analysed here preclude definitive conclusions about differences in concentrations of MPs and OPEs between countries or continents. This is because such concentrations are influenced not only by country/continent, but factors such as: organic matter content of sediment, proximity to point sources of MPs/OPEs, and hydrological factors like river size, catchment, and land use etc.. Nevertheless, the data presented here provide a valuable snapshot of MP and OPE contamination of freshwater sediments across the globe. Concentrations of OPEs in sediments are then compared to the abundance and distribution of MPs in the same samples to examine any possible relationships. The potential ecological risk of the OPEs to aquatic organisms at these study locations is also assessed and presented.

5.2 Spatial variation in concentrations of OPEs in freshwater sediments.

The concentrations of the eight target OPEs (TCEP, TCIPP, TBOEP, EHDP, TNBP, TDCIPP, TPHP, and TMTP) in each study location were used to calculate Σ_8OPEs. In locations such as the River Thur in Switzerland where more than one sampling point existed for the same river or Nepal where more than one river was sampled, the average concentration per river or country is used to allow comparison across locations, countries, and continents. As presented in Table 5.1 (The full datasets are provided in the supplementary information to this chapter), the highest concentration of Σ_8OPEs (92 ng/g) was recorded in the Dingam Creek, Canada. The Σ_8OPEs concentration for rivers from Europe is quite high with a mean $\sum_8$ OPEs of (62 ng/g) compared to river Tres Bazos in Chile (South America) where the lowest concentration was recorded (20 ng/g). This high concentration in the studied European rivers is largely influenced by the high concentration of OPEs recorded in the Chicheley Brook, UK which may have been impacted by effluent discharges from sewage treatment works (Harry et al., 2016). The $\sum_8$ OPEs in the studied rivers from Africa, Asia, and Europe are 35 ng/g, 53 ng/g, and 62 ng/g (dw) respectively. A comparison of these results to a selection of previous studies from the same regions is shown in Table 5.5. Notably, the average $\sum_8$OPEs concentration for samples collected from African rivers (47 ng/g), slightly exceeds the figure reported by Mekni et al., (2020) (45 ng/g) for sediment samples from Bizerte Lagoon in Tunisia. It is important, however, to stress that the referenced study was added chiefly due to scarcity of data on OPE concentrations in African freshwater sediments. Although the study furnishes an approximate understanding of OPE concentration in sediments derived from this region, the fact that it is a lagoon, renders the comparison indicative rather than definitive. The concentration reported in the present study is lower than the concentration reported for the Vaal River (South Africa) by Chokwe and Okonkwo (2019) (71 ng/g). For the samples from Asia, the concentration of $\sum_8$ OPEs in this study (53 ng/g) exceeds the reported concentration in two of the previous studies considered (Li et al., 2019a and Wang et al., 2018) but is much lower than the concentrations reported for both the Kathmandu Valley in Nepal (388 ng/g) (Yadav and Devi, 2020) and the concentration for Lake Shihwa in Korea (341 ng/g) (Lee et al., 2018). The concentration of $\sum_8$ OPEs in this study for the studied European rivers exceeds that measured in sediment from the Sava River (Slovenia, Croatia, Bosnia and Herzegovina and Serbia) (28 ng/g) as well as the Evrotas River Sediment in Greece (22 ng/g) (Giulivo et al., 2017). In contrast, concentrations reported for the Adige River in Italy and the River Besòs in Spain were 103 and 240 ng/g respectively (Cristale et al., 2013, Giulivo et al., 2017) thus exceeding that reported in this study for the studied European rivers. The $\sum_8$ OPEs concentration of 92 ng/g for the three rivers from Canada (Thames Ontario (Kintore Creek East), Thames Ontario (Dingman Creek) and Thames Ontario (Kintore Creek West)) is higher than the concentrations reported in 3 previous studies (Lake Ontario, Lakes Superior and Lake Michigan) which were 14 ng/g, 0.73 ng/g, and 3 ng/g respectively.

To test the potential statistical significance of the observed differences in $\sum_8$ OPEs concentrations across continents, ANOVA (with Tukey post-hoc test) was used and it shows that the high concentration observed in the American rivers studied here ($n = 3$) was significantly higher than the concentrations in samples from all other continents in this study (Asia ($n = 36$), Africa ($n = 6$), and Europe ($n = 18$)) with note that the sample number for the American rivers was very small. The concentration of $\sum_8$ OPEs in the European and Asian rivers studied here also exceed significantly, those in Africa, while the lack of data on OPE concentration in sediment across the world makes further comparison of these results with previous studies difficult.

Table 5. 1: Mean concentration of $\sum_8$ OPEs (ng/g) in international sediment samples for each location, country, and continent.

Continent	Country	River	$\sum_8$ OPEs /location	Mean $\sum_8$ OPEs /Country	Mean of $\sum_8$ OPEs /Continent
Africa (n=6)	Mozambique	Limpopo River	41	41	35
		Mkomati River	41		
	Nigeria	River Niger UPP	32	31	
		River Benue UPP	38		
		River Benue Down	24		
	Swaziland	Mbuluzi River	32	32	
Asia (n= 36)	Malaysia	Klang River1	25	25	53
		Klang River2	24		
	Nepal	Koshi River	21	95	
		Kali Gandaki River1	168		
	Republic of Korea	Geum River	13	13	
	India	Ganges River UPP 1	68	79	
		Ganges River UPP 2	61		
		Ganges River UPP 3	25		
		Ganges River UPP 4	154		
		Ganges River UPP 5	100		
		Ganges river UPP 6	46		
		Ganges river UPP 7	92		
		Ganges river UPP 8	38		
		Ganges river UPP 9	98		
		Ganges river UPP 10	160		
		Ganges river MID 1	56		
		Ganges river MID 2	80		
		Ganges river MID 3	90		
		Ganges river MID 4	43		
		Ganges river MID 5	56		
		Ganges river MID 6	52		

		Ganges river MID 7	64		
		Ganges river MID 8	76		
		Ganges river MID 9	41		
		Ganges river MID 10	90		
		Ganges river Low 1	82		
		Ganges river Low 2	152		
		Ganges river Low 3	54		
		Ganges river Low 4	61		
		Ganges river Low 5	88		
		Ganges river Low 6	68		
		Ganges river Low 7	83		
		Ganges river Low 8	98		
		Ganges river Low 9	111		
		Ganges river Low 10	92		
Europe (n = 18)	Switzerland	Thur river1	65	56	62
		Thur river2	51		
		Thur river3	57		
		Thur river4	46		
		Thur river5	63		
	Poland	Vistula River1	36	47	
		Vistula River2	53		
		Vistula River3	17		
	Greece	Kifisos river1	53	47	
		Kifisos river2	41		
	Spain	Canoves	125	91	
		Francoli River	56		
	UK	River Irwell C	75	70	
		River Itchen	34		
		River Irwell Site B	33		
		River Irwell Site A	91		

		Chicheley Brook Site 1	57		
		Chicheley Brook Site 2	130		
South America (n = 1)	Chile	Tres Bazos	20	100	20
North America (n = 3)	Canada	Thames Ontario (Kintore Creek East)	32	178	92
		Thames Ontario (Dingman Creek)	211		
		Thames Ontario (Kintore Creek West)	33		

5.3 OPE profiles and detection frequencies.

Considering the fact that OPEs are ubiquitous in the environment (Xing et al., 2018), a high detection frequency was anticipated for the 8 target OPEs (especially the chlorinated OPEs; TCEP, TCIPP and TDCIPP). All 8 target OPEs were detected in all continents except for TMTP which was not detected in any of the samples from the Americas (Figure 5.1). The detection frequencies in Africa ranged from 100% for TCEP, TCIPP, TBOEP, and EHDPP and TPHP to 50% for TNBP. In Asia, the detection frequencies for TCEP, TCIPP, TBOEP, and EHDPP were all 100% just like in Africa but for TNBP, TDCIPP, and TMTP it was 91%. Of the three chlorinated OPEs (Cl-OPEs) targeted in this study (TCEP, TDCIPP, and TCIPP), TDCIPP had 100% detection frequency in the Americas and 91, 90 and 83% in Europe, Asia, and Africa respectively while TCEP and TCIPP had 100% detection frequency in all four continents as shown in Figure 5.2. This high detection frequency of Cl-OPEs can be attributed to the high production and use of Cl-OPEs, their greater persistence in water and the fact that they are more resistant to degradation through indirect photolysis and/or hydrolysis than the alkyl and Aryl OPEs (Kim et al., 2017, Sundkvist et al., 2010, Regnery and Püttmann, 2010). Furthermore, longer characteristic transport distances (CTDs) have been associated with Cl-OPEs compared to the alkyl/aryl-OPEs (Suhring et al., 2016).

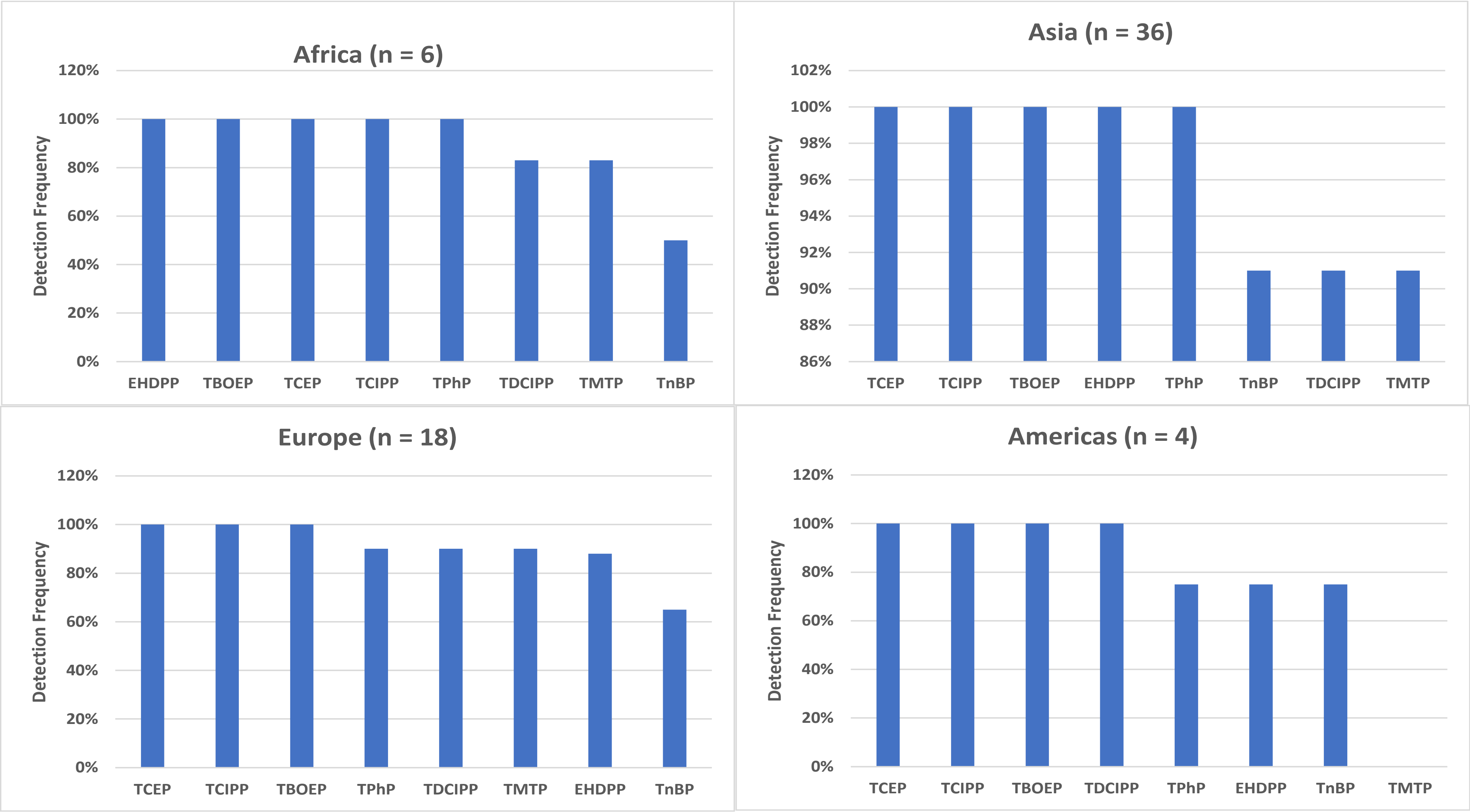


Figure 5. 1. Detection Frequency (%) of the Target OPEs in international sediment samples categorised by continent.

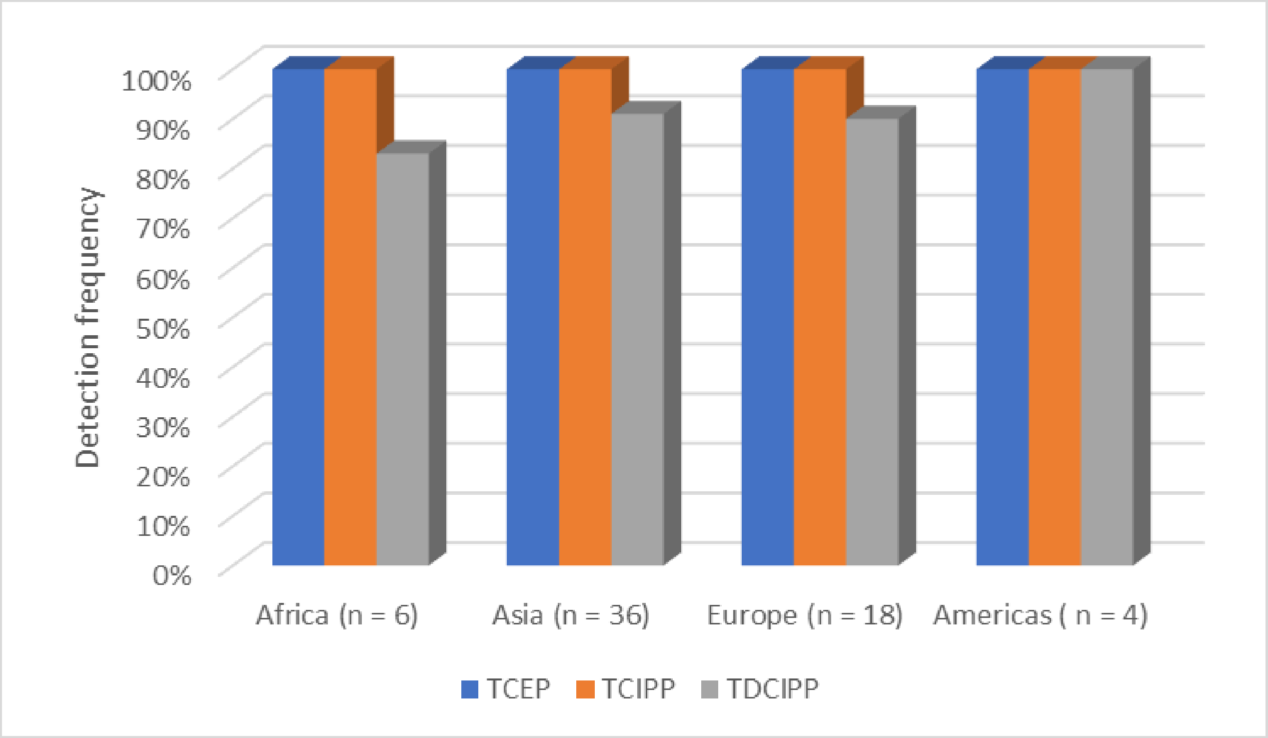


Figure 5. 2. Detection Frequency (%) of the target chlorinated OPEs in international sediment samples from different continents.

Figure 5.3 is the profile of the eight target OPEs in freshwater sediments from the various continents in this study and it shows the percentage contributions of the target OPEs to $\sum_8$ OPEs in the studied continents. The distribution of the 8 OPEs across the profile in Africa, Asia, and Europe further highlights the ubiquity of OPEs in the environment. The profile for the Americas appears to show TMTP but this is the percentage representing the value of 0.02 ng/g used for non-detects. TBOEP and TCIPP are the two most abundant OPEs in all four continents as shown on the profiles. For the three Cl-OPEs (TCEP, TCIPP, and TDCIPP), there is a clear representation of all 3 OPEs in all four continents with TCIPP being the most abundant.

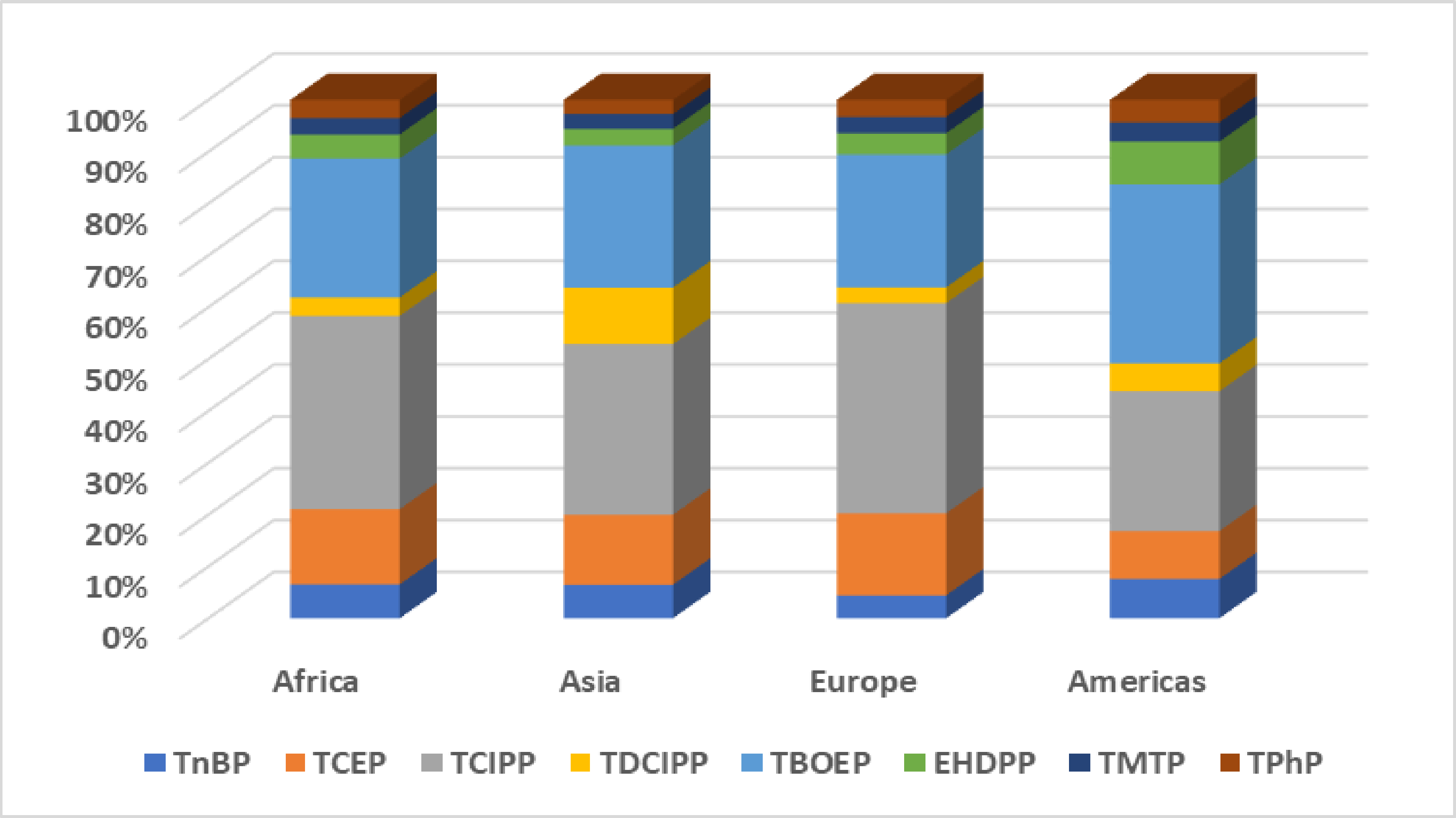


Figure 5. 3. Average profiles of target OPEs presented at percent contributions to Σ_8OPEs in the studied continents.

Looking at specific countries from where sediment samples were collected (Nepal, Greece, Poland, Swaziland, Spain, Mozambique, Switzerland, Canada, Malaysia, Republic of Korea, United Kingdom, Chile, Nigeria, and India), $\sum_8$ OPEs concentrations ranged from 13 ng/g in the Republic of Korea to 95 ng/g (dw) in Nepal (with TDCIPP (62 ng/g (dw)) contributing substantially to the high concentration of $\sum_8$ OPEs in Nepal). The number of samples collected from India allowed a comparison of OPE concentrations along the Ganges River and reveals a higher concentration of $\sum_8$ OPEs at the lower region of the river. However, this concentration at the lower region is only statistically significant at the higher region than the concentration of $\sum_8$ OPEs at the upper region and not the middle region which shows a direct inverse of what was observed with the concentration of MPs along the river. For chlorinated OPEs (TCEP, TCIPP, TDCIPP) alone, $\sum_3$OPEs range from 3 ng/g (dw) in Chile and Republic of Korea to 28 ng/g (dw) in Nepal. The concentration of $\sum_3$OPEs in Spain was also high (24 ng/g dw) and is over 50% higher than the concentrations of $\sum_3$OPEs in Greece (11 ng/g), Poland (11 ng/g), Switzerland (12 ng/g), Canada (11 ng/g) and the United Kingdom (10 ng/g). For individual target OPEs, concentrations varied across study locations as shown in Figure 5.4. TCEP was the most abundant OPE in most study locations, but TBOEP was the most abundant OPE in Canada and India. The detection frequency of all eight target OPEs in 11 out of the 14 countries was 100% while for the other three (Swaziland, Mozambique, and Republic of Korea), the detection frequencies were 80%. Such high detection frequencies of certain OPEs such as TCIPP, TMTP, and EHDPP in sediment have previously been traced to anthropogenic sources (Cristale et al., 2013c, Ricking et al., 2003, Takahashi et al., 2012).

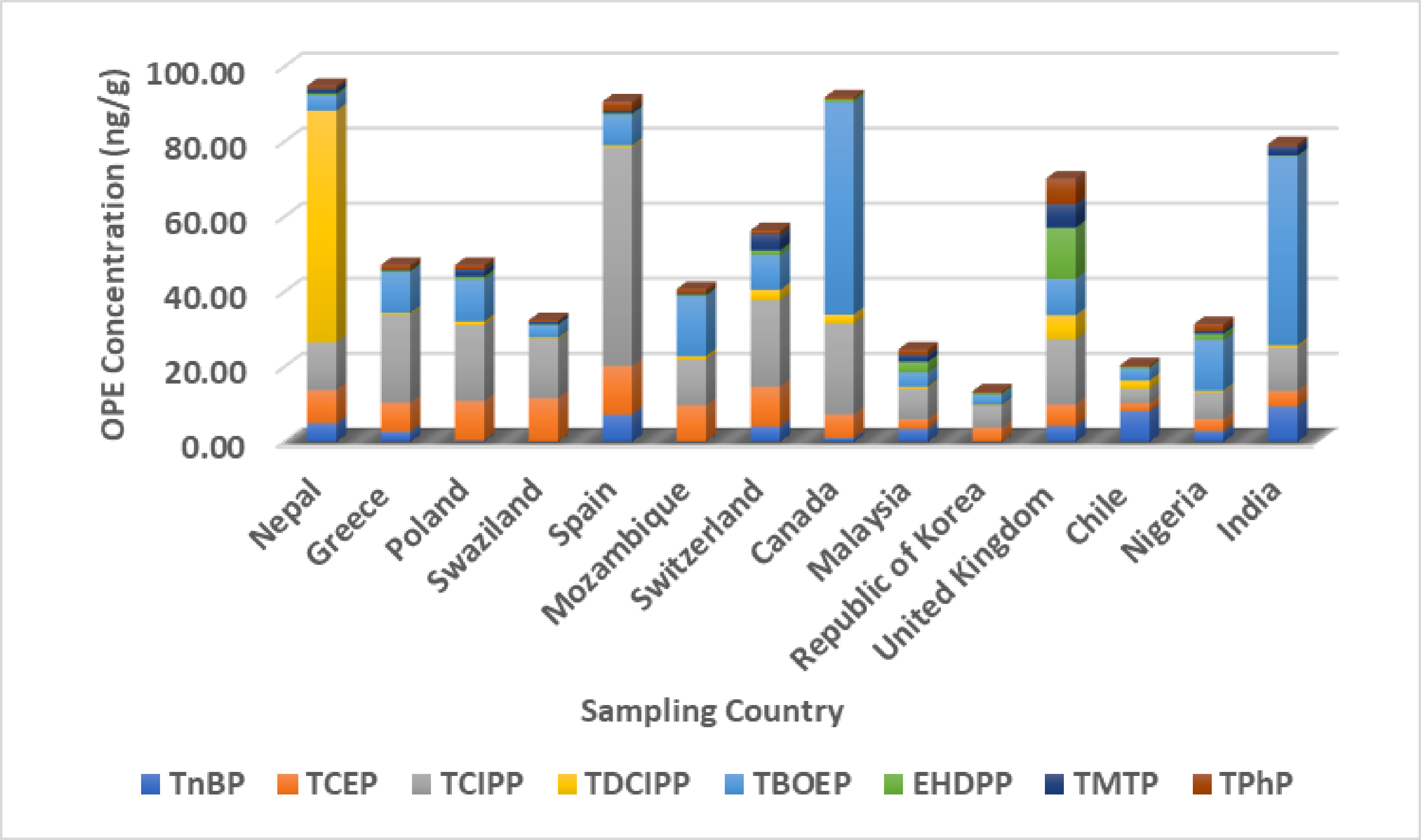


Figure 5. 4. Mean Concentration of individual target OPEs in each study country.

Table 5. 2. Comparison between OPE concentrations in surficial freshwater sediments for international samples in this study and previous data from the same region.

Continent	Study	Location	TNBP (ng/g)	TCEP (ng/g)	TCIPP (ng/g)	TBOEP (ng/g)	EHDP (ng/g)	TMTP (ng/g)	TPHP (ng/g)	TDCIPP (ng/g)
Africa	This study	Swaziland, Mozambique, and Nigeria	3.08	6.85	17.55	12.63	2.19	1.51	1.65	1.68
	(Chokwe and Okonkwo, 2019)	Vaal River, South Africa.	37.51	2.56	1.14	26.21	-	-	2.88	0.66
	(Mekni et al., 2020)	Bizerte Lagoon, Tunisia	8.02	5.66	8.05	0.38	8.09	/	12.29	2.05
Asia	This study	Nepal, Malaysia, Republic of Korea and India	3.41	7.14	17.37	14.49	1.69	1.51	1.44	5.76
	(Yadav and Devi, 2020)	Kathmandu valley, Nepal. (River Sediment)	76.41	18.46	112.59	-	135.60	-	39.60	5.66
	(Wang et al., 2018b, Liu et al., 2018b)	Lake Taihu, China	0.00	3.00	1.20	0.00	0.38	0.38	1.70	0.10
	(Lee et al., 2018)	Lake Shihwa, Korea	2.14	18.40	194.00	64.40			18.70	43.60
	(Li et al., 2019a)	Lian River (China)	0.00	0.00	0.00	-	-	-	-	0.10
Europe	This Study	Greece, Poland, Spain, Switzerland, and United Kingdom	2.28	8.13	20.82	13.18	2.10	1.64	1.68	1.56
	(Giulivo et al., 2017)	Evrotas River Sediment (Greece)	2.39	1.78	4.59	1.47	4.82	4.82	0.36	1.63
		Adige River (Italy)	5.52	2.51	14.93	2.36	37.31	37.31	0.86	2.31
		Sava River (Slovenia, Croatia, Bosnia and Herzegovina and Serbia)	7.65	0.79	6.60	3.16	4.76	4.76	-	0.36
	(Cristale et al., 2013)	River Besòs (Spain)	8.40	7.12	164.71	< LOD	37.71	-	13.26	8.74
North America	This Study	Thames River, Canada	0.94	6.31	24.09	56.60	0.74	0.04	0.44	2.57
	(Cao et al., 2017)	Lake Ontario	1.43	ND	0.71	7.29	0.31	0.31	2.62	1.43
		Lake Superior	0.34	0.15	0.15	0.03	0.01	0.01	0.04	-
		Lake Michigan	0.52	ND	0.39	1.52	0.03	0.03	0.17	-
South America	This Study	Tres Bazos, Chile	8.10	2.10	3.90	3.20	0.40	0.04	0.40	2.20

5.4 Concentrations of MPs in International Sediment Samples

Table 5.1 provides the mean concentration of MPs in each study location, each country, and each continent, to facilitate geographical comparisons, with the full datasets provided in the supplementary information to this chapter. The highest mean concentration of MPs was recorded in the samples collected from Europe (602 MPs/kg) while the lowest was recorded in the studied South American rivers (100 MPs/kg). This high concentration of MPs observed in this study for the studied European rivers is similar to the results of some previous studies such as: Horton et al., (2017), Sekudewicz et al., (2021), and Blair et al., (2019) who respectively reported mean concentrations of: 660 particles/kg (dw), 190 to 580 particles/kg (dw) and 161–432 particles/kg (dw) at different locations in Europe. Nevertheless, it is crucial to emphasise the notable discrepancies in methodologies utilized across various studies compared to the present study, which could profoundly affect the quantified microplastic concentrations reported. An example is Horton et al who processed sediments in three steps (visual inspection of whole sample, flotation using $ZnCl_2$ solution and further visual inspection of unfloated material). This method might favour the capturing of very dense plastics and other particles like paints as reported in the study (Horton et al., 2017b).

The second highest concentration of MPs was in the studied African rivers (420 MPs/kg) where the high concentration of MPs in Mozambique contributed substantially to the concentration. To test the statistical significance of this observed difference in concentration of MPs across continents, ANOVA (with Tukey post-hoc test) was used and the samples from North and South America were grouped together as “Americas” (n=3) to increase the sample size. The ANOVA test revealed that the differences are not statistically significant at $p < 0.05$ across all continents. This result was compared to MP concentrations reported in previous studies from the same continents (Africa: n = 3, Asia: n = 4, Europe: n = 3, Americas: n = 3) (Table 5.2) and the result revealed very similar trends except for the Americas where the high concentration of MP reported by Gerolin et al., (2020) in Brazil (417 to 8178 particles/kg (dw)) increased the value substantially. In North America, a previous study on the distribution of microplastic particles in benthic sediment of the Thames River in Ontario, Canada (One of the rivers in this study) reported a range of MP abundance covering what was reported in this study. For the second study in Canada, the MP abundance (220 particles/kg (dw)) was almost the same as the result in this study (178 particles/kg (dw)) while in South America, the MP abundance reported by Gerolin et al., (2020) as mentioned previously exceeds the concentration reported in this study.

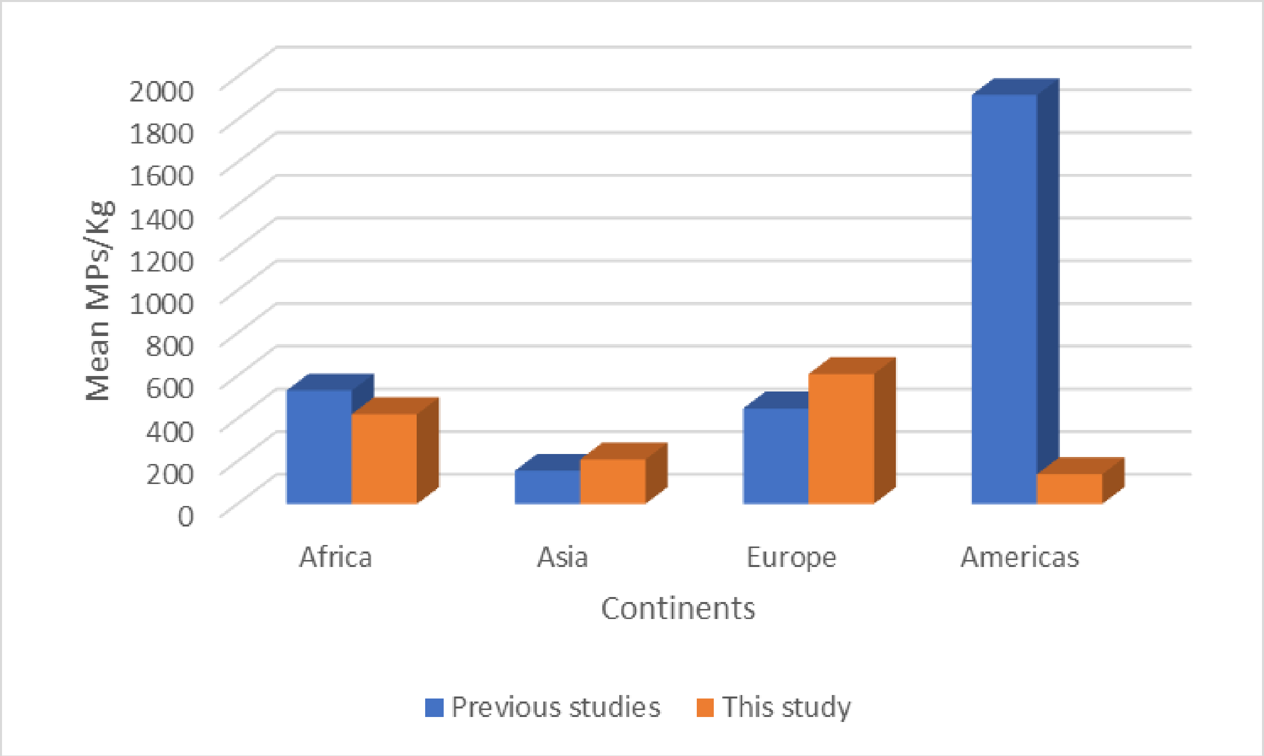


Figure 5. 5. Comparison between the concentrations of MPs by continent in this study and previous studies.

Analysis of Variance (with Tukey post-hoc test) was also used to test the statistical significance of any differences in concentrations of MPs observed in previous studies across continents and it revealed that the difference observed are statistically significant between all continents except between Asia and Americas.

Table 5. 3. Mean concentration of MPs (MPs/kg) in international sediment samples analysed in this study.

Continent	Country	River	Mean MPs/location	Mean MPs/Country	Mean MPs/Continent
Africa (n=6)	Mozambique	Limpopo River	267	217	420
		Mkomati River	167		
	Nigeria	River Niger UPP	167	111	
		River Benue UPP	100		
		River Benue Down	67		
	Swaziland	Mbuluzi River	933	933	
Asia (n= 36)	Malaysia	Klang River1	200	217	208
		Klang River2	233		
	Nepal	Koshi River	100	50	
		Kali Gandaki River1	50		
		Kali Gandaki River2	0		
	Republic of Korea	Geum River	233	233	
	India	Ganges River UPP 1	0	334	
		Ganges River UPP 2	267		
		Ganges River UPP 3	200		
		Ganges River UPP 4	0		
		Ganges River UPP 5	567		
		Ganges river UPP 6	167		
		Ganges river UPP 7	367		
		Ganges river UPP 8	67		
		Ganges river UPP 9	133		
		Ganges river UPP 10	133		
		Ganges river MID 1	1167		
		Ganges river MID 2	433		
		Ganges river MID 3	467		
		Ganges river MID 4	233		
		Ganges river MID 5	267		

		Ganges river MID 6	333		
		Ganges river MID 7	1333		
		Ganges river MID 8	533		
		Ganges river MID 9	167		
		Ganges river MID 10	233		
		Ganges river Low 1	867		
		Ganges river Low 2	100		
		Ganges river Low 3	333		
		Ganges river Low 4	300		
		Ganges river Low 5	200		
		Ganges river Low 6	667		
		Ganges river Low 7	0		
		Ganges river Low 8	133		
		Ganges river Low 9	100		
		Ganges river Low 10	267		
Europe (n = 18)	Switzerland	Thur river1	67	80	602
		Thur river2	67		
		Thur river3	100		
		Thur river4	67		
		Thur river5	100		
	Poland	Vistula River1	567	478	
		Vistula River2	400		
		Vistula River3	467		
	Greece	Kifisos river1	433	552	
		Kifisos river2	671		
	Spain	Canoves	0	1217	
		Francoli River	2433		
	UK	River Irwell C	300	683	
		River Itchen	333		
		River Irwell Site B	367		

		River Irwell Site A	300		
		Chicheley Brook Site 1	2200		
		Chicheley Brook Site 2	600		
South America (n = 1)	Chile	Tres Bazos	100	100	100
North America (n = 3)	Canada	Thames Ontario (Kintore Creek East)	133	178	178
		Thames Ontario (Dingman Creek)	400		
		Thames Ontario (Kintore Creek West)	0		

As shown in Table 5.1 and figure 5.6, the mean number of MPs per kilogram (dw) at the various countries from which samples were obtained ranged from 1217 MPs/kg (dw) in Spain (Francoli River and Cànoves stream) to 0 MPs/kg in some locations (Thames Ontario (Kintore Creek West), Canoves in spain and Kali Gandaki River in Nepal). This high MP pollution recorded in Spain is mainly due to Francoli River which is impacted by industrial harbour and activities around Tarragona and Barcelona (Eljarrat et al., 2001).

An ANOVA test (with Tukey post-hoc test) shows that the concentrations in Spain significantly exceed those in other European countries studied except Switzerland. The mean number of MPs/kg recorded in samples from Poland, Swaziland, and the United Kingdom all exceeded 400 MPs/kg while those for samples from Greece, Mozambique, Switzerland, Canada, Malaysia, Republic of Korea, Chile, and Nigeria were all lower than 300 MPs/kg. In comparing the results from Nigeria to results from two previous studies in Africa (including one from Nigeria), the results were similar in some respects. The mean number of MPs/kg in this study falls within the range reported by Olarinmoye et al., (2020) for a lagoon in Lagos, Nigeria and the concentrations reported by Dahms et al., (2020) for Braamfontein Spruit, in Johannesburg, South Africa (Table 5.2).

For Asia, five previous studies from the same continent were compared to the results of this study. One of these previous studies (Yang et al., 2021) reported MP data from one of the rivers in the current study (Koshi River in Nepal). Yang et al., 2021 reported 58 ± 27 MPs/kg (dw) which is less than the 100 MPs/kg reported in this study. For the other four previous studies in Asia, the MP abundance and other parameters reported in this study are not very different from those reported here. Our sampling points ($n = 30$) in India span the lower, middle, and upper parts of the Ganges River (from Devprayag to Noorpur) as well as some of its tributaries and distributaries such as the Hooghly River, Koshi River, Gandaki River, Kiul River, and Son

River. The lower area of the Ganges cover locations around Manihari - Bihar to Murshidabad - West Bengal, as well as the Kiul and Koshi rivers. Areas around Ghazipur, Uttar Pradesh to Patna District, Bihar as well as samples from Gandak and Sone rivers all fall within the middle area while areas around Uttarakhand to Kanpur Nagar, Uttar Pradesh were all grouped in the upper area. The mean concentration of MPs in India was 334 MPs/kg (dw) but the concentration of MPs varied substantially along the upper, middle, and lower regions of the Ganges. The highest average concentration of MPs was recorded in the middle region, and this is attributable to proximity to anthropogenic sources. Statistically, the higher concentrations in the middle region are not significant compared to the concentrations at the upper and lower regions of the river but concentrations in the upper region significantly exceed those in the lower region.

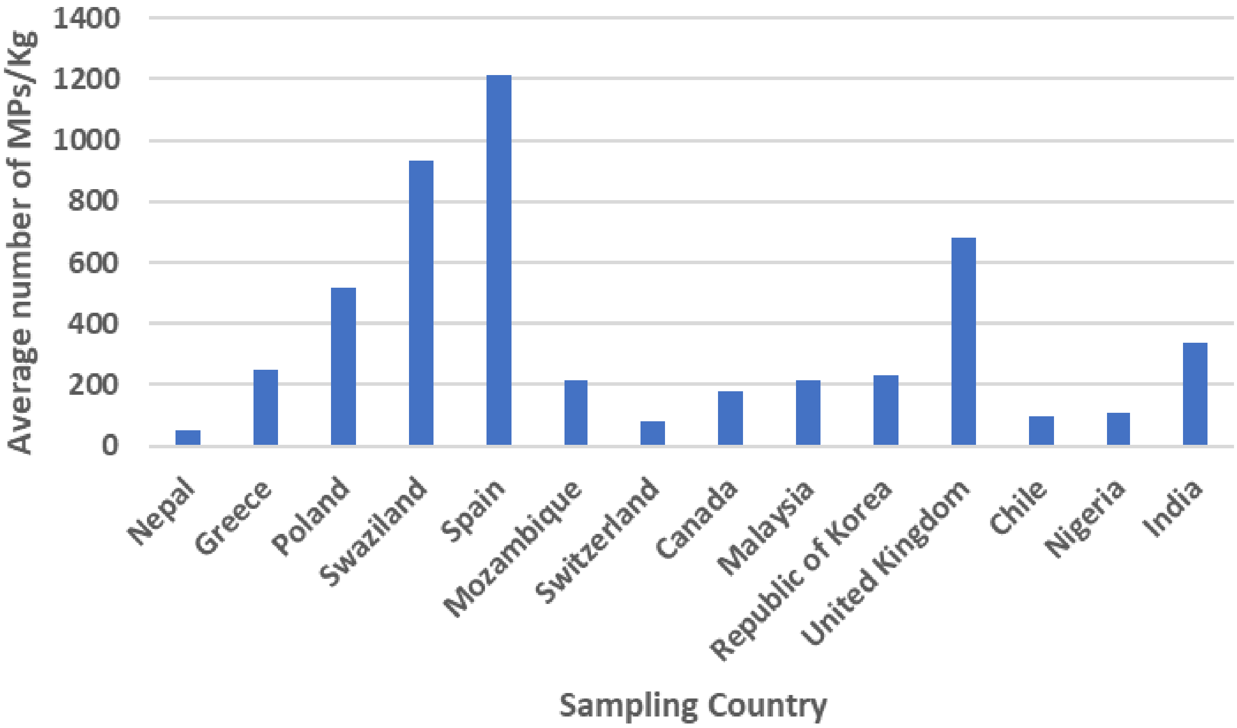


Figure 5. 6. Average concentrations of MPs (MPs/kg) in the various countries studied.

5.5 Morphological characteristics of MPs detected in riverine sediments.

The morphologies of particles in all study locations were identified using the Nikon SMZ-1000 stereo microscope fitted with a Nikon Intensilight C-HGF1 long-life mercury light source and a Nikon DS-Fi1 5-megapixel digital microscope camera head. The MPs were then grouped into fibres, fragments and spheres (Zhao et al., 2014). As shown in Figure 5.7, the most abundant morphological group across all countries are fragments. The percentage of fragments per kg of sediment (dw) range from 100% in Nepal to 40% in the Republic of Korea. The highest percentage of fibres was recorded in South Korea (60%) and the lowest recorded in India (2%). Spheres were only identified in four countries (Greece, United Kingdom, Nigeria, and India) with percentage abundances of 10%, 3%, 20% and 3% respectively. The average length of MPs in all the countries where sediment samples were collected was < 1500 µm. In fact, for 6 out of the 14 countries studied, average MP length was < 300 µm and was less than 1000 µm for all but one country.

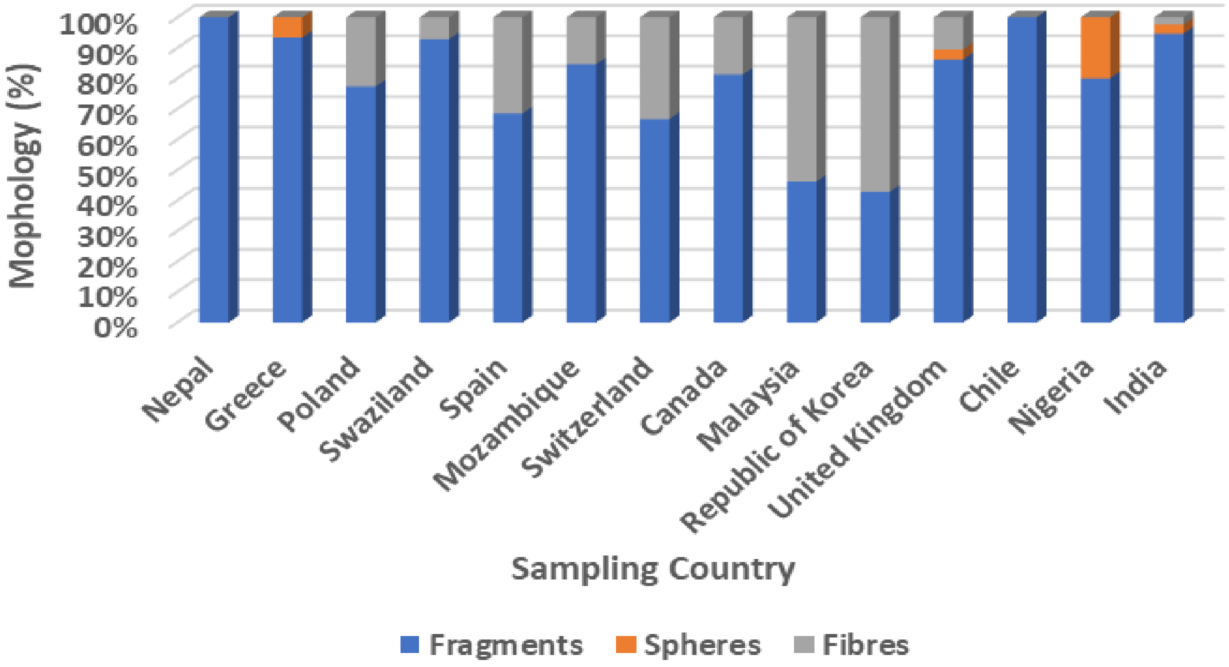


Figure 5. 7. Morphology of MPs in the various countries studied.

The size range and morphology in this study for samples from Europe are similar to most previous studies. The size range in this study is less than that reported in one study (Horton et al., 2017a) but similar to that reported in the other three (Sekudewicz et al., 2021, Margenat et al., 2021, Blair et al., 2019). In comparing the morphological characteristics of the isolated MPs for Asia in this study to previous studies from the same continent, the average length of MPs in this study (0.1 mm) falls within the size range reported by Yang et al, 2021 (< 1 mm) for the Koshi River in Nepal (one of the rivers in this study). However, in contrast to our study where the most abundant morphology was fragments, 98 % of the MPs in the study by Yang et al, 2021 were fibres (Yang et al., 2021). In North America, a previous study on the Thames River (one of the rivers in this study) reported a similar size range and dominant morphology to that reported in this study. In contrast, a second study in Canada reported the most abundant morphology to be microfibres as opposed to fragments in our study.

5.6 Variation of MP concentrations by location

MP abundance as well as characteristics varied across the various study locations but for ease of presentation, the average results from each country of study is presented here. As shown in Figure 5.8, the highest concentration of MPs (1217 MPs/kg) was recorded in Spain where sediment samples were collected from two locations (Canoves and Francoli rivers). All isolated MPs in these locations were fragments and the average length of MPs was 213 μm. The closest concentration to that measured in Spain was in Swaziland where a MP concentration of 933 MPs/kg was recorded of which 93% are fragments. The concentrations of MPs in the United Kingdom (683 MPs/kg) and Poland (521 MPs/kg) were the only other two locations at which concentrations of MPs exceeded 500 MPs/kg. The other locations had concentrations ranging from 334 MPs/kg (India) to 50 MPs/kg (Nepal) and the average length of MPs in all locations (except Malaysia) were < 1 mm. In Malaysia, the average length was 1.2 mm.

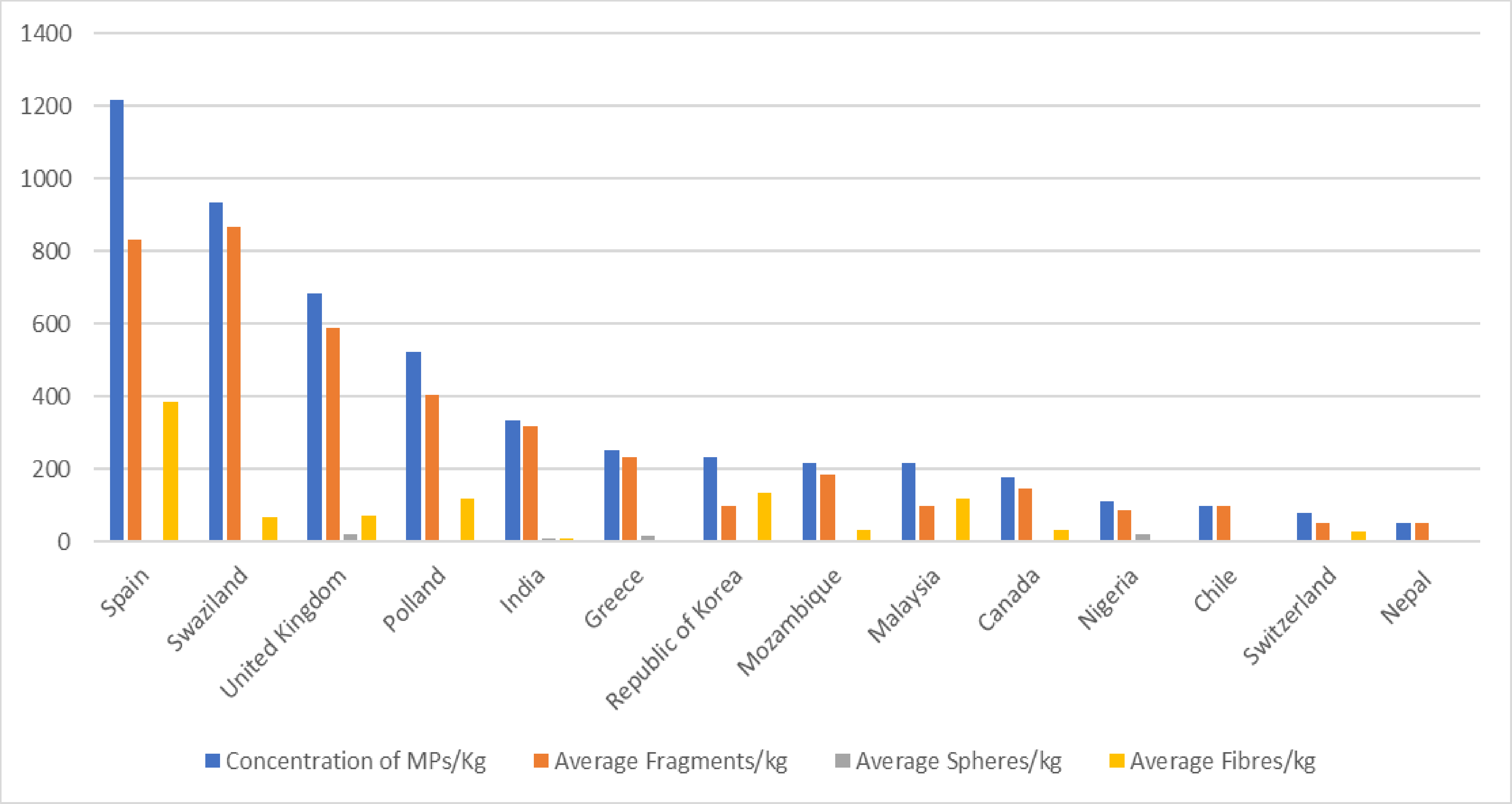


Figure 5. 8. Variation in MP concentrations (expressed as SMPs, as fragments, and as spheres) at all study locations.

The results obtained from this study were compared to other studies from the same region and in a few cases with previous studies from the same rivers as shown in Table 5.4. This comparison shows that results obtained in this study are not strikingly different from those of previous studies.

Table 5. 4. Comparison of concentrations, length, and morphology of MPs between this study and other studies from the same region.

Continent	Study	Location	MP Concentration (MPs/kg) dw	Average length (mm)	Dominant Morphology	MP Types	Notable finding
Africa	This study	Swaziland, Mozambique, and Nigeria	111	0.075	Spheres		
	(Olarinmoye et al., 2020)	A lagoon in Lagos, Nigeria	310–2319	0.1 – 1	Fibres and fragments	PP, PE and PS	
	(Dahms et al., 2020)	Braamfontein Spruit, in Johannesburg, South Africa	166.8	0.5 to 2	Fragments		Increased depth and the corresponding decreased flow encourage deposition of MPs in sediment
Asia	This study	Nepal, Malaysia, Republic of Korea and India	50 to 334	0.1 to 1.2			
	(Yang et al., 2021)	Koshi River (Nepal)	58 ± 27	Approximately 60% of MPs <1	98% Fibres	PE, PET, PA, PP and PS	Sewage effluents, air transport and deposition, Urbanization, agriculture, traffic, and tourism contributed to pollution
	(Sarijan et al., 2018)	Skudai and Tebrau rivers, (Malaysia)	200 ± 80 and 680 ± 140	Approximately 60% are 1 to 5	Fragments		
	(Ta and Babel, 2020)	Chao Phraya River, Thailand	91 ± 13	0.05–0.3	Fragments and fibres	PP, PE and PS	
	(Gopinath et al., 2020)	Red Hills Lake Tamil Nadu, India	27	0.3 to 2	Fibres (37.9%), fragments (27%)	PP, PE and PS	
	(Sruthy and Ramasamy, 2017)	Vembanad Lake, India	252.80 ± 25.76		Film and foam	LDPE, HDPE and PS	
Europe	This Study	Greece, Poland, Spain, Switzerland, and United Kingdom	80 to 1217	0.2 to 0.7	Fragments		

	(Horton et al., 2017a)	River Thames, UK	660	1 mm–4	Fragments and fibres	polypropylene, polyester and polyarylsulphone	Identified road marking paints as a source of microplastics pollution
	(Sekudewicz et al., 2021)	Vistula River in Poland	190 to 580	0.3–0.75	fibres	PS and PP	The highest MP pollution was in sampling points located near the WWTP
	(Margenat et al., 2021)	Cànoves stream (Spain)	1–601	0.02 to 2.5	95.3% fragments	PVC, PVCA and PE	
	(Blair et al., 2019)	River Kelvin in Glasgow, UK	161–432 MPs	0.25– 5	Fibres > 88%		
North America	This Study	Thames River, Canada	178	0.5	Fragments		
	(Vermaire et al., 2017)	Ottawa River, Canada	220		70 to 100 % microfibres		Plastic concentrations were significantly higher downstream of the wastewater treatment plant compared with upstream of the effluent output
	(Corcoran et al., 2019)	Thames River in Ontario, Canada	6 to 2444	0.063 to 2.38	Fragments and fibres	PE, PP, PVC, PET and PAN-polyacrylonitrile.	
South America	This Study	Tres Bazos, Chile	100	0.5	Fragments		
	(Correa-Araneda et al., 2022)	Biobío river, Chile (Water Samples)	37 ± 6		84% Fragments	PVC, PE, PET, PP and PS	
	(Gerolin et al., 2020)	Amazon rivers, Brazil	417 to 8178	0.063–5			

5.7 Ecological Risk Assessment

The risk quotient (RQ) method was used to assess the risk of our 8 target OPEs to aquatic organisms in the various sampling countries (Nepal, Greece, Poland, Swaziland, Spain, Mozambique, Switzerland, Canada, Malaysia, Republic of Korea, United Kingdom, Chile, Nigeria, and India) and continents. The RQ is calculated as a quotient of the measured environmental concentration (MEC) and the predicted no effect concentration (PNEC) and has been previously applied by several researchers (Shi et al., 2016, Gao et al., 2020, Yadav and Devi, 2020). The PNEC values used in this study were obtained from previous studies (Wang et al., 2019, Hu et al., 2021, Verbruggen et al., 2006). The potential risk from the OPE concentrations detected in this study to aquatic organisms were grouped such that: RQ values ranging from 0.01 to 0.1 were classed as low risk; RQ values between 0.1 and 1 as moderate risk; while RQ values above 1 were classed as high risk (Liu et al., 2017, Yan et al., 2017, Xing et al., 2018). As shown in Table 5.5, concentrations of all target compounds in all study locations in this study (except one) fall within the low risk. The one exception is the concentration of EHDPP in the United Kingdom (0.45 ng/g) can be considered to pose moderate risk as was the case for one of the study locations in the monthly samples from the Worcester Birmingham Canal presented in this study. This result is similar to the RQs reported by Cristale et al., (2013) for the River Aire in the United Kingdom where RQs for TCEP, TCIPP, TDCIPP, and TPHP all fell within the “no significant risk” range ($RQ < 1$) for aquatic organisms such as fish, daphnia, and algae (Cristale et al., 2013b, Yadav et al., 2018). Although ecological risk assessment for OPEs in sediments from other parts of the world (apart from China) are quite scarce, a few studies have reported similarly low risk ($RQ < 0.1$) values in other parts of the world. Pantelaki and Voutsa, (2021) reported low RQs ($RQ > 0.1$) for triethyl phosphate (TEP), TNBP, TCEP, TClPP, TDClPP, TBOEP, TEHP, and Triphenylphosphine oxide (TPPO).

In order to evaluate the overall effect of our target OPEs across the different countries and continents studied, the joint effect of target OPEs was calculated as a sum of individual RQs (∑RQs) as presented in Table 5.4. and this allowed the comparison of risk posed by the target OPEs across continents. Again, ∑RQ values across all five continents considered in this study fall within the "no significant risk" range (RQ < 1) (Cristale et al., 2013b). It is however important to note that the highest ∑RQ value (0.22) was recorded for Europe.

Table 5. 5. RQ values for target OPEs in sediment samples from Nepal, Greece, Poland, Swaziland, Spain, Mozambique, Switzerland, Canada, Malaysia, Republic of Korea, United Kingdom, Chile, Nigeria, and India grouped by continent.

Continents	Countries	Target OPEs										Mean ∑ RQs /continent
			TNBP	TCEP	TCIPP	TBOEP	EHDPP	TMTP	TPHP	TDCIPP	∑ RQs	
Africa	Mozambique											0.12
		MEC	0.02	9.64	12.25	15.95	0.36	0.32	1.25	0.87		
		PNEC	900	386	1700	2480	30	/	130	320		
		RQ	0.01	0.03	0.02	0.02	0.01	/	0.01	0.01	0.11	
	Nigeria											
		MEC	2.77	3.32	7.09	13.53	1.56	0.84	1.75	0.45		
		PNEC	900	386	1700	2480	30	/	130	320		
		RQ	0.01	0.02	0.01	0.02	0.05	/	0.01	0.01	0.13	
	Swaziland											
		MEC	0.02	11.6	15.7	3.3	0.19	0.67	0.47	0.39		
		PNEC	900	386	1700	2480	30	/	130	320		
		RQ	0.01	0.03	0.02	0.01	0.02	/	0.01	0.01	0.11	
Asia	Malaysia											0.17
		MEC	3.36	2.65	8.1	3.8	2.9	1.82	1.46	0.47		
		PNEC	900	386	1700	2480	30.2	/	130	320		
		RQ	0.01	0.02	0.02	0.01	0.1	/	0.01	0.01	0.18	
	Nepal											
		MEC	4.86	8.95	12.6	3.96	0.58	1.44	0.62	61.81		
		PNEC	900	386	1700	2480	30	/	130	320		
		RQ	0.02	0.02	0.02	0.01	0.02	/	0.02	0.19	0.3	
	Rep of Korea											
		MEC	0.02	3.7	5.8	2.8	0.42	0.04	0.4	0.2		
		PNEC	900	386	1700	2480	30	/	130	320		
		RQ	0.01	0.01	0.01	0.01	0.01	/	0.01	0.01	0.07	
	India											

		MEC	9.32	4.3	11.35	50.43	0.18	2.42	0.64	0.64		
		PNEC	900	386	1700	2480	30	/	130	320		
		RQ	0.01	0.01	0.02	0.02	0.02	/	0.02	0.01	0.11	
Europe	Switzerland											0.22
		MEC	4.05	10.6	23.04	9.34	1.19	4.27	1.09	2.71		
		PNEC	900	386	1700	2480	30	/	130	320		
		RQ	0.02	0.03	0.01	0.01	0.04	/	0.02	0.02	0.15	
	Poland											
		MEC	0.31	10.49	20.21	11.26	0.77	1.9	1.18	0.94		
		PNEC	900	386	1700	2480	30	/	130	320		
		RQ	0.01	0.03	0.01	0.02	0.03	/	0.02	0.01	0.13	
	Greece											
		MEC	2.56	7.72	23.45	10.95	0.32	0.28	1.27	0.51		
		PNEC	900	386	1700	2480	30	/	130	320		
		RQ	0.01	0.02	0.01	0.01	0.01	/	0.01	0.01	0.08	
	Spain											
		MEC	7.2	13	58.15	8.1	0.47	0.75	2.4	0.57		
		PNEC	900	386	1700	2480	30	/	130	320		
		RQ	0.02	0.03	0.03	0.01	0.02	/	0.02	0.01	0.14	
	UK											
		MEC	4.28	5.6	17.42	9.67	13.65	6.28	6.85	6.38		
		PNEC	900	386	1700	2480	30	/	130	320		
		RQ	0.02	0.02	0.01	0.01	0.45	/	0.05	0.02	0.58	
South America	Chile											0.1
		MEC	8.1	2.1	3.9	3.2	0.4	0.04	0.4	2.2		
		PNEC	900	386	1700	2480	30	/	130	320		
		RQ	0.02	0.02	0.01	0.01	0.01	/	0.01	0.02	0.1	
North America	Canada											0.12

		MEC	0.94	6.31	24.09	56.6	0.74	0.04	0.44	2.57		
		PNEC	900	386	1700	2480	30	/	130	320		
		RQ	0.01	0.02	0.01	0.02	0.03	/	0.01	0.02	0.12	

5.8 Correlation between MP and OPE concentrations in international sediment samples.

Visual observation of the relationship between mean number of MPs per kg, and mean concentration of all 8 target OPEs per location and $\sum_8$ OPEs reveal negative and positive correlations at different points as shown in Figure 5.9. A Pearson correlation test was then used to check the statistical significance of this observed relationship and the result is summarised in Table 5.6. The results show mostly weak and insignificant correlations except for the strong and significant correlations between average MPs/kg versus both TCEP and TCIPP and where the Pearson correlation values were 0.559 and 0.684 respectively while the p values were 0.038 and 0.007 respectively.

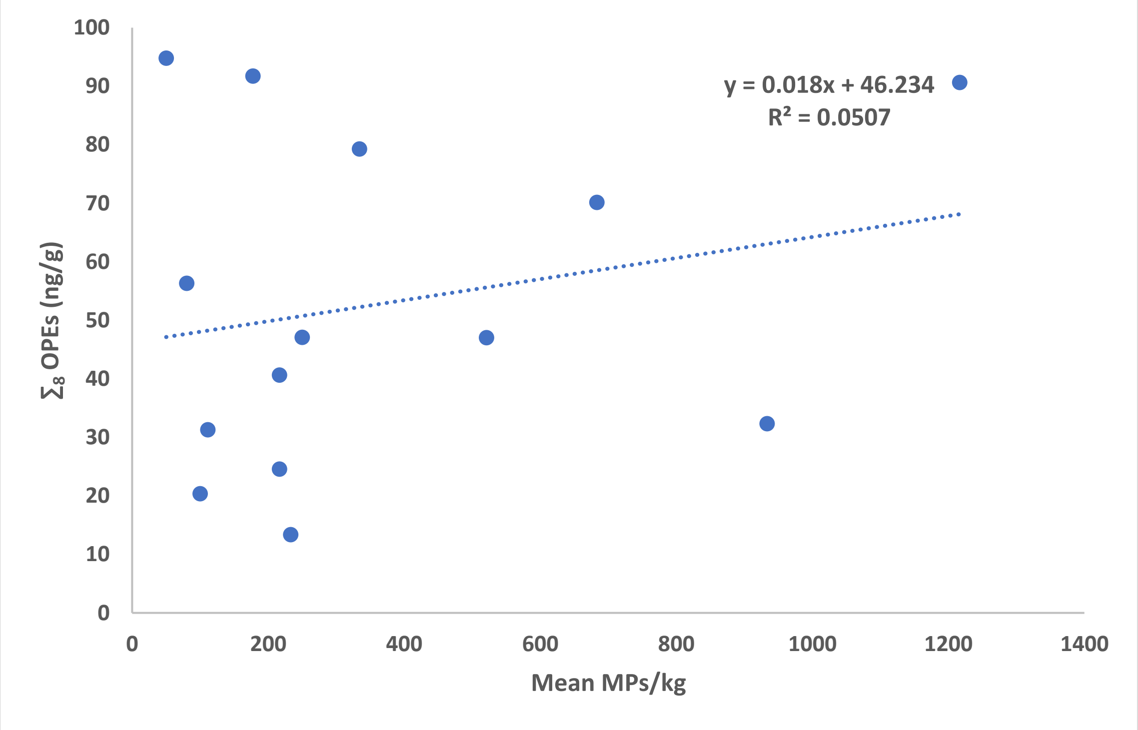


Figure 5. 9. Relationship between Mean number of MPs per kg and mean concentration of all 8 target OPEs per location and $\sum_8$ OPEs.

Table 5. 6. Correlation test between mean number of MPs per kg, mean concentration of all 8 target OPEs per location and $\sum_8$ OPEs.

Correlations

		Mean MPs/kg	**Average Length (µm)**	**Total Area**	**TNBP**	**TCEP**	**TCIPP**	**TBOEP**	**EHDPP**	**TMTP**	**TPHP**	**TDCIPP**	**Mean OPE Conc (ng/g)**	**$\sum_8$ OPEs**
Average MPs/kg	**Pearson Correlation**	1	-.197	.488	.050	.559*	.684**	-.147	.200	.117	.382	-.256	.225	.225
	Sig.		.499	.107	.866	.038	.007	.615	.493	.691	.177	.377	.439	.439
Average Length (µm)	**Pearson Correlation**		1	.601*	-.083	-.316	-.155	-.152	.077	.135	-.082	-.291	-.372	-.372
	Sig.			.039	.778	.271	.596	.603	.793	.646	.781	.314	.190	.190
Total Area (µm2)	**Pearson Correlation**			1	.308	-.039	.228	-.295	.476	.076	.330	-.281	-.160	-.160
	Sig.				.330	.903	.475	.352	.117	.814	.294	.376	.620	.620

*. Correlation is significant at the 0.05 level.

**. Correlation is significant at the 0.01 level.

CHAPTER 6

6.1 Summary, Discussion and Conclusions

Following the analysis of sediment samples collected from a range of locations across the world and from 3 rivers (and an urban canal) in the UK, the possibility of correlation between Microplastic concentrations and the concentration of OPEs in the same sediment samples was investigated to further understand the interaction between Microplastics and chemical plastic additives in freshwater sediments. Specifically, the overriding aim of this thesis was to investigate the relationship between abundance of MPs and concentrations of OPEs in sediment samples upstream and downstream of WWTPs (which previous studies have identified as a source of freshwater MPs), as well as in sediment samples from different parts of the world.

This was achieved using validated analytical methodologies for measuring the concentrations of OPEs, as well as quantifying/characterising MPs in sediments. Robust statistical tools were used to test observations and establish useful patterns and relationships.

The results revealed that the mean number of MPs varied across the UK study locations, with the highest mean number observed in the River Tame. Among the isolated microplastics, fragments were the most dominant morphological group, while fibres were the least abundant. All targeted OPEs were detected at the UK study locations, with varying detection frequencies. The concentration of Σ_8OPEs exhibited seasonal variations, with the highest concentrations observed during autumn months, and inversely correlated with river levels and flow rates. The overall potential risk posed by the target OPEs in the UK study locations was generally low, except for a moderate risk associated with 2-ethylhexyl diphenyl phosphate (EHDPP) in the Worcester-Birmingham canal.

In sediment samples from other countries, Σ_8OPEs concentrations ranged from 13 ng/g (dw) in the Republic of Korea to 95 ng/g (dw) in Nepal, with tris (1,3-dichloro-2 propyl) phosphate (TDClPP) contributing significantly to the high concentration in Nepal. The detection frequency of all target OPEs in most countries was 100%, with slightly lower detection frequencies in three countries.

MP concentrations varied among the countries studied, with Spain exhibiting the highest mean number of microplastics per kg of sediment (dw), while some locations showed no detectable microplastics. The percentage of fragments and fibres in sediment samples varied across countries, with South Korea having the highest percentage of fibres and Nepal having the highest percentage of fragments. Spheres were only identified in a few countries, with varying abundances. The average length of microplastics in sediment samples was generally below 1500 μm, with most countries having an average length below 300 μm.

6.2 Discussion

The occurrence and concentrations of OPEs in sediment samples from the four studied locations reveal that all four locations exhibited detectable concentrations of OPEs in the sediment, with a significant variation in concentrations in terms of both spatial and temporal dimensions. This substantial concentration of OPEs detected across the four study locations further confirms the previous assertion that sediments are a major sink for OPEs (Liao et al., 2020). It can also be attributed to their widespread application in everyday products such as polyurethane foam, textiles, furniture, electrical and electronic equipment, building materials, insulation materials, lacquers, glues, floor finish waxes as well as hydraulic fluids (Yang et al., 2019, Reemtsma et al., 2008). This perhaps also hints to a possible explanation for the fact that the highest average Σ_8OPE concentration was recorded in the Worcester-Birmingham canal (the most urban of all four-study location with the highest proximity to potential sources of OPEs and the only location without effluent from a WWTP). Higher concentrations of most individual target OPE were also recorded at the Worcester-Birmingham canal with this location providing the highest average concentration for 7 out of the 8 target OPEs analysed in the present study. Although other factors such as low flow rates in this waterway might also aid partitioning of OPEs to sediment thereby influencing the detection of higher concentrations of OPEs in sediments (Montuori et al., 2015).

Although evaluation of the impacts of WWTPs on OPE contamination was not a major objective of this study, the analysis of sediment samples collected upstream and downstream of the 3 WWTPs discharging to our target rivers, provided some insight into the possible impact of these WWTPs on OPE contamination of receiving sediments. With significantly higher concentration of Σ_8OPE and a number of individual targets OPEs observed downstream of only one study location and 3 target OPEs respectively, it could be argued that the WWTPs might not be contributing substantially to the concentration of OPEs in the sampled sediments. This is likely due to a combination of factors including: efficient removal of OPEs by the treatment operations of the WWTPs in this study, as well as potential degradation of parent OPEs (since they are not highly resistant to aerobic and anaerobic degradation) and their excretion as metabolites (di- and mono-esters) in humans and other animals, which were not quantified in the present or previous studies (Meyer and Bester, 2004, Pantelaki and Voutsa, 2022, Petropoulou et al., 2016).

This observed concentrations of OPEs was also influenced by seasonal variations and associated hydrodynamic factors (river level and flow rate) as the highest $\sum_8$OPE concentration for all study locations was recorded during the autumn months (September, October, and November), while the lowest concentrations were all recorded in the winter months (December, January, and February). The observed relationships between river level, flow rate, and OPE concentrations can be attributed to the diluting effect of higher river levels as a result of higher rainfall and the fact that slow flowrate facilitates contaminant partitioning/adsorption to sediment particles (Luo et al., 2021). Another possible explanation is the overflow caused by more abundant precipitation in winter which might lead to chemical loss in pipes before they reach the WWTPs.

Although the potential risk posed by the target OPEs in all study locations was found to be low (except for a moderate risk identified for EHDPP in the Worcester-Birmingham canal), it should be noted that risk assessments based on single chemicals, do not adequately account for possible synergistic and antagonistic effects of the complex chemical mixtures present in sediments.

Similar to OPEs, sediment samples from the four studied locations revealed that all four locations exhibited substantial concentration of MPs, with a significant variation in concentrations in terms of both spatial and temporal dimensions as well as morphological characteristics.

Unlike the OPEs where the highest concentration was observed at the most urban location without input from a WWTP, the highest concentration of MPs was recorded at the study location receiving effluent from the WWTP servicing the largest population. This might be suggestive of a possible relationship between the population serviced by a WWTP and MP pollution as suggested by some previous studies (Li et al., 2018) but other factors such as proportion of industrial and domestic wastewater in the influent and treatment processes must be carefully considered before reaching such a conclusion.

Analysis of the mean quantity of MPs per kg (dw) for all four research locations revealed notable variations in MP concentrations, with the highest mean number of MPs/kg observed in the River Tame. The Worcester -Birmingham Canal exhibited a significantly greater abundance of MPs compared to the River Severn and River Tame, while the MP abundance in the River Tame surpassed that of the River Sowe. Fragments (71%) were the most prevalent morphological group among the isolated MPs, whereas fibres (9%) were the least abundant.

The higher prevalence of fragments observed across the four study locations indicates that the predominant sources of these microplastics (MPs) are more likely to be secondary in nature, specifically arising from plastic fragmentation processes, as opposed to primary sources. The abundance of fragments in freshwater sediments, which has also been documented by other researchers (Margenat et al., 2021; Ramírez-Álvarez et al., 2020b), is often attributed to the continuous input of plastic waste into freshwater environments from diverse origins, including urban runoff, industrial discharges, and inadequate waste management practices. As a consequence of these inputs, plastic fragments tend to sink and accumulate in riverbeds (Wang et al., 2017). The settling behaviour of fragments can also provide some explanation for their abundance. Fragments possess a lower surface-to-volume ratio compared to fibres, making them more likely to descend to the riverbed (Lin et al., 2018).

Although previous studies have pinpointed WWTPs as potential contributors to MP pollution in freshwater environments (Kay et al., 2018, Wu et al., 2022), the difference in MP abundance upstream and downstream of WWTPs across all four study locations in this study were not statistically significant. This possibly implies that there might be other important sources of MPs such as atmospheric deposition, sewage sludge used for agricultural purposes, and breakdown of larger plastic items. The study finds that the highest number of MPs per kg occurred during the winter months for all four study locations, with one potential explanation being the increased wash-off of terrestrial MPs into the rivers due to higher rainfall and corresponding higher flow rate in winter. Additionally, the study reveals a strong positive correlation between the total number of MPs/kg and both river level and river flow rate at the River Severn, emphasizing the importance of considering hydrological factors when studying the distribution and transport of MPs in aquatic environments.

A more global approach to understanding the research questions that informed this study was achieved using the results obtained from the analysis of sediment samples from Nepal, Greece, Poland, Swaziland, Spain, Mozambique, Switzerland, Canada, Malaysia, Republic of Korea, United Kingdom, Chile, Nigeria, and India.

OPEs were detected across all five continents, with their elevated detection frequency signifying their extensive use and production. The variation in OPE concentration across the various locations can be attributed to a number of factors one of which is the consumption patterns of OPEs in form of flame retardants and/or plasticizers across the different countries from where sediment samples were collected (Guo et al., 2022). The high concentrations of OPEs in North America, (particularly in Dingam Creek, Canada), and Europe (largely influenced by the high concentration of OPEs recorded in the Chicheley Brook, UK) would not come as a surprise seeing that North America and Western Europe accounts for 34 % and 29 % respectively of the global consumption of flame retardants (Yang et al., 2019). The high concentration at Chicheley Brook in the UK also supports the previous assertion that WWTPs might be a contributor to environmental pollution with OPEs thereby highlighting the need for enhanced sewage treatment work management to curtail OPE release into the environment.

This global approach also revealed significant variations in MP concentrations across continents, with the highest concentration documented in the Francoli River, Spain. Besides the impact of an industrial harbour and other industrial activities around Tarragona and Barcelona (Eljarrat et al., 2001) which can account for this increased MP concentration, similarly high concentration of MPs in Spain has been previously associated with the extensive use of sewage sludge for agricultural purposes as 65% of Spain's sewage sludge production is recycled through agricultural soils (Roig et al., 2012) .

Other factors that might be responsible for the variation in concentration, size, and morphology of the MPs across different countries include disparities in plastic waste generation and management practices across continents, as well as fluctuations in environmental factors such as river flow and proximity to plastic pollution sources. Differences in MP morphology across countries could also be attributed to the type and age of plastic debris, and exposure to environmental factors like UV radiation and wave action.

6.3 Key findings

While in some instances, higher OPE concentrations were observed downstream of WWTPs compared to upstream, suggesting a possible impact of WWTPs on environmental pollution with OPEs; our data shows that urbanisation and proximity to potential anthropogenic sources of contamination might have a greater influence on freshwater contamination with OPEs than WWTPs. This is because the highest average $\sum_8$OPEs concentration over the 12 months period was recorded in the Worcester and Birmingham canal (the most urban location and the only location in this study that does not receive direct input from a wastewater treatment plant)

The situation with MPs appears to be different as MP concentrations in each of the same four study locations in which OPEs were measured, were highest in the River Tame, the location with the WWTP servicing the highest population of people of all 4 locations. This suggests that in contrast to what was observed with OPEs, WWTPs might have a greater influence on freshwater contamination with MPs.

Although requiring further investigation, the results of this study suggest a possible relationship between the size of the population serviced by the respective wastewater treatment plants and concentrations of OPEs in sediment receiving discharge from that WWTP. The relative abundance of OPEs by location was: WWTP1 (servicing the highest population of approximately 2.5 million people) > WWTP 2 (serves a population of 420,470) > WWTP3 (services a total population of 50,000 to 200,000 people).

Freshwater contamination with OPEs is influenced by seasonal variations and corresponding hydrodynamic factors (flow rate and river level) as the highest concentration of $\sum_8$ OPEs for all study locations was recorded in the autumn months (September, October, and November) that coincided with the lowest flow rates and river levels.

In contrast, seasonal variation of MPs showed the highest concentrations to occur in the winter months at all four sampling locations. This may possibly be attributed to the washing of terrestrial MPs into the target water body as a result of higher rainfall during winter.

The potential risk posed by the target OPEs in all study locations were found to be low except for EHDPP in the Worcester-Birmingham Canal which can be considered to pose moderate risk. However, the possibility of overestimation or underestimation of risk has been cited as one of the limitations of the PNEC approach used in this study to assess risk.

Although this study is the first report of OPEs in UK freshwater sediments, most of the key findings such as the concentration of OPEs, seasonal trends in OPE concentrations, the role of WWTPs on freshwater contamination with OPEs, and the potential risk of the target OPEs to aquatic organisms are broadly consistent with those reported in previous studies in other parts of the world.

A lower percentage of the smaller size range (63 to 199 µm) of MPs was observed in locations receiving discharge from WWTPs compared to the Worcester-Birmingham Canal - the only site without a WWTP near the sampling point. This might be suggestive of a possible role of WWTPs in sediment MP size variation.

Most of the MPs identified in this study may have originated from secondary sources as fragments were found to be the most dominant morphological group (71%) of the MPs found at our 4 study locations.

The low percentage of MP fibres detected in this study, confirms the results of some previous studies where the low number of fibres was attributed to factors such as reduced settlement due to shape and low density (Hoellein et al., 2019) as well as the Nile Red method not being very effective in detecting fibres and microfibres (Tamminga et al., 2017, Shim et al., 2016, Nel et al., 2021).

Our observation of a negative correlation between mean MPs number and OPE concentrations in sediment indicates that the sources of MPs and OPEs are different and pending further investigation, might be a possible indication that MPs may be adsorbing OPEs in the studied samples.

In line with some previous studies, the concentrations of MPs and $\sum_8$ OPEs recorded in sediments from different continents shows the highest and lowest concentrations to be present in Europe and North America respectively.

The need for a more global approach to the study of environmental pollution was highlighted by the lack of data on freshwater MP pollution in some parts of the world. This is an important omission as the second highest concentration of MPs recorded in this study was in Africa – a continent hitherto poorly characterised with respect to MPs.

6.4 Research gaps and future perspectives

This study makes a valuable contribution to the current understanding of the role of WWTPs in environmental pollution and the relationship between OPEs and MPs in freshwater sediment. One of the intended approaches to this study was to investigate a possible correlation between concentrations in sediments of: MPs, OPEs in MPs isolated from sediments, and OPEs in natural sediment particles. However, a limitation of this study is that polymer types were not identified and the concentration of OPEs in isolated MPs could not be determined as the number of MPs isolated per location was insufficient to provide a measurable mass of isolated MPs. Further study is therefore recommended to understand the role of MPs as a source or a sink of organic chemicals to the aquatic environment. Studies are required to understand the fate and behaviour of hazardous MP chemical additives and the factors influencing their release to the freshwater environment from MPs. The impact on additive bioavailability of factors such as plastic polymer type, concentration, and physicochemical properties of chemical additives, the organic content of sediment and the temperature, is also unknown.

The lack of definitive information about removal efficiency of treatment operations in WWTPs, as well as the potential degradation of parent OPEs and their excretion as metabolites makes it difficult to obtain conclusive evidence on the role of WWTPs as sources of OPEs to the environment; and further research in this area is encouraged. Also, the interaction between MPs and chemical pollutants under natural environmental conditions, requires further study to facilitate the development of technologies to effect removal of pollutants from aquatic environments. Furthermore, as the PNEC approach adopted for ecological risk assessment in this study can lead to overestimation or underestimation of risk, research into more reliable approaches suitable for large data sets is strongly recommended. Also, the fact that most current risk assessments are based on single chemical exposures, does not consider possible synergistic or antagonistic effects that might result from chemical mixtures.

Finally, use of radiometrically dated freshwater sediment cores for studies on temporal trends of MPs (and associated additives such as OPEs) is encouraged, as this will not only provide information on historical trends but also enable modelling and prediction of future trends and associated risks to the aquatic environment.

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Appendices

Concentrations of MPs/Kg and target OPEs (ng/g) in the studied international sediment samples.

Country	Continent	Location	Mps/Kg	Average Length (μm)	Fragments/kg	Spheres/kg	Fibres/kg	TNBP (ng/g)	TCEP (ng/g)	TCIPP (ng/g)	TBOEP (ng/g)	EHDPP (ng/g)	TMTP (ng/g)	TPHP (ng/g)	TDCIPP (ng/g)	∑8 OPEs (ng/g)
Nepal	Asia	Unnamed rivers (Nepal)	100	102	100	0	0	0.02	6.60	9.80	1.92	0.20	0.60	0.67	1.61	21.42
Greece	Europe	River Kifissos Attika ATT 2	67	671	67	0	0	0.02	11.90	32.50	6.00	0.22	0.51	1.57	0.49	53.21
Poland	Europe	River Vistula	567	175	567	0	0	0.02	12.40	7.70	12.40	0.42	0.99	1.29	0.37	35.59
Swaziland	Africa	Mbuluzi River	933	292	867	0	67	0.02	11.60	15.70	3.30	0.19	0.67	0.47	0.39	32.34
Spain	Europe	Francoli River	2433	425	1667	0	767	0.69	18.90	89.10	11.30	0.63	0.60	3.00	0.93	125.15
Switzerland	Europe	Thur river catchment	100	939	67	0	33	0.02	20.56	13.70	9.80	0.28	16.30	1.24	2.75	64.65
Poland	Europe	River Vistula	400	937	167	0	233	1.50	9.10	23.80	15.80	0.43	1.25	0.25	0.98	53.11
Mozambique	Africa	Mkomati River	167	83	167	0	0	0.02	11.30	11.80	14.90	0.25	0.04	1.33	1.00	40.64
Switzerland	Europe	Thur river catchment	67	165	67	0	0	0.02	9.90	18.80	20.90	0.23	0.29	0.81	0.11	51.06
Mozambique	Africa	Limpopo River	267	132	200	0	67	0.02	7.97	12.70	17.00	0.47	0.59	1.16	0.74	40.65

Canada	North America	Thames Ontario (Kintore Creek East)	133	1127	100	0	33	1.00	6.40	16.38	4.10	0.02	0.04	0.89	3.00	31.83
Malaysia	Asia	Thames Ontario (Dingman Creek)	200	896	133	0	67	0.02	3.30	9.60	4.40	5.50	0.04	2.51	0.03	25.40
Poland	Europe	Thames Ontario (Kintore Creek West)	467	224	433	0	33	0.02	3.90	3.07	9.90	0.02	0.04	0.03	0.03	17.01
Switzerland	Europe	Thames Ontario (Kintore Creek East)	67	905	33	0	33	0.02	10.45	37.40	6.70	0.02	0.35	1.00	1.00	56.94
Canada	North America	Thames Ontario (Dingman Creek)	400	360	333	0	67	0.02	7.04	41.90	159.00	1.70	0.04	0.03	1.10	210.83
Canada	North America	Thames Ontario (Kintore Creek West)	0	0	0	0	0	1.80	5.48	14.00	6.70	0.50	0.04	0.40	3.60	32.52
Republic of Korea	Asia	River Geum	233	527	100	0	133	0.02	3.70	5.80	2.80	0.42	0.04	0.40	0.20	13.38
Nepal	Asia	Unnamed rivers (Nepal)	0		0	0	0	9.70	11.30	15.40	6.00	0.95	2.27	0.56	122.00	168.18
Greece	Europe	River Kifissos Attika ATT 1	433	110	400	33	0	5.10	3.54	14.40	15.90	0.42	0.04	0.96	0.53	40.89

United Kingdom	Europe	River Irwell C	300	351	233	0	67	0.98	3.90	25.30	12.90	7.30	2.06	2.40	20.40	75.24
Switzerland	Europe	Thur river catchment	100	1230	33	0	67	8.90	5.30	19.20	4.80	0.40	2.90	1.50	3.20	46.20
Malaysia	Asia	Klang River 2	233	1475	67	0	167	6.70	2.00	6.60	3.20	0.30	3.60	0.40	0.90	23.70
United Kingdom	Europe	River Itchen	333	216	300	0	33	8.40	2.50	8.00	7.80	1.20	4.30	1.30	0.90	34.40
United Kingdom	Europe	River Irwell Site B	367	592	233	0	133	0.02	3.50	15.00	2.20	5.10	3.60	1.20	2.80	33.42
Switzerland	Europe	Thur river catchment Thur 1	67	174	67	0	0	11.30	6.80	26.10	4.50	5.00	1.50	0.90	6.50	62.60
Chile	South America	Tres Bazos	100	518	100	0	0	8.10	2.10	3.90	3.20	0.40	0.04	0.40	2.20	20.34
United Kingdom	Europe	River Irwell Site A	300	122	300	0	0	7.90	5.20	25.20	7.80	7.90	2.20	29.70	5.10	91.00
Spain	Europe	Canoves slow	0	0	0	0	0	13.70	7.10	27.20	4.90	0.30	0.90	1.80	0.20	56.10
United Kingdom	Europe	Chicheley Brook Site 1	2200	354	1900	133	167	1.30	7.80	9.00	11.10	6.40	7.80	5.30	8.40	57.10
United Kingdom	Europe	Chicheley Brook Site 2	600	394	567	0	33	7.10	10.70	22.00	16.20	54.00	17.70	1.20	0.70	129.60
Nigeria	Africa	Niger Upstream	167	93	167	0	0	1.20	2.10	5.19	17.55	2.25	0.66	2.36	0.73	32.04
Nigeria	Africa	Benue Upstream	100	42	33	67	0	2.81	5.27	8.75	14.88	1.90	1.38	2.19	0.58	37.76
Nigeria	Africa	Benue downstream	67	90	67	0	0	4.31	2.58	7.32	8.16	0.53	0.48	0.70	0.02	24.10
India	Asia	Ganges/Hooghly system	0	0	0	0	0	1.90	1.20	7.30	54.00	0.20	3.30	0.40	0.05	68.35
India	Asia	Ganges/Hooghly system	267	166	267	0	0	1.40	0.80	3.80	53.00	0.30	1.15	0.60	0.30	61.35

India	Asia	Ganges/Hooghly system	200	488	200	0	0	1.20	0.80	5.50	11.00	0.40	4.40	0.30	1.10	24.70
India	Asia	Ganges/Hooghly system	0	0	0	0	0	1.10	1.10	4.30	136.00	0.40	4.30	0.60	6.30	154.10
India	Asia	Ganges/Hooghly system	567	251	533	0	33	1.00	0.80	4.60	89.00	0.30	3.10	0.60	0.70	100.10
India	Asia	Ganges/Hooghly system	167	214	167	0	0	1.20	0.70	5.00	36.00	0.10	0.25	2.40	0.80	46.45
India	Asia	Ganges/Hooghly system	367	308	333	0	33	1.40	0.90	5.50	82.00	0.20	0.55	0.30	0.70	91.55
India	Asia	Ganges/Hooghly system	67	135	67	0	0	1.80	0.80	6.10	28.00	0.10	0.35	0.30	0.70	38.15
India	Asia	Ganges/Hooghly system	133	366	133	0	0	2.40	3.50	9.30	81.00	0.30	0.80	0.30	0.70	98.30
India	Asia	Ganges/Hooghly system	133	268	133	0	0	3.20	1.90	10.50	139.00	0.30	3.30	0.40	1.10	159.70
India	Asia	Ganges/Hooghly system	1167	255	1100	33	33	21.70	5.50	7.90	19.40	0.10	0.30	0.20	0.96	56.06
India	Asia	Ganges/Hooghly system	433	170	367	67	0	23.30	6.70	7.60	38.30	0.10	3.75	0.15	0.42	80.32
India	Asia	Ganges/Hooghly system	467	176	467	0	0	33.60	6.40	15.20	33.60	0.09	0.33	0.28	0.47	89.97
India	Asia	Ganges/Hooghly system	233	318	233	0	0	4.90	3.90	5.20	28.60	0.15	0.10	0.26	0.25	43.36
India	Asia	Ganges/Hooghly system	267	138	267	0	0	19.00	5.10	9.70	21.30	0.07	0.05	0.30	0.38	55.90
India	Asia	Ganges/Hooghly system	333	471	300	33	0	9.00	3.60	6.70	25.90	0.10	5.90	0.40	0.20	51.80
India	Asia	Gandak	1333	172	1333	0	0	16.00	5.40	7.90	27.90	0.10	6.20	0.35	0.41	64.26
India	Asia	Ganges/Hooghly system	533	175	533	0	0	21.60	10.60	16.50	24.90	0.34	0.50	1.20	0.35	75.99
India	Asia	Sone	167	161	167	0	0	10.98	2.60	6.20	20.60	0.07	0.09	0.30	0.44	41.28

India	Asia	Gandak	233	236	233	0	0	19.40	3.77	11.60	49.30	0.06	5.20	0.22	0.18	89.73
India	Asia	Koshi	867	318	800	0	67	4.30	3.37	17.10	37.70	0.25	15.66	2.70	0.49	81.57
India	Asia	Ganges/Hooghly system	100	118	100	0	0	35.70	14.60	49.00	51.00	0.19	0.62	0.54	0.22	151.87
India	Asia	Ganges/Hooghly system	333	155	333	0	0	4.56	3.30	13.80	30.80	0.16	0.34	0.22	0.39	53.57
India	Asia	Ganges/Hooghly system	300	168	300	0	0	4.70	3.00	1.50	50.70	0.31	0.45	0.59	0.12	61.37
India	Asia	Ganges/Hooghly system	200	197	167	0	33	4.10	4.20	16.00	60.90	0.17	0.10	2.28	0.05	87.80
India	Asia	Kiul	667	251	667	0	0	5.80	6.70	18.65	33.50	0.01	0.83	1.18	0.38	67.05
India	Asia	Ganges/Hooghly system	0	0	0	0	0	5.30	3.70	17.20	55.90	0.12	0.56	0.45	0.22	83.45
India	Asia	Ganges/Hooghly system	133	125	67	67	0	5.50	5.70	15.97	68.60	0.12	0.46	0.42	0.30	97.07
India	Asia	Ganges/Hooghly system	100	389	100	0	0	8.10	9.20	20.00	65.20	0.11	7.52	0.29	0.32	110.74
India	Asia	Ganges/Hooghly system	267	209	133	100	33	5.30	9.10	14.90	59.70	0.21	2.30	0.53	0.23	92.27