



A spatio-temporal record of  
microplastic particles and natural  
textile fibres in the River Trent and its  
tributaries, and in atmospheric  
deposition across the Trent catchment

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BSc (Hons), MRes

Thesis submitted to the University of Nottingham for the degree of  
Doctor of Philosophy

December 2019

## **Acknowledgements**

I am incredibly grateful for the support and encouragement I have received from friends, family and colleagues since the start of my PhD. My first thanks go to Dr Rachel Gomes and Dr C. Paul Nathanail who have guided me through the three years of my PhD with so much enthusiasm, and who have ensured that I have been able to take full advantage of every opportunity.

Thanks also go to Teresa Needham for her tireless support both in and out of the laboratory; to Dr Bill Macnaughtan for his assistance and training in FTIR spectroscopy; and to Dr Amanda O'Byrne (Amanda Burson) for the instruction in fluorescence microscopy. In addition to supporting me in their respective areas of expertise, Teresa, Bill, and Amanda's willingness to discuss ideas and engage with my research has been invaluable. The fieldwork and laboratory work conducted throughout this PhD has also been supported by Clare Templey, Ian Conway, and University of Nottingham Estates.

This PhD has been financially supported by the University of Nottingham's Sir Francis Hill Scholarship, the School of Geography, and the Papplewick Pumping Station Water Education Trust. The opportunities, both professional and personal, that this support has provided have greatly enhanced my postgraduate experience.

I would also like to recognise the support of my colleagues and friends in the University of Nottingham, School of Geography, who have made my postgraduate studies so enjoyable.

A special thank you to must go to my parents, Wendy and Ray, for all that they have done for me, and who assisted in all bar one of the freshwater sampling occasions conducted for this research.

Finally, my biggest thanks go to my supervisor Dr Matthew Johnson, who has coached me throughout both my MRes and PhD. His support has ensured that my PhD has been as fulfilling an experience as possible, and has given me the confidence to pursue a career in academia.

*The most significant barrier to developing and implementing a viable environmental strategy appears to be the environmental debate itself. It has long left its home territory of science and ventured into the less predictable public arena of green groups, public interests, and public watchdogs. Politicization of environmental issues has lead [sic] to a degree of polarization among various interests that seriously interferes with objective scientific evaluation of the key issues*

Andrady (2003)

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## **Extended Abstract**

### **Context**

The study of microplastic (<5 mm) pollution has, over the past 15 years, emerged as an emotive topic of environmental concern. The environmental impacts of microplastic particles are known to be varied and potentially severe. Freshwater systems are known to be pathways for the transport of microplastic particles from their sources, ultimately to the marine environment. Despite this, efforts to study freshwater microplastic pollution have been limited by the methodological approaches taken to collect and process samples, and identify particles within them. Many freshwater microplastic studies are therefore unable to provide a comprehensive assessment of their concentrations.

This thesis presents a systematic and objective assessment of freshwater and airborne microplastic pollution in and around the River Trent across Staffordshire, Nottinghamshire and Leicestershire.

### **Methods**

Microplastic pollution was quantified in three rivers and in atmospheric deposition across the Trent Catchment, UK. Over a 12 month period, freshwater samples were collected every four weeks on the Rivers Trent, Leen, and Soar, and in atmospheric deposition at four sites across the University of Nottingham's UK campuses every two weeks.

This sampling campaign was supported by robust techniques to categorise microplastic particles isolated from environmental samples. This included the development of a comprehensive methodology for the categorisation of textile fibres, and a thorough assessment of the use of Nile red in microplastic quantification.

### **Results**

This work has identified microplastic particles at all freshwater and atmospheric sites sampled. However, the concentrations of microplastic particles quantified in this research have been some of the lowest recorded in

the literature; not once exceeding 0.4 particles / L across the freshwater sampling network. The systematic nature of this sampling campaign has generated the one of the most temporally comprehensive dataset of any freshwater microplastic sampling campaign. By assessing temporal variation this work has identified the extent to which previous freshwater microplastic extrapolations reported in peer reviewed literature could have been misleading.

Furthermore, the methodological developments in the identification of microplastic particles made during the course of this work cast doubt on the findings of previous studies that have relied on the visual identification of microplastic textile fibres, and those that have used Nile red in microplastic quantification.

### **Main conclusions**

Microplastic particles are one of many anthropogenic pressures on environmental systems, and there is a need for pragmatic assessment of their prevalence. Without temporal sampling, freshwater microplastic studies are unlikely to be representative of the bodies of water sampled. Moreover, the methodologies employed to identify microplastic particles in environmental samples are subject to unquantified levels of error. The categorisation of textile fibres in particular has also provided further evidence to support emerging concerns for the potential ecological harm of natural textile fibres such as cotton and wool, alongside their plastic analogues.

Environmental concern for the ubiquity of microplastic pollution is well founded, and efforts to minimise microplastic emission should be encouraged. However, the research presented in this thesis highlights the importance of robust methodologies in microplastic sampling and identification, and recognises the need for objectivity in the interpretation and dissemination of microplastic research.

## Publications, presentations, and interviews

### Publications

Three chapters of this thesis have formed the basis of multi-authored manuscripts submitted to, and in two cases published in, peer reviewed journals. Authorship is shared with my supervisory team and additional colleagues without whom this research would not have been possible. Author contributions to the associated manuscript are stated at the end of each chapter. The title of each of these chapters is shared with its respective manuscript. The manuscript's stage of publication is outlined below.

Chapter 4 includes the text of the published manuscript and additional work that was not included in the manuscript due to the word limits of the chosen journal. The text, figures, and tables in chapter 5 are the same as those of the published manuscript. The text, figures, and tables in chapter 6 are the same as those in the manuscript associated with this chapter that has been submitted for peer review.

Thesis chapter	Title	Status (Date accepted)	Journal or (Target Journal)
4	Exploring the Efficacy of Nile Red in Microplastic Quantification: A Costaining Approach <a href="https://doi.org/10.1021/acs.estlett.9b00499">https://doi.org/10.1021/acs.estlett.9b00499</a>	Published September 2019	<i>Environmental Science and Technology Letters</i>
5	Freshwater and airborne textile fibre populations are dominated by 'natural', not microplastic, fibres <a href="https://doi.org/10.1016/j.scitotenv.2019.02.278">https://doi.org/10.1016/j.scitotenv.2019.02.278</a>	Published February 2019	<i>Science of the Total Environment</i>
6	Spatiotemporal variation of microplastic particles in atmospheric deposition and freshwater systems	Submitted November 2019	<i>(Environmental Pollution)</i>

### Presentations and interviews

Throughout the course of this PhD, I have communicated the research conducted and the broader issue of plastic pollution to a variety of public, professional, and academic audiences, detailed below.



### **Conference presentations**

**Stanton, T.**, Johnson, M., Nathanail, P., MacNaughtan, W. and Gomes, R.L. Current methodologies for the isolation of microplastic pollution cannot account for all domestic microplastics. *SETAC Europe 27<sup>th</sup> Annual Meeting* 7-11 May 2017. Brussels, Belgium. Oral presentation.

**Stanton, T.**, Johnson, M., Nathanail, P., MacNaughtan, W. and Gomes, R.L. Fluffy Rivers: Microplastic Particles and Textile Fibres in Upstream River Catchments *Nottingham Environment Conference*. 25-26 June 2018. Nottingham, UK. Oral presentation.

**Stanton, T.**, Johnson, M., Nathanail, P., MacNaughtan, W. and Gomes, R.L. Assessing the contribution of wastewater treatment to microplastic pollution *European Wastewater Management Conference* 17-18 July 2018. Manchester, UK. Poster presentation.

**Stanton, T.**, Johnson, M., Nathanail, P., MacNaughtan, W. and Gomes, R.L. Microplastic pollution in upstream river catchments. *MICRO 2018*, Arrecife, Lanzarote. 19-23 November 2018. Oral presentation

### **Invited speaker (academic / professional)**

**Stanton, T.** Microplastic pollution and wastewater treatment: State of knowledge and future directions *Foundation for Water Research Wastewater Forum*, 14<sup>th</sup> June 2017. London, UK. Oral presentation.

**Stanton, T.**, Johnson, M., Nathanail, P., MacNaughtan, W. and Gomes, R.L. Microplastic pollution in upstream river catchments. *Institute of Water Welsh Area Autumn Forum*, 31<sup>st</sup> October 2018. Cardiff, UK. Oral presentation.

**Stanton, T.**, Johnson, M., Wood, H. Plastics in the Environment *Institute for Mechanical Engineers 'Closing the plastics cycle – A Review of Current Best Practice Around The Whole Cycle'*, 2<sup>nd</sup> July 2019, London. Oral presentation.

### **Invited speaker (public)**

**Stanton, T.** The macro problem of a 'micro' pollutant *Papplewick Pumping*

*Station annual lecture*. 7<sup>th</sup> November 2018. Nottingham, UK. Oral presentation.

**Stanton, T.** *Plastic, Pollution, and People*. Pint of Science. 21<sup>st</sup> May 2019. Nottingham, UK. Oral presentation.

**Stanton, T.** Microplastic pollution in the Trent catchment *PubhD*, 17<sup>th</sup> July 2019. Nottingham, UK. Oral presentation given using only a flipchart.

**Stanton, T** The unnatural natural: plastic alternatives and the problems they pose *University of Nottingham Science Public Lecture Series*. 18th July 2019 - Nottingham, UK. Oral presentation.

### **Invited panellist**

Plastic Panel *Nottingham Environment Conference*, 24<sup>th</sup> June 2019. Nottingham, UK.

Microplastics in Wastewater: Responsibilities & Challenges *European Wastewater Management Conference*, 16<sup>th</sup> July 2019. Birmingham, UK.

### **Interviews**

Interviewed about my PhD research for the BBC's East Midlands Today regional news programme (Aired August 2019)

Interviewed for a BBC Wildlife Magazine fact file on microplastic pollution (<https://www.discoverwildlife.com/people/facts-about-microplastics/>)

Interviewed about my research for a World Textile Information Network podcast (Aired November 2019)

## 1. Context

In 1972 scientists sampling the Sargassum community of the Sargasso Sea reported the unexpected incidence of plastic particles smaller than 5 mm in their neusten net trawls (Carpenter and Smith, 1972). This was the first publication to document the environmental incidence of what is now known as microplastic pollution. Later that same year, a survey of similarly small plastic particles in coastal waters made the cover of *Science* (Carpenter et al, 1972). Despite these early accounts of the presence of small plastic particles in the marine environment, the prevalence of small plastic particles was not of great scientific concern until Thompson et al. (2004) reported the ubiquity of what they called 'microplastics' in predominantly coastal sediments and waters around the UK. Five years later, Arthur et al. (2009) defined microplastic particles as pieces of plastic smaller than 5 mm in their largest dimension, which included those observed by Carpenter and Smith (1972) and Carpenter et al. (1972). Since the start of this modern era of microplastic pollution studies, the ubiquity of microplastic particles, and their environmental impacts have garnered extensive environmental concern from public, political, regulatory, industrial, and scientific stakeholders.

The mass production of plastic polymers began in the mid-twentieth century (Thompson et al. 2009). Since then plastic production and consumption has increased rapidly, and in 2017 348 million tonnes of plastic was produced globally (Plastics Europe 2018). The populations of all but the most isolated contemporary civilisations exploit plastic products in their day-to-day lives. Despite a growing evidence base for the environmental harm associated with plastic waste, global reliance on plastic has not diminished, and plastic pollution continues to present a varied and complex threat to organisms in a wide range of environments across the planet.

Plastic waste has the potential to compromise ecosystems and the organisms that inhabit them. In its many forms, plastic waste can entangle animals as large as sharks and cetaceans (Stelfox et al. 2016). It can also cause gastrointestinal blockages if ingested (Lusher et al. 2015) and can block other orifices, such as

nasal passages (Robinson and Figgner, 2015). These consequences of plastic pollution have been shown to have both lethal and sub-lethal effects on organisms. But the impacts of plastic pollution go beyond this visible harm.

Through time, plastic pollution does not biodegrade, instead breaking down to ever-smaller pieces (Scott, 1972). Eventually this plastic debris will break down to pieces <5 mm in their largest dimension: the size of microplastic particles. Microplastic pollution can compromise the physical, chemical and biological function of the environments it pollutes. It is known to alter the thermal properties of sediments (Carson et al. 2011), and act as a vector for bacteria (McCormick et al. 2016; Arias-Andres et al. 2018). Pollutants including heavy metals (Vedolin et al. 2018) and persistent organic pollutants (Frias et al. 2010) can adhere to their surfaces, and plastics can also leach chemicals used in their production, such as dyes and plasticisers (Massos and Turner, 2017). Moreover, if ingested microplastics can block and abrade gastrointestinal tracts (Lusher et al. 2013), potentially exposing organisms to the aforementioned transported and leached chemicals (Browne et al. 2013).

Microplastic particles <50 µm have even been identified in human stools (Liebmann et al. 2018), though it is not known whether microplastic particles can harm humans when ingested, or if they are retained in the human gut. However, the inhalation of microplastics particles has been associated with pulmonary illnesses in employees of synthetic textile factories (Pimentel et al. 1975) – though the concentrations at which textile factory employees are exposed to are not environmentally representative.

Though microplastic pollution has been shown to have complex and diverse environmental effects, significant knowledge gaps persist in our appreciation of the environmental prevalence of microplastic particles. Current understanding of microplastic pollution is limited by inconsistencies in the methodologies used to collect samples and analyse the particles isolated from them. Microplastic particles are known to be ubiquitous across marine (e.g. Thompson et al. 2004), freshwater (e.g. Horton et al. 2017), atmospheric (e.g. Dris et al. 2016), and terrestrial (e.g. Scheurer and Bigalke, 2018) systems.

However, as a result of these inconsistencies, the findings of microplastic surveys cannot be universally compared.

Moreover, the environments in which microplastic pollution is studied are not equally represented in the scientific literature. The majority of microplastic pollution studies have documented their presence in the marine environment, and their impact on marine organisms. Although freshwater systems have been identified as considerable sources of plastic pollution (Schmidt et al. 2017; Lebreton et al. 2018), they remain underrepresented in microplastic research.

Freshwater environments, especially rivers, are of considerable environmental importance. Incorporating a variety of anthropogenic landscapes in their catchments, rivers are recipients of numerous pollutants, which they transport downstream. However, whilst we know that microplastic particles are present in freshwater systems, and that they have the potential to cause complex environmental harm, our understanding of their abundance, concentration, and spatio-temporal distribution in these systems is lacking.

Atmospheric deposition of microplastic particles has been studied even less. As of 12/10/2019, just seven publications document the prevalence of microplastic pollution in atmospheric deposition (Dris et al. 2016; Cai et al. 2017; Dris et al. 2017; Allen et al. 2019; Bergman et al. 2019; Klein and Fischer, 2019; Stanton et al. 2019a). Of these, only one study (Stanton et al. 2019a (Chapter 5)) considers the atmospheric contribution of microplastic particles alongside largely open freshwater systems.

Least represented in the peer-reviewed literature is the prevalence of terrestrial microplastic pollution in soils (Scheurer and Bigalke, 2018; Liu et al. 2018; Zhang and Liu, 2018). The disparate spatial coverage of microplastic research is a considerable limitation to the comprehensive understanding of microplastic pollution in the environment, hindering consideration of sources, pathways, and appropriate methods of remediating microplastic pollution.

Nevertheless, in lieu of a comprehensive scientific understanding of microplastic pollution in the environment, key political, public, and industrial

stakeholders have effected change on the evidence that is available to reduce the amount of plastic pollution, of any kind, that enters the environment. Visible efforts to achieve this include the widespread phasing out by industry of microplastic particles used in certain cosmetic products, which are particularly prevalent in facial scrubs (Fendall and Sewell, 2009). This was later legislatively reinforced across North America and the UK (Xanthos and Walker, 2017). Plastic carrier bags have also been the subject of extensive efforts to minimise plastic pollution from consumers. Restrictions on their use have been implemented across Europe since 2002, and a recorded decrease in their incidence in European marine waters has been observed since the onset of these restrictions (Maes et al. 2018).

Beyond industrial and political efforts, public efforts to minimise plastic consumption include the use of reusable containers such as coffee cups in cafes and food containers at supermarkets and takeaways. Consumers may also elect to purchase non-plastic alternatives to common plastic products such as drinks containers and synthetic-fibred clothing. However, though well intended, this environmental action can have detrimental environmental impacts that are not widely communicated to consumers. For example, plastic packaging is known to reduce food waste (Williams and Wikström, 2011; Barlow and Morgan, 2013), and glass and aluminium drinks containers have been shown to have greater global warming potentials than their plastic analogues (Pasqualino et al. 2011; Amienyo et al. 2013).

Plastic products enable the convenient consumption that modern societies are seemingly, and often inadvertently, dependent on. Plastic products have proven their ability to facilitate this consumerism. However, the ability of plastic alternatives to do this has not been similarly assessed. The route of the plastic pollution problem therefore lies not in the plastic itself, but people's relationship with it. Where there is positive action to minimise plastic pollution by stakeholders, it is important that this action is properly informed and does not exacerbate other forms of environmental degradation.

In order to produce a comprehensive assessment of freshwater microplastic pollution, the aim of this thesis is therefore to produce an objective spatio-temporal record of riverine and atmospheric microplastic pollution, using the River Trent catchment, UK, as a case study.

Specific objectives to achieve this aim are:

1. To review published methods for the collection of microplastic particles from aquatic environments and atmospheric deposition, and published methods for the identification of microplastic particles in environmental samples.
2. To evaluate methodologies for the identification of microplastic particles in environmental samples.
3. To develop a suitable method for the collection of freshwater and atmospheric samples for microplastic analysis.
4. To systematically sample rivers and atmospheric deposition throughout the Trent catchment for microplastic particles over a 12 month period.
5. To discuss the relative exposure of 'natural' and microplastic textile fibres in the environment.

## **2. Plastic, Pollution, and People**

### **2.1. Plastic**

#### **2.1.1. The Plastic Age**

Historians have categorised broad stages of humanity's history – the Stone, Bronze and Iron ages – by the materials that dominated societies at their respective points in time (Brydson, 1999). Since the first century AD – recognised as the end of the Iron Age (Joy, 2011) – it has not been possible to use such a method of classification. The populations that followed Iron Age civilisations have utilised a variety of materials such that no single material has dominated (Brydson, 1999). However, in more recent history, a diverse group of mostly synthetic polymers, known as plastics, has rendered redundant many materials on which societies had relied for millennia.

Plastics have facilitated over 100 years of scientific and industrial innovation, societal development, economic growth, and environmental protection. Their diverse applications have revolutionised human populations worldwide (Thompson et al. 2009), touching the lives of all but the planet's most remote citizens. But plastics are also a victim of their own success.

At the end of its useful life, plastic that is not correctly disposed of is known to burden environments across the world, where it is designated a pollutant. Plastic pollution poses physical and chemical threats to the environment and the organisms that inhabit it. Public, political, and scientific concern for the breadth and severity of consequences associated with plastic pollution has resulted in extensive efforts to better understand its distribution and impacts at a global scale. Of particular concern are the risks associated with small plastic particles known as microplastics. Smaller than 5 mm in their largest dimension, microplastic particles present particularly complex environmental problems.

The presence and prevalence of plastic and microplastic waste in the environment has been reported since the early 1970s (Heyerdahl, 1971; Carpenter et al. 1972). Moreover, the ubiquity of plastic, and particularly microplastics, throughout contemporary sedimentary deposits, has been



identified as a possible stratigraphic indicator of the onset of the Anthropocene (Zalasiewicz et al. 2016). Alongside plastic's integration into modern day society, the prevalence of plastic and microplastic litter in the environment has therefore led some to categorise the current period of human history as the Plastic Age (Thompson et al. 2009).

Plastics are a heterogeneous group of polymers, which are compounds derived from the polymerisation of multiple repeating units known as monomers (Halden, 2010). They are defined by the Oxford English Dictionary as *organic polymers of high molecular weight, now usually based on synthetic materials, and may be moulded, extruded, or cast when they are soft or liquid, and then set into a rigid or slightly elastic form*. Organic polymers can be natural, such as cellulose and latex rubber. Prior to World War II, before the use of petrochemicals that dominates plastic production today, many plastics – including those that are so familiar today, such as polyethylene – were produced from vegetable sources, and later the products of the destructive distillation of coal (Brydson, 1999). Nowadays 'plastic' is largely synonymous with polymers synthesised from chemical components of crude oil or natural gas (Rubin, 1990).

Patented in 1909, the American Chemical Society (ACS) recognise Bakelite as the first completely synthetic plastic, synthesised from phenol and formaldehyde (ACS, 1993). Plastics often contain additives, introduced to the polymer during production, that enhance their stability and tune their properties as desired (Elias and Mülhaupt, 2016).

Throughout this thesis, unless otherwise stated, *plastic* refers to these synthetic polymers, which can be divided into two broad categories: thermoplastics and thermosets. Thermoplastics are those that, though rigid and brittle under the conditions at which they are designed to be used, will soften at high enough temperatures that they can be reworked. Thermosets will maintain their form at high temperatures such that they cannot be remelted or reshaped (Elias and Mülhaupt, 2016).

### **2.1.2. Plastics in society**

Plastic, in its many guises, now fades into invisibility in people's day-to-day lives (Meikle, 1995). However, the anthropogenic modification of natural polymers dates back to the purification of latex rubber by Mesoamerican people as early as 1600 BC who enhanced its elasticity and shaped it into balls for recreational activities (Hosler et al. 1999; Grove, 2014). The civilisations that followed increasingly relied on the utilisation and modification of natural polymers such as horn and waxes, until the development of modern thermoplastics in the nineteenth century (Andrady and Neal, 2009). It was not until the mid-1900s though, with the onset of the mass production of plastic (Thompson et al. 2009), that plastics began to have the far reaching societal influences that are both exploited and vilified in the present day.

Plastics are cheap, lightweight, and durable (Hopewell et al. 2009), and can be mass produced (Frias and Nash, 2019). As a result, plastic products have benefited society greatly. Durable packaging reduces food waste, and its inert properties make it ideal for the safe storage of food and drink as well as multiple medical applications (Andrady and Neal, 2009). Plastic has also driven down product costs, and its durability has been exploited across numerous municipal sectors including energy, sewerage and transport.

The convenience and affordability of short-lived plastic products has also long facilitated a disposable 'on-the-go' lifestyle. Though in some societies this has recently evolved into a reusable 'on-the-go' lifestyle – with the promotion of bags for life, keep cups, refillable water bottles, and much more – as of 2017 disposable packaging still accounted for approximately 40% of plastic demand in the European Union plus Norway and Switzerland (Plastics Europe, 2018). Public reliance on plastic products is reflected by their reported incidence in the environment, where plastic is particularly prevalent amongst marine (Maes et al. 2018) and coastal (Nelms et al. 2017) anthropogenic litter. Once it reaches the environment, plastic's durability compounds its current designation as a pollutant.

## **2.2. Pollution**

### **2.2.1. Plastic pollution**

Plastic is not metabolised by the environment in which it ends up, meaning plastics do not biodegrade. Any degradation that does occur is usually initiated by exposure to ultraviolet light (photodegradation) (Webb et al. 2013). Plastic waste in environments with limited exposure to ultraviolet light, such as pelagic and benthic environments of aquatic systems, are therefore even less likely to degrade (Webb et al. 2013). As a result, plastic accumulates in the environment, where it has been described as one of the most salient pressures on aquatic ecosystems (Wagner et al. 2014). Plastic waste that is not recycled or incinerated will likely end up in one of two places; relatively confined to landfill, or unrestrained as a result of irresponsible waste disposal or accidental spillage. In landfill, persistent plastic waste is just one of several threats to the immediate environment, and it is concentrated into small geographical areas. However, loss of plastic waste to environments not designated for waste management – whether deliberate or accidental – presents a multitude of threats to a diversity of environments, and the wellbeing of organisms that inhabit them. This includes humans, to whom there are additional economic and social consequences.

It is in the marine system that plastic pollution has been documented in the greatest depth. Here, plastic pollution threatens organisms of all sizes, as well as their habitats. An estimated 10% of all floating marine debris is lost or discarded fishing gear (Stelfox et al. 2016), most of which is now made from synthetic materials (Bjordal, 2002). Entanglement in fishing gear, such as ghost nets is a constant threat to aquatic organisms, and is known to threaten aquatic megafauna including cetaceans and sharks (Stelfox et al. 2016), as well as smaller organisms such as diving birds (Schrey and Vauk, 1987). The impacts of this entanglement include drowning, starvation, and inhibited predator avoidance (Derraik, 2002; Gregory, 2009). Entanglement is not exclusive to fishing gear, but also includes smaller pieces of plastic waste such as plastic bags and six / four pack rings (Ryan, 2018).

Plastics are also known to have an affinity with certain toxins including polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE) (Mato et al. 2001). These toxins can adhere to the surface of plastics and concentrate there (Wang et al. 2018). Plastics in the environment could therefore act as a vector for the transport of these chemicals (Zarfl and Matthies, 2010). Additionally, chemicals associated with plastic materials can be leached from plastic particles in the environment. Phthalates, a group of plasticisers, are known to contaminate the soft tissues of aquatic organisms, and have been used as a proxy for plastic exposure in both whales (Fossi et al. 2012; 2014) and sharks (Fossi et al. 2014).

Plastics also present a threat to the organisms that ingest them, with potential to block and / or abrade gastrointestinal tracts, and subsequently bioaccumulate. Plastic ingestion has been documented in organisms as large as whales (Lusher et al. 2015a). Plastic material can also block more than the gastrointestinal tract, and has potential to cause obstruction and blockages of other orifices. For example, particularly emotive images have been circulated in recent years of turtles having a plastic straw (Robinson and Figgner, 2015), and a plastic fork (Robinson et al. 2016) removed from their nasal passages.

Much of the concern for plastic pollution is directed towards its fate in marine environments, often attributed to the representation of plastic pollution in the media, including the BBC's Blue Planet II. However, the freshwater environment is recognised as a major source of plastic in the seas and oceans. Predicting plastic emissions from these rivers is challenging given the underrepresentation of plastic pollution studies in freshwater environments (Blettler et al. 2018). Efforts to produce representative estimates of plastic fluxes that account for the variability of freshwater environments are particularly lacking. For example, such reliable estimates must account for variability in plastic concentrations through time, and throughout the profile of freshwater systems from their surface waters to their sediments. Nevertheless, estimated contributions of riverine plastic pollution to the marine system

include 67% of all marine plastic from 20 rivers (Lebreton et al. 2018), and 88-95% from just 10 rivers (Schmidt et al. 2017).

Whilst large pieces of plastic pollution are a salient reminder of society's impact on the environment, the plastic waste that can be easily observed is greatly outnumbered by that which is not (Law and Thompson, 2014).

### **2.2.2. Microplastics**

Thompson et al. (2004) are widely recognised as having coined the term microplastic in reference to 'microscopic plastic fragments'. Though they themselves do not define these particles by size, Thompson et al. (2004) include particles in the size range 20-2000  $\mu\text{m}$  in their assessment of microscopic plastic particle ingestion, favouring a non-literal definition of 'micro'. There is a clear argument for defining microplastic particles as those smaller than 1 mm, in line with the International System of Unit's definition of the prefix 'micro' (Browne, 2015). However, since its first use in 2004, it is the <5 mm definition which has been most widely used, and was adopted by the US National Oceanic and Atmospheric Administration Marine Debris Programme in Arthur et al. (2009). Perhaps more importantly though, extensive public dissemination of microplastic research has characterised microplastic particles as those smaller than 5 mm in their largest dimension.

Present in every shape, colour, polymer, and a wide range of densities, microplastics are a heterogeneous group of particulate pollutants. Microplastics can be broadly divided into two categories: primary and secondary. Primary microplastics are those that are manufactured at sizes 5 mm or smaller in their largest dimension, and include the infamous microbeads that are present in some cosmetic products, and which have been banned in some countries including the USA in 2015, and the UK in 2018. Secondary microplastics are those derived from the breakdown of plastic material larger than 5 mm in its largest dimension. This degradation is facilitated by both biotic and abiotic mechanisms (Klein et al. 2018).

The physical degradation of plastics, ultimately to microplastics, in the environment can occur by a variety of means, often initiated exposure to ultraviolet (UV) light (photodegradation) (Webb et al. 2013). Other classes of polymer degradation including thermal, oxidative, radiative, mechanical and ultrasonic, chemical, and biological (Tewarson, 2003). These processes are often slower in aquatic environments than on land due to higher temperatures and greater exposure to solar radiation on land (Cooper and Corcoran, 2010).

The degradation of plastic by these means is a result of the weakening, and eventual breaking, of covalent bonds within the structure of the plastic polymers, known as chain scission (Gewert et al. 2015). This chain scission can occur at any point within a polymer's structure, with the potential to cleave monomers from the inert polymer, many of which can cause environmental harm themselves (Lithner et al. 2011).

In search of a comprehensive definition of 'microplastic' that accounts for these variations in size, morphology, and origin, Frias and Nash (2019) define microplastic particles as *any synthetic particle or polymeric matrix, with regular or irregular shape and with size ranging from 1  $\mu\text{m}$  to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water*. Whilst this definition is semantically more complete than those that refer only to particle size, it is inappropriate in its assumption that all synthetic polymers are plastic. Rayon, for example, is synthesised from the polymer cellulose, and is therefore a non-plastic synthetic particle.

Microplastic particles are therefore defined more simply here as:

*Pieces of plastic of anthropogenic origin in the size range 1  $\mu\text{m}$  to 5 mm in their largest dimension, with particles smaller than 1  $\mu\text{m}$  being classified as nanoplastics.*

Though the term 'microplastic' was first used in 2004 (Thompson et al. 2004), the first published record of the environmental prevalence of plastic particles <5 mm dates back to 1972 (Carpenter et al. 1972; Carpenter and Smith 1972).

The majority of research has quantified microplastic particles in the marine environments where they have been identified in water, sediment, and biota sampled from every ocean. However, there is an extensive and growing body of literature that has recorded plastic and microplastic pollution in freshwater environments including in rivers (Castañeda et al. 2014; Klein et al. 2015; Hurley et al. 2018a) and lakes (Eriksen et al. 2013; Imhof et al. 2013; Free et al. 2014; Vaughan et al. 2017). Microplastic particles are also known to be deposited from the atmosphere (Dris et al, 2016; Stanton et al. 2019a) but, though the study of airborne microplastic particles is limited (Allen et al. 2019), it is in the terrestrial environment that microplastic prevalence is least understood (Rillig, 2012).

### **2.2.3. Microplastics as particulate pollutants**

Microplastic particles in environmental samples have been assigned to multiple broad categories including fibres, fragments, films, and spheres (e.g. Yonkos et al. 2014; Klein et al. 2015; Horton et al. 2017a; Wang et al. 2017a). Fibrous particles that may tangle following ingestion, have been identified as particularly harmful (Lusher et al. 2013; Wright et al. 2013).

In aquatic systems microplastics are known to exert a variety of pressures on the environment and the organisms that inhabit them. Microplastic particles can also alter the abiotic characteristics of an environment. They have been experimentally shown to alter the flow of water through sediments, and to decrease their maximum temperature and the rate at which they warm, with the potential to affect organisms with temperature-dependent sex-determination eggs such as sea turtles (Carson et al. 2011).

Microplastic particles are also known to be ingested by organisms as small as zooplankton (Cole et al. 2013) and, in the same manner as larger pieces of plastic, may partially or completely block the gastrointestinal tracts of organisms (Lusher et al. 2013). Whilst some organisms are able to eject microplastic particles as pseudofaeces, retained microplastics have the potential to compromise feeding and digestion (Lusher et al. 2015b). Particles

that are retained may also bioaccumulate through higher trophic orders, however, due to their small size, the physical harm of ingested microplastic particles is likely to be lower in larger organisms.

Bacterial communities are also known to colonise the surface of microplastic particles, with the potential to be transported throughout aquatic systems. McCormick et al. (2014) found less diverse but significantly different bacterial assemblages colonising the surface of microplastic particles when compared to suspended organic matter and the water column. Pathogenic bacteria that colonise microplastic particles collected from the marine environment include *Aeromonas salmonicida* (Viršek et al. 2017) and *Vibrio parahaemolyticus* (Kirstein et al. 2016). In laboratory studies plasmid transfer in bacterial assemblages has also been found to be higher in communities that colonise microplastic particles when compared to free-living communities, with potential implications for the spread of antimicrobial resistance (Arias-Andres et al. 2018).

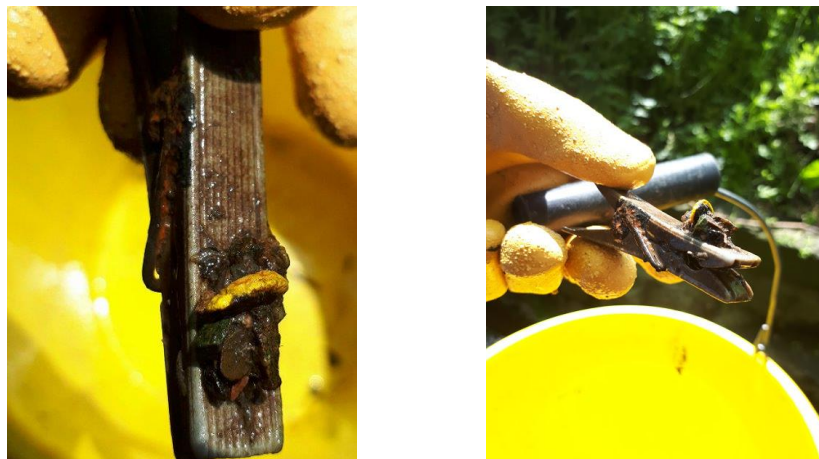
Despite these varied physical impacts of microplastics, particularly in aquatic systems, microplastic particles are not the only small anthropogenic particle within aquatic environments. Other anthropogenic particulates common to freshwater sediments include glass, metal, and ceramics, and non-plastic anthropogenic textile fibres (Jaspers and Grieken, 1997; Stanton et al. 2019a). Moreover, atmospheric deposition is also a known pathway for the introduction of particularly small anthropogenic particles into aquatic environments including Spheroidal Carbonaceous Particles (SCPs) (Rose, 2008) and particulate matter (PM<sub>10</sub> and PM<sub>2.5</sub>) (Verma et al. 2013).

SCPs are known to act as vectors for pollutants including trace metals and organic pollutants (Martins et al. 2010). Particulate matter is also known to be associated with heavy metals, and laboratory exposure of particulate matter (PM<sub>2.5</sub>) has been shown to alter the behaviour of freshwater organisms including freshwater snails (Hartono et al. 2017). The effects of these different anthropogenic particulates have not yet been compared, and so it is not possible to comment on the relative threats they pose.



As well as being one of many anthropogenic particles, microplastic particles are components of an aquatic system's diverse deposited and suspended sedimentary material, and ought to be considered as such in the assessment of their risk. The definition of microplastics as plastic particles smaller than 5 mm in their largest dimension does not align with standard measurements of sediment grain size, and the diversity of shapes in which microplastics are found further complicates efforts to understand microplastic pollution alongside other particulate threats to the aquatic environment.

However, though there is potential for microplastic contamination of sediments to alter environmental processes (de Souza Machado et al. 2018a; Rillig et al. 2019), these processes can also exploit plastic and microplastic in the environment. For example, microplastic particles have been identified in the cases of caddisfly (Ehlers et al. 2019) (Figure 2.1).



**Figure 2.1: A caddis fly case constructed on a plastic clothes peg, incorporating a yellow fragment that is thought to be a fragment of road paint, found during a litter survey of Saffron Brook, Leicester, UK. The yellow fragment is larger than 5 mm, and so is not a microplastic particle.**

Microplastic particles, in their many shapes, are known to exert multiple pressures on freshwater environments. However, the extent of this harm in relation to the complex biological and environmental impacts of anthropogenic and natural particulates in aquatic environments is unlikely to be consistent through space and time. Therefore, whilst the physical environmental impacts of microplastic particles are unquestionable, there is a need to place these

impacts in a broader context of the diverse particulate threats to aquatic systems.

#### **2.2.4. Microplastics as a chemical pollutant**

Plastic polymers themselves are not regarded as hazardous chemicals to the environment because their large molecular size makes them biochemically inert (Lithner et al. 2011). Though the fundamental molecular structure of different plastic polymers is similar, this structure can be modified to give plastics a variety of physical and chemical properties. Early plastic pollution discourses even promoted the use of additives that accelerated the breakdown of plastic in the environment (Scott, 1973). However, this chemical tailoring of plastic products does not only influence the physical threat that plastic pollution poses.

Plastics that are in the environment can also act as sorbents for chemical pollutants. The affinity of synthetic polymers has been exploited for clean-up of various, particularly hydrophobic, pollutants. For example, woven plastic fibres, have been used in the clean-up of oil spills in aquatic systems, with the plastic fibres acting as oil sorbents. The efficacy of different fibre morphologies and polymers in this application has been shown to vary. Zhu et al. (2011) demonstrated greater sorption capacities in an electrospun polyvinyl chloride / polystyrene material compared to a commercially available polypropylene oil sorbent patch. The physical structure of fibrous oil sorbents also influences their sorption capacity, with porous fibres exhibiting higher sorption capacities than non-porous fibres (Lin et al. 2012). Unlike non-plastic fibres such as cotton and wool, whose potential to act as oil sorbents has also been demonstrated (Choi and Cloud, 1992; Lim et al. 2007; Radetic et al. 2008), these physical properties can be refined in the production of plastic fibres. Another plastic oil sorbent, butyl rubber, has also been shown to have potential applications in the removal of PAHs from the environment (Ceylan et al. 2009). PAHs are a group of pollutants with carcinogenic and mutagenic effects that are designated as priority pollutants by the European Water Framework Directive (Li et al. 2019)

The use of plastics as sorbents in the remediation of environmental contamination is only temporary, with the plastic sorbents being removed from the environment for cleaning and reuse after they have served their purpose. However, microplastic particles in the environment share this property, and will also act as chemical sorbents. Microplastics in the environment have been contaminated with various pollutants including persistent organic pollutants (POPs) such as dichlorodiphenyltrichloroethane (DDT), PAHs, and PCBs (Frias et al. 2010; Rochman et al. 2012; Bakir et al. 2014a; Tan et al. 2019). It has also been suggested that microplastic particles can act as vectors of heavy metals (Vedolin et al. 2018), though these metals may have been sourced from the manufacturing of the plastics themselves (Wang et al. 2017), for example from dyes (Massos and Turner, 2017).

The role of microplastics as chemical pollutants is not limited to chemical pollutants in the environment. Chemicals added to plastics during their production, including plasticisers, pigments, and fire retardants, could also be leached from plastics in the environment (Rochman, 2015; Gallo et al. 2018). These potential leachates include bisphenol A, phthalate esters, and formaldehyde (Rochman, 2015), and heavy metals (Massos and Turner, 2017; Wang et al. 2017b). Moreover, though the polymers are not considered hazardous to the environment, polymerisation reactions seldom proceed to completion (Araujo et al. 2002). Residual monomers can persist within plastic material post-production and have the potential to cause harm, with some monomers classified as mutagens and / or carcinogens (Lithner et al. 2011). Microplastics therefore act as both a source and a vector of harmful chemicals in the environment.

The chemical threat of plastic pollution is inversely correlated to the size of the particle, with smaller particles, possessing larger surface areas to which chemicals can adhere and from which they can be leached, relative to their volumes. The chemical threat of microplastics is therefore greater for fibrous particles such as synthetic textile fibres than it is for fragments (Cesa et al. 2017).

Though plastics are not the only route by which organisms are exposed to harmful chemicals in the environment, ingestion of plastics to which these chemicals are sorbed to the surface is a known pathway by which organisms are exposed to chemical pollution (Gallo et al. 2018). Chemicals adhered to the surface of microplastic particles may desorb from microplastic particles, with detrimental effects. For example, following ingestion by lugworms, the leaching of pollutants from microplastic particles has been found to increase their susceptibility to pathogens, with potentially lethal effects (Browne et al. 2013). The desorption of POPs has also been found to be greater in simulated physiological conditions than in seawater (Bakir et al. 2014b). The dynamics of pollutant sorption to microplastic particles is not consistent (Rochman et al. 2012; Bakir et al. 2014b; Wang et al. 2015; Brennecke et al. 2016; Wang and Wang, 2018). For example, Bakir et al. (2012) report an interference in the sorption of one toxin, phenanthrene, in the presence of another, DDT. Whilst microplastic particles of different polymers are known to have an affinity with a variety of toxins, this affinity is therefore dependent on both the polymer and toxin present in the environment.

The consequences of these chemical pollutants associated with microplastic particles is not limited to the organisms that ingest them. Where the desorbed chemicals become incorporated into the tissues of organisms that are preyed upon, these chemicals can bioaccumulate within organisms of higher trophic orders (Teuten et al. 2009; Cole et al. 2011; Wang et al. 2015).

Just as microplastics are not the only particulate threat to freshwater environments, they are also not the only chemical-carrying particulates in the freshwater system. Anthropogenic activity has altered the relationship between freshwater systems and their sediment at scales much larger than microplastic pollution (Wilkes et al. 2019). Land use changes and modifications to river courses and bed structures have altered the erosive and depositional properties of affected systems, negatively affecting habitat quality (Wood and Armitage, 1997). This has altered sediment availability and fluxes, and suspended sediment particles are also known to facilitate the transport of

various toxins including polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and heavy metals (Rügner et al. 2019).

### **2.2.5. Riverine microplastic pollution**

Not all plastic waste within a freshwater catchment will reach the marine environment. Some will be removed from the system through refuse collection, and in some cases by atmospheric transport to adjacent catchments. Plastic waste can also be locked in the catchment for long periods of time, becoming incorporated into soils and lacustrine sediments.

Microplastic particles have been isolated from freshwater sediments (e.g. Klein et al. 2015; Horton et al. 2017a) and, following various biotic interactions outlined by Woodall et al. (2014), including biofouling and ingestion, all microplastic particles have the potential to settle onto, and be incorporated into, aquatic sediments. However, the variety of polymers from which plastic products are made, and the different shapes into which plastic particles can degrade means that the timescales over which this occurs are unlikely to be consistent across all particle sizes and polymers.

Nevertheless, rivers are an important pathway for the transport of terrestrial debris, including plastic, through the freshwater system. They connect the anthropogenic activity within catchment to the marine environment. Freshwater microplastic research has associated microplastic abundance with centres of population (Eerkes-Medrano et al. 2015; Peters and Bratton, 2016; Horton et al. 2017a; Tibbetts et al. 2018) and wastewater treatment plants (WWTPs) (Murphy et al. 2016; Mintenig et al. 2017; Talvitie et al. 2017; Ziajahromi et al. 2017).

Microplastic removal from WWTP influent has been found to exceed 99% (e.g. Talvitie et al. 2017). Despite these high levels of microplastic removal, the volume of water released by wastewater treatment plants means that they still have the potential to release large numbers of microplastic particles in their liquid effluent (Mason et al. 2016). Furthermore, the microplastic particles that do not pollute the effluent of WWTPs are incorporated into the solid products

of the wastewater treatment process, forming a component of the WWTPs sludge (Mahon et al. 2017). Where this sludge is applied to land, microplastic particles are directly introduced to the terrestrial environment, and may subsequently be washed into aquatic environments during periods of rain (Li et al. 2018a). Sources of microplastics associated with centres of population include point sources, such as industrial effluent (Lechner and Ramler, 2015), and diffuse sources such as urban dust (Dehghani et al. 2017) and tyre wear particles (Kole et al. 2017).

However, current understanding of the prevalence and distribution riverine microplastic pollution prevalence and distribution is spatially limited. The majority of freshwater microplastic studies have been conducted at a small number of sites on rivers across Europe, North America, and China (see chapter 6), rarely accounting for river catchments in their entirety. Consideration of less developed reaches of river systems is also disproportionate to developed systems, and is key to understanding the relative contributions of different microplastic sources.

Moreover, despite the mobility of rivers, current understanding of riverine plastic pollution is further limited by infrequent assessment of temporal variation (Schmidt et al. 2017). Microplastic studies that do not account for this intra-site variability over representative periods of time therefore cannot broaden scientific understanding of microplastic prevalence and distribution beyond the time of sample collection. As the majority of freshwater microplastic research only reports the presence of microplastic pollution at few points in space and time, few publications are able to report findings that are representative of the sampled system. As a result, the true extent of the threats that microplastic pollution poses to freshwater environments remains poorly understood.

#### **2.2.6. Atmospheric microplastic pollution**

Current understanding of freshwater microplastic pollution is further limited by a widespread lack of consideration for atmospheric deposition to what is a

predominantly open system. At the time of writing (12/10/2019), the atmospheric transport and deposition of microplastic particles had only been reported in seven studies. In chronological order these were Dris et al. (2016), Dris et al. (2017), Cai et al. (2017), Stanton et al. (2019a) (Chapter 5), Allen et al. (2019), Bergmann et al. (2019), and Klein and Fischer, (2019). Wright et al. (2019) also outline an approach for the identification of inhalable microplastic particles within particulate matter samples under controlled conditions.

These publications have identified microplastic particles in urban (Dris et al. 2016; Cai et al. 2017; Bergman et al. 2019; Klein and Fischer, 2019; Stanton et al. 2019a), and remote (Allen et al. 2019; Bergman et al. 2019) atmospheric deposition. Airborne microplastic particles are therefore a likely source of microplastic particles in some parts of the freshwater system. However, the sampling regimes conducted in these studies have been similarly limited in their spatial and temporal extent.

Sources of airborne microplastic particles are less obvious than some freshwater sources, and little is known of the mechanisms that entrain microplastic particles. Understanding the entrainment of microplastic particles into the atmosphere and their transport through it is challenging given the variety of shapes, sizes, and densities that microplastic particles can be found in. With particular reference to smaller microplastic particles, it is possible to infer similarities to other airborne particles, which remain in the atmosphere for different lengths of time. Often sourced from anthropogenic activities such as road traffic and energy production (Keuken et al. 2013), airborne particles with aerodynamic diameters  $<10 \mu\text{m}$  ( $\text{PM}_{10}$ ) are of particular concern to human health, being small enough to be inhaled, with particles  $<2.5 \mu\text{m}$  ( $\text{PM}_{2.5}$ ) having the potential to reach the deep lung (Wright et al. 2019). Even in the absence of precipitation, particles of this size do not remain airborne for long; the airborne residence times of particles with an aerodynamic diameter of  $1\text{-}10 \mu\text{m}$  is reportedly as low as 10-100 hours (Esmen and Corn, 1967; Whelpdale, 1974), with sea salt particles  $>50 \mu\text{m}$  having very short atmospheric lifetimes (Athanasopoulou et al. 2008).

The introduction of other anthropogenic particles to the atmosphere is clearer. SCPs, for example, are introduced during the high temperature combustion of coal and oil (Wik and Renberg, 1996). The deposition of SCPs has been identified in aquatic sediments globally, and particularly in the northern hemisphere (Rose, 2001). The study of SCPs is largely concerned with their abundance in the lacustrine environments into which they are deposited, and the sediments in which they are preserved. Here their presence is interpreted as an indicator of atmospheric deposition from the industrial combustion of coal and oil.

Though the atmospheric residence times of particles  $<10\ \mu\text{m}$  is reportedly in the order of hours (Whelpdale, 1974) SCPs have been observed in lacustrine sediments far from sources of industrial activity. In lakes that are isolated from heavy industry and large populations by seas and oceans, Rose et al. (1999) observed SCPs in lake sediments from Svalbard which. Though predominantly  $<10\ \mu\text{m}$  in diameter, some of these were as large as  $25\text{-}35\ \mu\text{m}$ . Rose et al. (1999) do not consider industrial activity in Svalbard to have been the source of these SCPs.

Similarly, Allen et al. (2019) and Bergmann et al. (2019) both report the presence of microplastic particles deposited from the atmosphere in remote regions demonstrating that microplastic particles can also remain airborne and travel long distances from their source. Allen et al. (2019) report that the majority of particles they observed were  $\leq 50\ \mu\text{m}$ , and Bergmann et al. (2019) report that 80% of the particles they identified were  $\leq 25\ \mu\text{m}$  – though this is reported for all particles that Bergmann et al. (2019) identify, including from samples collected in urban environments.

Though the long-distant transport of airborne microplastic particles has been observed, mechanisms of microplastic entrainment into the atmosphere are currently largely theoretical. Some microplastic particles, like SCPs, are thought to be ejected into the atmosphere from chimneys during the incineration of waste (Dris et al. 2016). Microplastics may also be blown from landfill sites (Duis and Coors, 2016) and from soils treated with biosolids (Environmental



Audit Committee, 2016). Understanding the nature of the introduction of microplastic particles into the atmosphere is necessary in order to comprehensively understand atmospheric microplastic pollution.

In addressing this knowledge gap, future research can be guided by similar fields of study. In considering the atmospheric transport of micro-charcoal particles, for example, Clark (1988) details the known physics of the airborne transport of mineral particles. Specific to quartz, with a density of  $2.65 \text{ g cm}^{-3}$ , Clark (1998) describes the three methods by which wind transports particles; briefly, these are 1) suspension (particles  $<60 \text{ }\mu\text{m}$ ), 2) saltation (particles  $60\text{--}2000 \text{ }\mu\text{m}$ ), and 3) traction. Clark also identifies particles of  $100 \text{ }\mu\text{m}$  in size as the most easily entrained due to the cohesive forces and aerodynamic properties of smaller particles and the mass of larger particles respectively. From this it is possible to infer that, due to the lower density of common plastic materials compared to quartz, larger particles of plastic may be more easily entrained than mineral particles. However, a greater understanding of the physics behind this is necessary in order to substantiate this assumption.

### **2.2.7. Natural textile fibres – microplastic’s overlooked analogue?**

Following observations of synthetic textile fibres in beach sediments collected across the globe, Browne et al. (2011) confirmed the presence of synthetic textile fibres in the effluent of washing machines used to wash synthetic fibred garments. They reported that the sampled garments shed  $>1900$  fibres per wash. Subsequent work has demonstrated that the number of fibres shed is influenced by fabric type and washing conditions (temperature, type of detergent, presence of conditioner), with findings varying greatly. Napper and Thompson (2016) estimate that the washing machine effluent of an average 6kg load of acrylic garments could release as many as  $>700\,000$  fibres, whilst De Falco et al. (2018) estimate a release of  $>6\,000\,000$  fibres from a typical 5 kg wash of polyester garments. Hernandez et al. (2017), reporting fibre release as a mass of fibres per mass of garment washed, found approximate fibre loss ranged from 0.025 to 0.1 mg of fibres per 1 g of fabric washed.

As awareness of the impacts of plastic and microplastics pollution has grown, efforts by stakeholders from industry, politics, and the general public have been made to minimise plastic consumption and emission. This has included a move by some to plastic alternatives. Natural textile fibres, such as cotton and wool, are alternatives to synthetic fibres. However, very little work has considered the potential environmental harm of these non-plastic textile fibres.

Where the emission of microplastic fibres from the washing of synthetic textile fabrics has been repeatedly quantified, there is also very little complementary work that quantifies the relative release of fibres from the washing of natural textile fibred garments. In a comparative study of fibre shedding during garment washing, Sillanpää and Sainio, (2017) report that the polyester garments studied (two types of fleece, one softshell, and one technical sports shirt) shed between 210 000 and 13 000 000 fibres per kg of washed garments, whilst cotton garments (jeans and a shirt) shed between 3 600 000 and 4 600 000 fibres per kg of garment washed.

Natural textile fibres have been documented in environmental samples collected for microplastic pollution, including atmospheric deposition (Dris et al. 2016; 2017; Stanton et al. 2019a (Chapter 5)), river water (Dris et al. 2018; Stanton et al. 2019a), wastewater (Talvitie et al. 2017) and in the gastrointestinal tract of fish (Compa et al. 2018), terrestrial birds (Zhao et al. 2016) and macrocrustaceans (Remy et al. 2015). Within these studies, natural textile fibres are regularly quantified in higher abundances than synthetic textile fibres.

Moreover, beyond the field of environmental pollution the study of textile fibre populations within forensic science has long reported the dominance of natural over synthetic textile fibres (e.g. Kelly and Griffin, 1998; Cantrell et al. 2001; Palmer and Oliver, 2004). Natural fibres have also been shown to persist in the environment over long periods of time in certain environments. Chen and Jakes (2001) reported no morphological differences between new cotton fibres and those recovered from waistcoat retrieved from a shipwreck found in the

Atlantic Ocean that had been submerged for 133 years. Older fibres, including woollen fibres, have also been analysed from textile samples buried in sediments of from the Cave of Letters, in modern day Israel (Müller et al. 2006). Though these fibres were reportedly more brittle than modern fibres, indicating a degree of degradation, Müller et al. (2006) note their resemblance to modern woollen fibres, with surface scales (see Stanton et al. 2019a) evident in the SEM images.

Nevertheless, despite their prevalence and potential longevity, Ladewig et al. (2015) speculate that dismissal of natural textile fibres as environmental pollutants is rooted in the assumption that natural fibres will degrade in the environment. Ladewig et al. (2015) also highlight the fact that, like microplastics, natural textile fibres have the potential to sorb chemical pollutants from their environment, and that the degradation of natural fibres will release chemicals associated with the textile industry into the environment more rapidly than slower-degrading synthetic fibres. A comprehensive understanding of the environmental fate and impact of textile fibres cannot be achieved without the consideration of natural textile fibres as both standalone anthropogenic particles, and relative to microplastic fibres.

In addition to their potential environmental persistence, the production and processing of some types of natural fibre, particularly cotton, is not without environmental consequences. A lot of cotton growth takes place in countries with little legislation protecting the environment and human health (Suran, 2018). For example, the shrinking of the Aral Sea due to irrigation of the rivers that feed it, is associated with cotton agriculture. In terms of crop production, land use, and water consumption, cotton is one of the largest crops in this region (Zhang et al. 2019).

It is likely that natural textile fibres will degrade in the environment eventually, however, there is insufficient evidence to state that this happens over time scales that preclude natural fibres from presenting an environmental threat similar to that of synthetic textile fibres. Moreover, the environmental consequences of natural textile fibre degradation are not sufficiently

understood. As natural textile fibres have been marketed by some as an alternative to synthetic fibres, framed around the fact that they are not plastic, it is vital that the relative environmental harm of all fibre types is considered in future research. Without this, stakeholders cannot make informed decisions as consumers, manufacturers, and legislators.

### **2.3. People**

Plastics are materials that are designed to be durable (Hopewell et al. 2009), and though their morphology may change, they will persist in any environment that they are lost to, or are released to, at the end of their useful life. Plastic pollution is therefore a tangible phenomenon. It happens in real time, and can be related directly to the day-to-day lives of average citizens.

Consequently, 'plastic' is becoming increasingly synonymous with plastic pollution, propagating efforts by stakeholders to minimise plastic consumption. As one of the most salient anthropogenic environmental pressures, it is a problem that has been prioritised by societies, politicians, and even scientists who have been distracted from environmental and social challenges that are of equal, if not greater, severity. It is therefore imperative that the realities of plastic pollution are not misrepresented, particularly in the public dissemination of environmental research.

Within the study and communication of plastic pollution, the methods used for the isolation and identification of microplastic particles in different environmental samples are varied. Consequently, the findings of microplastic studies are vulnerable to misrepresentation, and are not always representative of the environments or organisms under investigation.

The number of plastic pieces already in the environment is innumerable. In response to the environmental threats posed by this plastic, particularly in the marine system, efforts to reduce the amount of plastic entering the environment and clean up what is already there have come from science and engineering, society, politicians and industry. Efforts to remove plastic,

amongst other forms of anthropogenic litter, from different environments range from feats of marine engineering to committed volunteer groups.

For example, The Ocean Cleanup is a non-governmental organisation whose efforts to collect marine litter have made international headlines, with their System 001 currently undergoing its first mission to collect anthropogenic litter from the Great Pacific Garbage Patch (The Ocean Cleanup, 2019). In contrast, at the local scale, efforts to remove coastal anthropogenic waste through beach cleans are popular the world over, and in the UK are coordinated annually by the Marine Conservation Society (Nelms et al. 2017). Though the amount of anthropogenic litter collected at such events is not of the same order as large scale engineering projects, their significance is undeniable given the longevity of the plastic waste that is removed, and the community engagement and education that complements them.

Efforts have also been made from the plastics industry, such as the development of bioplastics. Bioplastics can either be bio-based or biodegradable. European Bioplastics (nd) defines bio-based plastics, as those which are derived from natural sources, predominantly plants, as was used in the production of some plastics in the early 20<sup>th</sup> century (Brydson, 1999). Bioplastics perform the same as traditional plastics but have a lower oil demand than conventional plastics. However, their production does incur other environmental costs particularly for the land requirements of plant-based bioplastic. Biodegradable plastics on the other hand, are those which are designed to degrade by the action of living organisms including bacteria, fungi, and algae (Gross and Kalra, 2002). Plastics that, in theory, biodegrade do not pose the same physical threat as conventional plastics at the end of their useful life. However, the biodegradability of bioplastics is often dependent on the environment in which they end up (Emadian et al. 2017). If they find their way into many natural environments, the efficacy of biodegradable plastics, with a particular focus on carrier bags, has been found to be limited (O'Brine and Thompson, 2010; Accinelli et al. 2012; Napper and Thompson, 2019).

Political efforts are also being pursued to reduce the amount of plastic waste that enters the environment in the present and in the future. Legislation prohibiting the manufacture and sale of wash-off cosmetic products containing microplastic particles in the USA, Europe and beyond has also reduced microplastic pollution from this industry. Though cosmetic microplastic particles represent only a small proportion of the microplastic particles in the marine environment (Boucher and Friot, 2017), awareness of their abundance in everyday consumer products, facilitated by extensive media coverage, has contributed to the public concern for plastic pollution more broadly.

Across multiple European countries levies on plastic carrier bags, informally termed the 'plastic bag tax' in the UK, have also incentivised the use of reusable bags by consumers. This has been associated with an appreciable drop in the incidence of plastic carrier bags in the North Sea and Celtic Sea since the mid-2000s (Maes et al. 2018).

In lieu of industrial and legislative efforts to minimise pollution from some plastic products, the use of alternative materials to plastic has also become increasingly fashionable beyond the use of natural fibres. For example, consumers can now buy drinking water in cans and boxes, as well as plastic bottles. CanO water, a brand which sells water in the former, even identifies its products as 'a solution to plastic pollution' (Figure 2.2). Promotion of products as ones that do not use plastic is not unique to CanO water. Plastic-free toiletries include toothpastes and dental floss (e.g. Geoorganics), and deodorant (e.g. Ben and Anna). Plastic-free products are often sold at a premium though, and therefore may preclude efforts to minimise plastic consumption by those who are unable to afford it.

Furthermore, alternatives to plastic packaging plastic are not free of environmental impact. Global Warming Potential (GWP) is a measure of a product's emission of the greenhouse gasses that contribute to global warming (Pasqualino et al. 2011). Glass and aluminium drinks containers, have been repeatedly shown to have GWPs greater than their plastic analogues (Pasqualino et al. 2011; Amienyo et al. 2013), whilst paper based plastic

alternatives including aseptic cartons (Meneses et al. 2012) and egg containers (Zabaniotou and Kassidi, 2003) have been shown to have lower GWPs than plastic alternatives. However, scaling up the use of paper packaging to replace plastic would also have considerable environmental consequences associated with the clearing of land.

Plastic pollution is a tangible consequence of everyday life. Graphic images of animals in distress following exposure to common products of questionable necessity, such as plastic cutlery (e.g. Robinson and Figgner, 2015; Robinson et al. 2016), are therefore very emotive. However, the realities of the environmental consequences of plastic alternatives are less well communicated to consumers.



**Figure 2.2: Advertising from CanO water for their water in a can, stating that their product is ‘a solution to plastic pollution’.**

This has contributed to a culture of anti-plastic discourses, which dominates public environmental concern. Concerning headlines from UK news organisations include ‘People eat at least 50,000 plastic particles a year, study finds’ (Carrington, 2019a: The Guardian online), ‘Microplastic pollution revealed ‘absolutely everywhere’ by new research’ (Carrington, 2019b: The

Guardian online), 'Average person swallows plastic equivalent to a credit card every week, report finds' (Knapton, 2019: The Telegraph online), and 'Scientists warn we could be breathing in microplastic particles laden with chemicals' (Johnston, 2016: The Independent online).

The alternatives to plastic pollution may not represent the solutions they are sometimes marketed as or assumed to be. The complexities of plastic pollution are also compounded by the extensive and often far removed environmental impacts of plastics and their alternatives, that are difficult to communicate to key stakeholders. However, public engagement with environmental threats is integral to positive environmental action, and the environmental activism that is routed in efforts to minimise plastic pollution should not be quashed. Great care must therefore be taken when disseminating plastic pollution research, and the scientific community have a responsibility to present their findings in a critical and considered manner.



### **3. Microplastic sampling, extraction, identification, and quantification**

A number of techniques are available to sample different environments for microplastic pollution. The isolation of microplastic particles from these samples, and their subsequent identification, can also be achieved through multiple means. The methodologies chosen for sample collection and processing, and for particle characterisation, will have a bearing on each study's findings. Inconsistencies in these methodologies therefore hamper the comparability of studies (Stock et al. 2019), and in turn our understanding of microplastic prevalence. There have therefore been repeated calls for methodological harmonisation in the study of microplastic pollution (Li et al. 2018b; Mai et al. 2018; Prata et al. 2019a; Stock et al. 2019).

Methods used in the study of microplastic pollution vary depending on the environment being sampled, the matrix being collected, and the equipment available. Microplastic sampling has predominantly been undertaken in aquatic (marine, estuarine and freshwater) environments, and a small number of studies have quantified microplastic pollution in atmospheric deposition and terrestrial soils. The methodological standardisation that has been called for is unlikely to be realised in a field where no single sequence of sampling and analysis is applicable across this variety of matrices. This section provides an overview of the variety of methodologies used to study microplastic pollution and considers their merits and limitations.

#### **3.1. Microplastic sampling**

##### **3.1.1. Water**

Collection of water samples for suspended microplastic analysis can follow one of two approaches: the collection of bulk, typically smaller volume, samples, and the concentration of larger volumes of water onto a mesh (e.g. nets and sieves). The unexpected observation of microplastic particles by Carpenter and Smith (1972) followed the trawling of neuston net with a 330 µm mesh aperture net through the surface waters of the Sargasso Sea. Trawling of nets

continues to be the most common method of sampling suspended and floating microplastics (Stock et al. 2019), affording microplastic samplers a simple methodology for concentrating large volumes of water onto the net's surface.

Neuston and manta nets are frequently used to collect microplastic particles on the water surface (Mai et al. 2018). Bongo nets and plankton nets are used to collect sub-surface samples, and can be deployed for both horizontal and vertical trawls (Prata et al. 2019a). The use of nets has also been used in large freshwater systems including lakes (Eriksen et al. 2013; Free et al. 2014) and large rivers (Mani et al. 2015; Dris et al. 2018). But the use of nets is not suitable for all aquatic environments, imposing practical limitations such as the requirement of boats or bridges from which the nets can be suspended, and a water depth sufficient for the net to be deployed. Moreover, established means of calculating the volume sampled using net trawls, required for the calculation of microplastic concentrations, are not always reliable (Rivers et al. 2019).

The principles of volume-reducing samples onto meshes is not exclusive to the trawling of nets though. Analogous techniques include the passing of water through fine (10 µm) cartridge filters (Mintenig et al. 2017; Wolf et al. 2019), an individual sieve (Stanton et al. 2019a) or a set of stacked sieves of different mesh apertures (Mason et al. 2016; Talvitie et al. 2017; Ziajahromi et al. 2017). Large volume bulk samples, such as those collected at specific depths of deep aquatic systems, can also be concentrated onto meshes in this way after sample collection (Courtene-Jones et al. 2017).

These techniques allow for the more accurate collection of known water volumes by manual measurements of water sampled or the setting of known flow rate using water pumps. They can also be used in the sampling of smaller aquatic systems, including streams and wastewater treatment plants, where the sampled channel can be shallower and / or narrower than standard trawling nets. However, the aperture of the mesh onto which samples are concentrated has a considerable bearing on a study's ability to representatively determine the concentration of microplastic particles in a collected sample,

with studies unable to account for microplastic particles smaller than the aperture of the mesh they use (Setälä et al. 2016).

The collection of single (grab) and agglomerated (composite) bulk volumes of water at single points in time, which are not volume-reduced prior to sample analysis, is another approach used in the sampling of aquatic environments (Li et al. 2018b). The volume of sample collected and filtered in this way has been reportedly as large as 100 L (Song et al. 2014), and as small as 100 ml (Dubaish and Liebezeit, 2013). These samples can account for the entire size range of microplastic particles, but must compromise between processing time and the acquisition of samples that are representative of the site at which they are collected.

### **3.1.2. Sediment**

The sampling of aquatic sediment regularly involves the collection of bulk samples using a variety of apparatus including a spoon (Klein et al. 2015; Horton et al. 2017a), a shovel (Zhang et al. 2016), a sediment grab (Castañeda et al. 2014; Sruthy and Ramasamy, 2017), and a sediment corer (Woodall et al. 2014; Matsuguma et al. 2017). It is also possible to sample freshwater sediments using the cylinder resuspension technique. Hurley et al. (2018a) used this approach, in which a section of sediment is isolated from the flow of water using a cylinder, and agitated to incorporate the fine sediment into the water that is also isolated by the cylinder, to sample microplastics in sediments of the upper Mersey and Irwell catchments, UK. Hurley et al. (2018a) later volume-reduced the turbid water that this sampling isolated onto a 63 µm sieve.

### **3.1.3. Atmospheric deposition**

Of the seven atmospheric microplastic publications, Dris et al. (2016), Cai et al. (2017), Allen et al. (2019), Klein and Fischer, 2019, and Stanton et al. (2019a) (Chapter 5) all followed a similar approach in which deposited particles were collected in a receptacle, whilst Dris et al. (2017) used a dust sampler to extract airborne particulates from a measurable volume of air (Table 3.1).

**Table 3.1: A summary of the sampling approaches taken for the collection of airborne microplastic particles (A) and deposited microplastic particles (D) in six of the seven studies to report the presence of atmospheric microplastics. The methods used by Bergmann et al. (2019) are discussed separately.**

Study	Sampling device	Sampling area	Sampler location	Number of sites	Collection frequency and period
Dris et al. (2016) (D)	Stainless steel funnel to glass bottle	0.325 m <sup>2</sup>	Rooftops	2	Sporadic, rainfall dependent. One site Feb 2014 to March 2015, the other Oct 2014 to March 2015.
Dris et al. (2017) (A)	Dust sampler	N/A	Rooftop	1	Triplicate samples collected in Feb, May, July, and Oct
Cai et al. (2017) (D)	Glass bottle, no funnel	0.0177 m <sup>2</sup>	~15 m above the ground	3	Oct (31 days), Nov (30 days), and Dec (31 days) 2016.
Allen et al. (2019) (D)	Rain sampler	0.014 m <sup>2</sup>	Raised above ground using adjustable stand. Height not stated.	1	Five different sample durations of 12, 19, 34, 41, and 34 days from Nov 2017 to March 2018.
	Particulate fallout collector	0.03 m <sup>2</sup>			
Klein and Fischer (2019) (D)	Bulk sampler	0.0113 m <sup>2</sup>	1 m above ground level	18	Biweekly for 12 weeks from Dec 2017 to Feb 2018
Stanton et al. (2019a) (D)	Glass funnel to glass bottle	0.0113 m <sup>2</sup>	Rooftops	4	Fortnightly from Nov 2017 to Oct 2018

It is known that concentrations of airborne particulates increase closer to the ground (Prata, 2018). Therefore, in addition to the frequency with which samples are collected, the height at which samples are collected will have a bearing on the abundance, size and type of microplastic particle that is likely to be collected. Parker-Jurd (2019, pers. comm.) report that the abundance of microplastic particles deposited at ground level was 'much higher' than the abundance of microplastic particles in atmospheric deposition collected from rooftops by Stanton et al. (2019a) (Chapter 5).

In contrast to the collection of deposited and airborne particles above ground level, Bergmann et al. (2019) sampled settled snow. They collected the surface layer of settled snow from Arctic ice floes; parked cars, public land, a snowfield, a village, and country lanes across Europe; and five unspecified locations in Svalbard. The sampled snow included temporally undefined 'freshly deposited' snow, snow that had fallen two days prior to sampling, and snow that was not temporally restrained at all. The potential for ground level contamination of even the freshly deposited snow that Bergmann et al. (2019) analyse is not quantified. It is therefore possible that the microplastics quantified by Bergmann et al. (2019) incorporated ground level particulate matter, inflating the reported airborne microplastic concentrations. Despite this, the findings of Bergmann et al. (2019) were reported in the context of microplastic particles in snowfall, and were widely distributed across multiple media platforms.

#### **3.1.4. Soil**

The presence of certain types of microplastic particle in soils, even in concentrations as low as 2% of soil, has been shown to alter physical and biological properties of some soil types including water retention, bulk density, structure and function, and microbial activity (de Souza Machado et al. 2018b). Polyethylene particles <150 µm in soil concentrations >0.4 % of soil increased mortality and reduced growth rate in some earth worms (Lwanga et al. 2016). However, though microplastics are likely to be abundant in soils globally (Rillig et al. 2012; Nizzetto et al. 2016; Horton et al. 2017b), the presence, abundance,

and distribution of microplastic pollution in the terrestrial system is poorly understood.

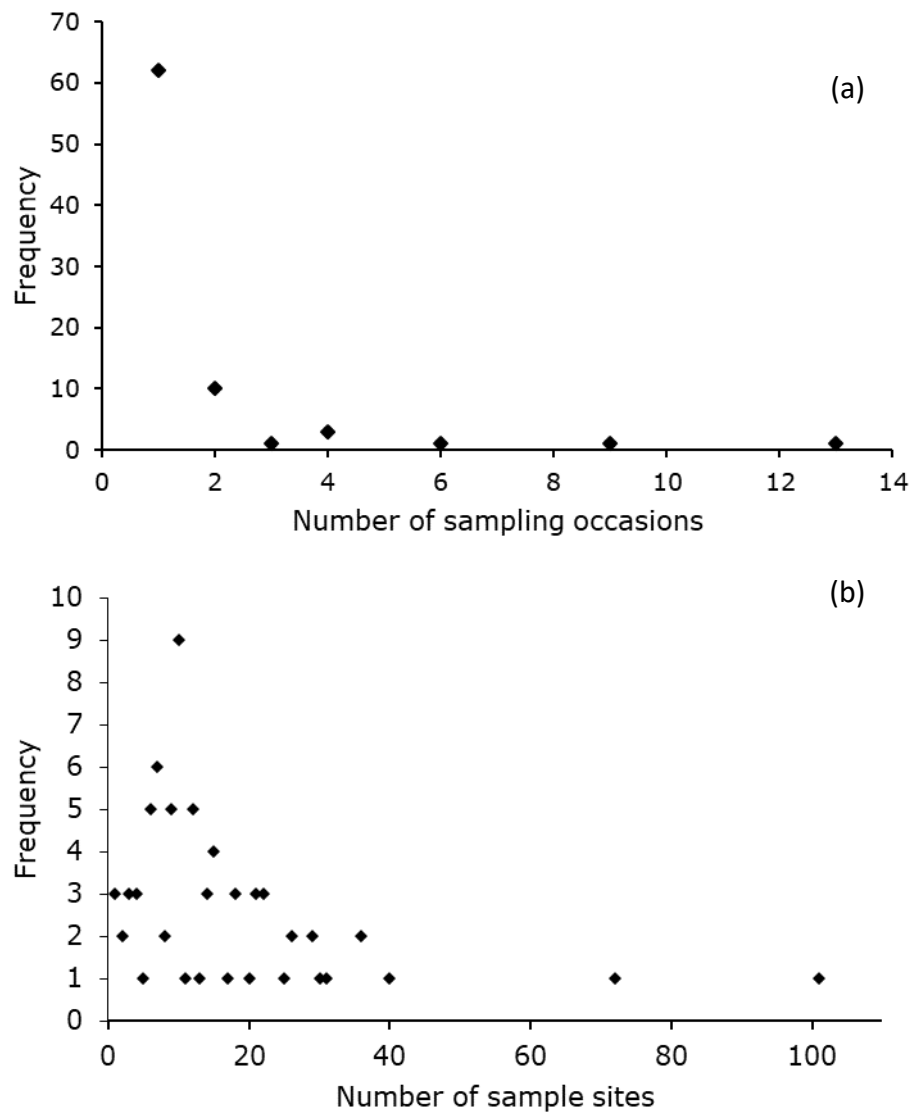
Zubris and Richards (2005), used synthetic textile fibres as a marker of anthropogenic activity in agricultural soils, where their presence was associated with WWTP solids that are applied to agricultural land as fertiliser. This first study of microplastic particles in soils sampled soil profiles at varying depths (0-50 cm), but did not state how the soil profiles were collected or sampled. Nevertheless, it demonstrated that microplastic particles persist in terrestrial environments. More recently, the presence of microplastics in soils has been investigated in floodplain soils (Scheurer and Bigalke, 2018) and agricultural soils (Liu et al. 2018; Zhang and Liu, 2018). Soil samples in these studies were collected using steel tools (Scheurer and Bigalke, 2018) and a spade (Zhang and Liu, 2018). Liu et al. (2018) do not state how their samples were collected.

### **3.1.5. Microplastic sampling design**

Microplastic research is not only influenced by the techniques used to collect and process samples, and to identify particles within them. Sample design can have a considerable bearing on the conclusions that a microplastic study is able to draw. In the freshwater environment, microplastic surveys regularly sample multiple sites to account for spatial variation (Figure 3.1a). However, with microplastic surveys usually sampling freshwater systems just once (Figure 3.1b), the influence of sampling frequency has been shown to influence microplastic concentrations greatly. Over a twelve month sampling campaign, Stanton et al. (2019a) found that flux extrapolations of freshwater textile fibres could vary by as much as 8 orders of magnitude throughout their sampling period.

In the study of atmospheric microplastic deposition (Table 3.1), Dris et al. (2016) is the only study not to clearly define the frequency with which samples were collected. They state that sample collection took place at various, unspecified, frequencies that were dependent on cumulative rainfall. Though environmental factors limited access for sample collection in Allen et al. 2019, they do state the exact frequency between the collection of samples. In contrast, Cai et al. (2017), Klein and Fischer (2019), and Stanton et al. (2019a) are the only studies to conduct a time-dependent sampling campaign, over three months, 12 weeks, and 12 months respectively.

The use of a temporally systematic sampling campaign in the collection of airborne particles affords studies greater scope to assess the contributions of environmental factors such as precipitation and wind speed and, when done at high temporal resolution, can allow studies to account for temporal variations within the limits of the sampling frequency. For example, neither Klein and Fischer (2019) or Stanton et al. (2019a) identify a correlation between precipitation and particle abundance, though Klein and Fischer do identify a relationship between wind direction and microplastic deposition.



**Figure 3.1: The number of sampling sites, of 79 freshwater microplastic studies produced for publication in chapter 6 of this thesis. Web of Knowledge parameters used to produce this figure are detailed in chapter 6.**

### 3.2. Microplastic isolation

Environmental samples are complex matrices formed of multiple organic and inorganic, and natural and anthropogenic components. With few exceptions (e.g. Dubaish and Liebezeit, 2013; Song et al. 2014), a processing step is required in order to isolate microplastic particles from other particulates within environmental samples. Inorganic particles can be isolated from microplastics and organic material by density separation because plastics of different polymers have different densities (Table 3.2) – though the buoyancy of these



polymers can vary in the environment, such as when biofilms form on plastics (Napper and Thompson, 2019). Microplastic particles are regularly extracted from environmental samples, and particularly sedimentary samples, using liquids of known density. These liquids include water ( $\text{H}_2\text{O}$ ,  $1.0 \text{ g cm}^{-3}$ ) (e.g. Zubris and Richards, 2005), sodium chloride ( $\text{NaCl}$ ,  $1.2 \text{ g cm}^{-3}$ ) (e.g. Peng et al. 2017), zinc chloride ( $\text{ZnCl}$ ,  $1.5\text{-}1.7 \text{ g cm}^{-3}$ ) (e.g. Horton et al. 2017a), and sodium iodide ( $\text{NaI}$ ,  $1.6\text{-}1.8 \text{ g cm}^{-3}$ ) (e.g. Claessens et al. 2013).

In the first study to extract microplastic particles from soils, Zubris and Richards (2005) isolated synthetic fibres from 3 g soils samples using a water-based density separation. After disaggregating samples into an aqueous solution in a conical flask, and filling the flask nearly to the top, they left the sample to settle. The water level was then raised dropwise until to the water surface, on which the synthetic fibres and other buoyant particles had surfaced, reached the brim of the flask. This surface water was pipetted onto microscope slides and mounted for analysis.

**Table 3.2: Density and European resin demands of common plastic polymers. European demand is taken from Plastics Europe (2018). Densities are taken from Nuelle et al. (2014).**

Polymer	European demand (%)	Density (g cm <sup>-3</sup> )
Polypropylene	19.3	0.89–0.91
Low density polyethylene		
Linear low density polyethylene	17.5	0.89–0.94
Medium density polyethylene		
High density polyethylene	12.3	0.94–0.97
Polyvinyl chloride	10.2	1.3–1.58
Polyurethane	7.7	1.17–1.28
Polyethylene Terephthalate	7.4	1.29–1.40
Polystyrene		1.04–1.08
Expanded polystyrene	6.6	0.015–0.03a
Polyamide		1.07–1.08
Acrylic	NA	1.17–1.20

This principle of density separation, or modifications of it, continue to be used in studies that extract microplastics from solid matrices, mixing samples with water or salt solution before leaving them to settle and decanting (Peng et al. 2017) or overflowing (Horton et al. 2017a) the surface of the mixture. It is recommended that the process of mixing, sedimentation, and extraction be repeated three times to ensure maximum extraction of microplastics from samples (Claessens et al. 2013; Horton et al. 2017a).

The density of the liquid used will have a bearing on the types of plastic polymers that can be isolated from samples, with only polymers of lower density than the density separation liquid being isolated from the sample. Lower density solutions such as water (H<sub>2</sub>O, 1.00 g cm<sup>-3</sup>) and sodium chloride (NaCl, 1.2 g cm<sup>-3</sup>) are cheaper and do not require safe handling and disposal measures. They are also dense enough to isolate polymers that account for a large proportion of polymer production including polyethylene and polypropylene (Plastics Europe, 2018) (Table 3.2). However, polymers denser than 1.2 g cm<sup>-3</sup>, including polyethylene terephthalate and polyvinyl chloride,

also represent a considerable proportion of the polymers that are produced in Europe (Plastics Europe, 2018) (Table 3.2). Though the proportions of polymers produced does not necessarily reflect the proportion of polymers found within the environment, these denser polymers are still of environmental importance. Denser salts such as zinc chloride ( $\text{ZnCl}_2$ , 1.5-1.7  $\text{g cm}^{-3}$ ) and sodium iodide (NaI, 1.6-1.8  $\text{g cm}^{-3}$ ) can therefore be used to enable studies to account for microplastic particles made from these denser polymers, which  $\text{H}_2\text{O}$  and NaCl are not able to isolate (Van Cauwenberghe et al. 2015). Density separation using salt solutions can also be applied to the retained material of volume-reduced water samples, such as samples collected from rivers with a high suspended solid load.

Particles can also be density separated by elutriation. Elutriation mixes sediment samples with a liquid in a vertical column. A gas or liquid is introduced to the bottom of the column opposing the direction of sedimentation (Claessens et al. 2013). This fluidises the sample, and carries buoyant particles to the top of the column with the rising water. In order to reduce the sample volume, the rising water then overflows onto a mesh where the retained material can undergo further processing by density separation or chemical treatment. The application of elutriation in microplastic analysis primarily fluidises sediments using water (Claessens et al. 2013; Zhu 2015; Kedzierski et al. 2016; Hengtsmann et al. 2018). Imhof et al. (2012) developed a similar separation device called the Munich Plastic Sediment Separator, though further development of this method of extracting microplastic particles from sediments has been recommended (Zobkov and Esiukova, 2017).

Organic material is common in water samples, and is of a similar density to many plastic polymers. As a result, it is often separated from the inorganic fraction of sediment samples during density separation. Removal of organic matter is not always included in the processing of samples for microplastic analysis. For example, Song et al. (2014) filtered their 100 L samples through large diameter (15 cm) filter papers, with no reported chemical treatment to remove organic material. However, organic material can obscure particles, and

where spectroscopic analytical techniques are used to identify particles, can affect the quality of spectra produced (Löder et al. 2017). Furthermore organic matter can slow the vacuum filtration of samples, increasing the processing times for each sample. Chemical treatment to remove organic matter therefore often follows the isolation of microplastic particles from samples.

A number of chemical treatments are available for the removal of organic material from environmental samples. Organic matter can be broadly categorised as biogenic (of plant or animal origin) or humic (originating from the decomposition of biogenic organic matter). Hydrogen peroxide ( $H_2O_2$ ) is commonly used to oxidise organic matter (Hurley et al. 2018b), however, used in different concentrations,  $H_2O_2$  has been found to alter the colour and morphology of microplastic particles, both of which are characteristics used in the visual identification of microplastic particles (Table 3.3). Nuelle et al. (2014) assessed the impacts of 30% and 35%  $H_2O_2$  treatments. They observed discolouration and a decrease in size and / or thinning for particles of polyamide, polycarbonate, and polypropylene >1 mm following a 30%  $H_2O_2$  treatment. This treatment also caused a browning of colour for polyethylene terephthalate, and fragmentation of linear low density polyethylene in particles >1 mm. For particles <1 mm exposure to 30%  $H_2O_2$ , Nuelle et al. (2014) reported discolouration and a decreases in size or thinning for particles of Polyvinyl chloride, polyethylene terephthalate, polyamide, polyurethane, polypropylene, and low and linear low density polyethylene. At a concentration of 35%,  $H_2O_2$  treatment changed the size of polyethylene and polypropylene particles <1 mm.

$H_2O_2$  treatment can be catalysed using an iron ( $Fe^{2+}$ ) catalyst (Fenton's reagent), however, the efficacy of this approach requires further testing (Hurley et al. 2018b). Acidic (Hydrogen chloride, HCl), basic (Sodium hydroxide, NaOH) and enzymatic (Proteinase-K) treatments have also been shown to be effective in the removal of >85% biogenic material (Cole et al. 2014). However, these reagents can also degrade plastic polymers (Hurley et al. 2018b). Dehaut et al. (2016) tested the impact of 6 treatment protocols on common polymers.

The reagents used in each protocol were 1) potassium hydroxide (KOH), 1b) KOH incubated at 60°C for 24 hours 2) pepsin enzyme prepared in HCl, 3) nitric acid (HNO<sub>3</sub>), 4) a mixture of HNO<sub>3</sub> and perchloric acid (HClO<sub>4</sub>), 5) NaOH, 6) peroxydisulfate potassium (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) and NaOH. Of these, protocols 1 and 2 did not degrade plastics but, respectively, were too time consuming and too ineffective in the removal of biogenic organic matter. Protocols 3 and 4 both affected all polymers, and protocols 1b, 5 and 6 had no effect on the polymers that were exposed to them.

Despite the limitations of H<sub>2</sub>O<sub>2</sub>, and the availability of alternative chemical treatments, it remains the most popular method to digest organic material in microplastic studies (Li et al, 2018b).

### **3.3. Microplastic identification**

The identification of microplastic particles following sample processing can be achieved in a variety of ways following subjective, semi-objective, and objective techniques. Subjective microplastic analysis is based solely on the visual analysis of particles, identified as microplastic by eye following established identification criteria (Table 3.3).

**Table 3.3: Published criteria for the visual identification of microplastic particles. Each publication expands on those that preceded it**

Publication	Criteria
Norén (2007)	<ul style="list-style-type: none"> <li>• No cellular or organic structures are visible in the plastic fragment/fibre</li> <li>• If the particle is a fibre it should be equally thick, not taper towards the ends and have a three-dimensional bending (not entirely straight fibres which indicates a biological origin)</li> <li>• Clear and homogeneously coloured particles (blue, red, black and yellow)</li> <li>• If it is not obvious that the particle/fibre is coloured, i.e. if it is transparent or whitish, it shall be examined with extra care in a microscope under high magnification and with fluorescence microscopy in order to exclude an organic origin.</li> </ul>
Nor and Obbard (2014)	<ul style="list-style-type: none"> <li>• Exclude particles that are shiny</li> <li>• Exclude fibres that are segmented or resemble twisted flat ribbons</li> </ul>
Marine and Environmental Research Institute (n.d)	<ul style="list-style-type: none"> <li>• Synthetic fibre will occasionally split or fray</li> <li>• Inconsistent colouring by design or as a result of weathering</li> </ul>
Stanton et al. (2019a)	<ul style="list-style-type: none"> <li>• Microplastic fibres may have bulbous ends</li> </ul>

Visual identification following these criteria can be further aided by tests to see if particles melt or deform following the application of heat, often with a hot needle (Hendrickson et al. 2018). However, visual identification alone has the potential to both over- and under-estimate microplastic concentrations (Song et al. 2015). Its reliability has also been found to decrease with decreasing particle size, and be more effective in the identification of textile fibres than microplastic fragments (Lenz et al. 2015). Microplastic research will therefore often follow a tiered semi-objective approach to microplastic identification in which visual identification is followed by confirmatory, often spectroscopic, analysis of a subset of particles (Stanton et al. 2019b (Chapter 4)).

Identification of microplastic particles from their chemical composition can be achieved by various techniques including Fourier transform infrared (FTIR)

spectroscopy, (Harrison et al. 2012; Primpke et al. 2018), Raman spectroscopy (Araujo et al. 2018; Schymanski et al. 2018), scanning electron microscopy with energy dispersive X-Ray spectroscopy (SEM/EDS) (Su et al. 2016; Wang et al. 2017c), and pyrolysis gas chromatography coupled to mass spectrometry (Pyr-GC/MS) (Fries et al. 2013; Hendrickson et al. 2018). These analytical techniques give an indication of the proportions of different polymers within a sample, and the accuracy of the preceding visual analysis.

These techniques are all able to produce spectra that reflect the chemical composition of the analysed particle and which can be identified by comparison to a spectral library of known polymers. Analysis by FTIR and Raman spectroscopy are the most common forms of microplastic identification. They do not require particle pre-treatment like SEM/EDS, and they are non-destructive techniques, unlike Pyr-GC/MS, allowing further analysis where necessary. Raman spectroscopy enables the analyst to identify smaller particles than FTIR spectroscopy (Käppler et al. 2016). However, the versatility of the different FTIR spectroscopy techniques available can lead to more rapid particle analysis.

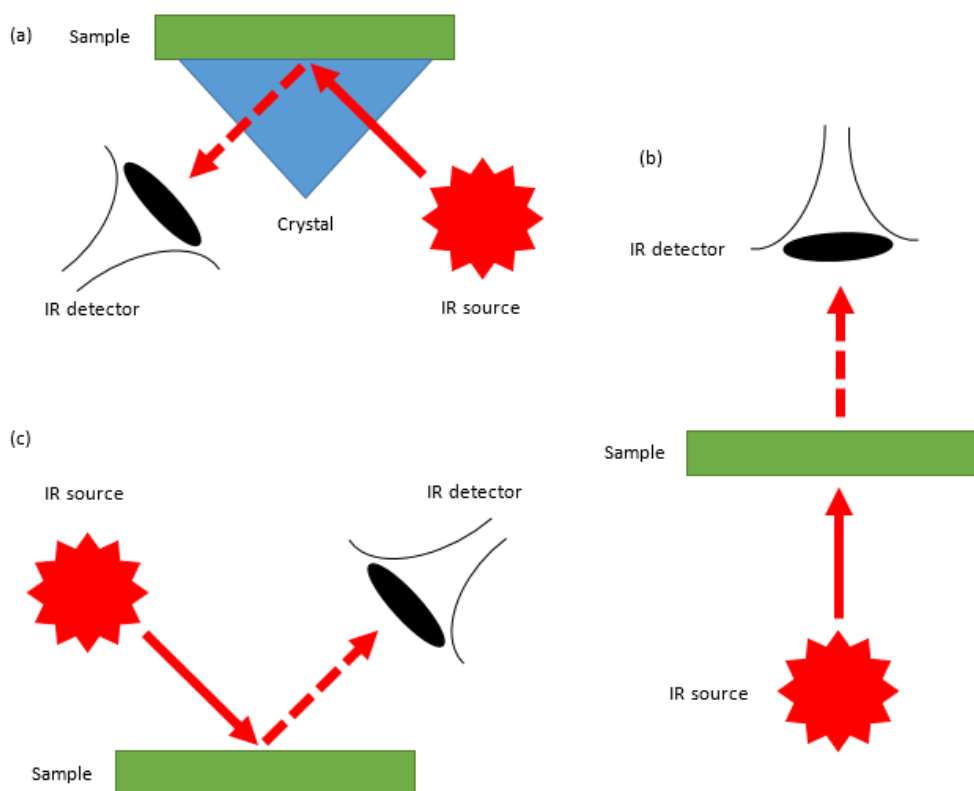
For all types of FTIR spectroscopy, an infrared (IR) beam is passed from a source to a detector via the sample. The detector is able to determine the chemical composition of the particle from the absence of certain wavelengths of light absorbed by the particle. Three semi-objective variations of FTIR spectroscopy – Attenuated Total Reflectance (ATR), transmission, and reflectance FTIR spectroscopy – can be used to characterise particles in microplastic research.

Particles that can be handled can be analysed by ATR-FTIR spectroscopy. This method transfers the sample onto a crystal through which the IR beam will pass (Figure 3.2a). This sample is therefore in direct contact with the IR beam, meaning less time is needed to generate a clear spectrum.

Successful transmission FTIR spectroscopy requires the transfer of the particle onto a material through which the IR beam can pass unchanged and, with the IR source below the sample, is only possible on samples thin enough for the IR

beam to pass through them (Figure 3.2b).

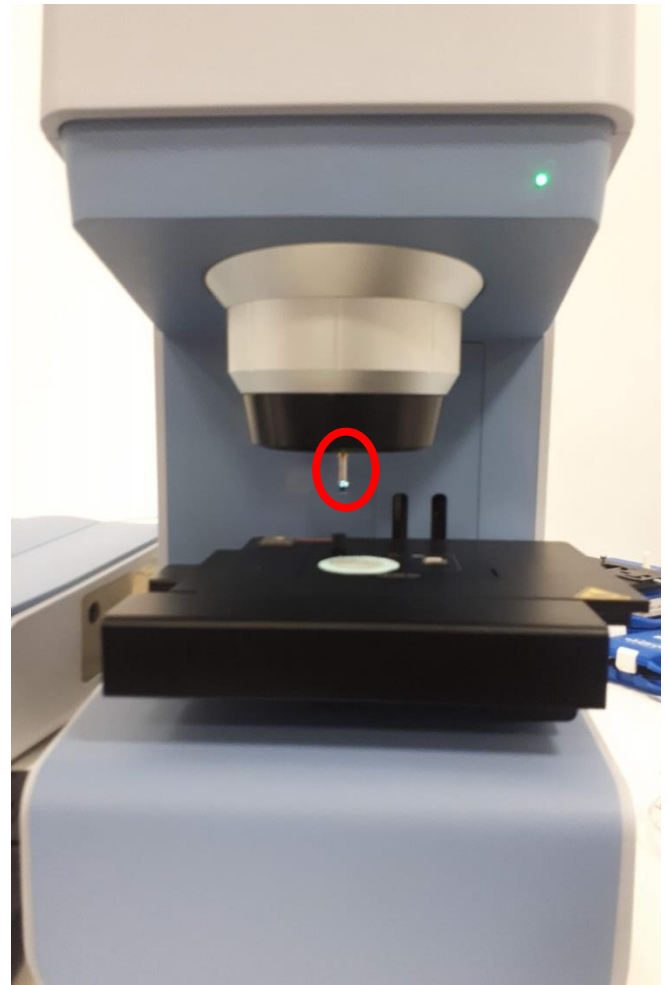
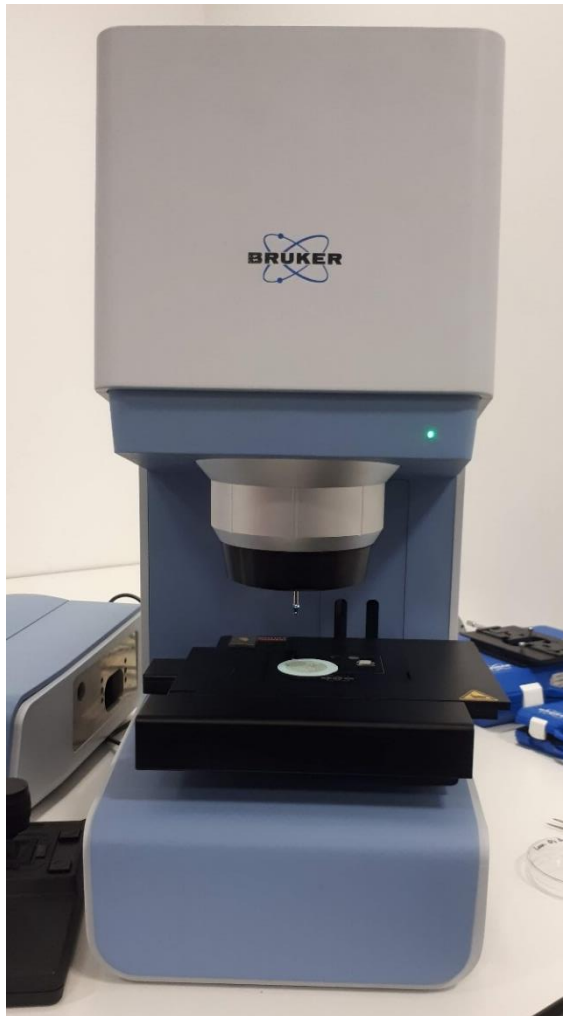
Reflectance FTIR spectroscopy does not require the particles to be handled by the analyst, and so can be used to analyse particles too small to be manipulated. The IR source and detector are both sourced from above the sample for reflectance FTIR spectroscopy (Figure 3.2c).



**Figure 3.2: Principles of ATR (a), reflectance (b), and transmission (c) FTIR spectroscopy.**

The use of an ATR-FTIR objective, a modification of the ATR-FTIR principle, can rapidly produce FTIR spectra for samples without requiring their transfer to an ATR-FTIR crystal. An ATR-FTIR objective is a crystal that can be lowered to a sample on a stage, providing the clarity and rapid spectrum generation of ATR-FTIR spectroscopy for particles too small to handle (Figure 3.3).



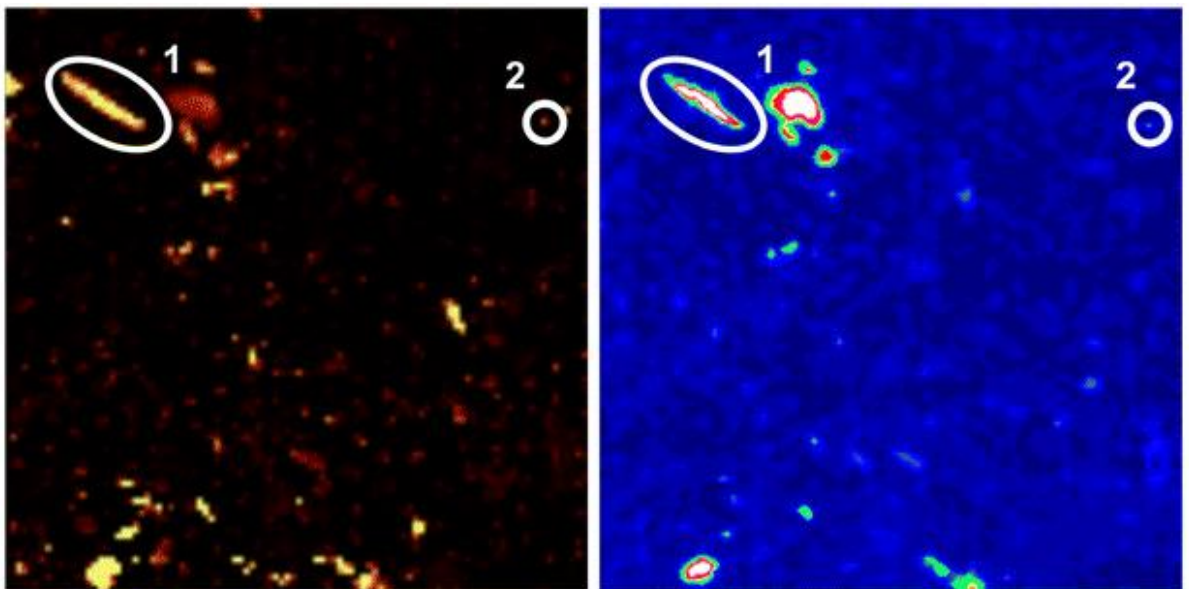


**Figure 3.3: A Bruker Lumos FTIR spectrometer with an ATR objective. An ATR crystal is fixed to the bottom of the ringed arm, to which the stage with the sample on it is raised.**

Though these techniques all have the ability to confirm the visual identification of particles, they are all limited by their restriction to only particles that are preselected by the analyst. They therefore rely on the ability of the analyst to visually identify microplastic particles. Furthermore, spectroscopic analysis can be time consuming, and so often microplastic analysts will only subject a subsample of particles to spectroscopic analysis. They may then extrapolate according to the identification error within this subsample (e.g. Dris et al. 2016).

Objective microplastic analysis encompasses techniques for microplastic identification that are not dependent on the visual preselection of particles.

Following vacuum filtration, Raman imaging (Araujo et al. 2018; Sobhani et al. 2019) and Focal Plane Array (FPA) FTIR spectroscopy (Tagg et al. 2015; Mintenig et al. 2017) can be used to analyse an entire area of a filter paper for microplastic particles. These techniques scan the sample from above to produce a spectra for each pixel in the given area (Tagg et al. 2015). The spectrum generated for each pixel can be analysed and illustrated on a false-colour image to produce a visualisation of the distribution of microplastic particles across the analysed area (Figure 3.4). This approach removes the need for particle preselection, but increases the time taken to analyse a sample. As a result, microplastic abundance across just a subsample of a filtered area is often analysed using these techniques (Imhof et al. 2016; Mintenig et al. 2017).



**Figure 3.4: A Raman (left) and FPA-FTIR (right) false colour image of the same field of view of a sample analysed for microplastic particles. Taken from K appler et al. (2016).**

An alternative technique that has been praised by some as a rapid and objective technique for microplastic identification, which does not require expensive equipment, is the use of the fluorescent dye Nile red. Nile red is a lipophilic stain (Erni-Cassola et al. 2017) that has an affinity to plastics due to their similar molecular structure to plastic polymers. It therefore has the potential to fluorescently label microplastic particles in environmental

samples, though this method is unable to determine the polymer composition of particles that it stains (Shim et al. 2017).

Furthermore, Nile red also has an affinity to natural lipid containing particles that are also in environmental samples. It is therefore, susceptible to false positives; though this affinity has been found to vary following treatment to remove biological material (Erni-Cassola et al. 2017; Maes et al. 2017; Wiggin and Holland, 2019). Nevertheless, Renner et al. (2018) describe the use of Nile red as a suitable standalone technique for microplastic identification. The limitations Nile red in microplastic analysis are explored in more detail in chapter 4 of this thesis.

The potential standardisation of microplastic identification is further therefore limited by the variety of analytical approaches that studies can take, which may be governed by the availability of equipment and a study's finances.

### **3.4. Microplastic quantification**

The number of microplastic particles within an environmental sample does not necessarily reflect the risk of microplastic pollution to the environment from which the sample was collected. This is because the relative environmental risks of microplastic particles of different shape, size, polymer, and additive compositions have not been sufficiently quantified. Synthetic textile fibres (Wright et al. 2013) and small microplastic particles (<10 µm) (Wright et al. 2019) are two types of microplastic particle that have greater potential to cause environmental harm due to their shape, with the potential to penetrate deeper into pulmonary and gastrointestinal systems respectively (Wright and Kelly, 2017). Samples that contain comparable numbers of microplastic particles may therefore have different environmental implications depending on the proportion of microplastic types within them. This can be accounted for by studies categorising microplastic particles by size (e.g. <50 µm, 50-100 µm, 100-500 µm, 500 µm-1 mm, >1mm) (e.g. Zhao et al. 2015) and / or shape (e.g. fibre, film, fragment, sphere etc.) (e.g. Eriksen et al. 2013; Wessel et al. 2016), though there is some subjectivity in the latter.

An alternative approach is to present numerical microplastic concentrations alongside a mass per volume concentration (e.g. Hendrickson et al. 2018). This approach brings microplastic pollution in line with other forms of pollution, such as heavy metals and pharmaceuticals, where concentrations are presented as unit mass of sample per unit volume in aquatic matrices (e.g. Hughes et al. 2013), and unit mass per unit mass of sample in solid matrices (e.g. Čelić et al. 2019; Sun et al. 2019). However, presenting microplastic concentrations as a mass alone does not aid the interpretation of the prevalence of microplastic particles of different types. Moreover, due to the small size of many microplastic particles, mass cannot always be determined for single particles. Weighing multiple microplastic particles in order to record a detectable mass will provide a mass of microplastic particles within a sample, but does not afford studies the ability to consider variations in particle type and size (Rivers et al. 2019), with implications for source attribution.

Providing a complementary measure of size along with particle abundance has been recommended by Rivers et al (2019), who identify total surface area as the most appropriate complementary metric; though total surface area is also unable to account for variations in particle shape. Rivers et al. (2019) also only calculated the area of the upward facing surface of the particle, and were therefore unable to assess particle size in its entirety.

A comprehensive quantification of microplastic particles would provide detail of the proportion of all particles in categories of shape, size, and polymer. However, such analysis would be a time-consuming endeavour that would require access to specific equipment for the identification of small microplastic particles.

The methods of sample collection, processing, and analysis are dependent on the resources available to each study. Moreover, whether or not the methods used are appropriate is, to some extent, dependent on the aim of the study and the context into which the findings are placed. Given the heterogeneity of microplastic particles present in the environment, the matrices in which they are studied, and methodologies available for each stage of their environmental

assessment, it is hard to identify a single method for microplastic research that is better than others. Despite the repeated and well-justified concerns over the lack of comparability between studies, it is therefore hard, and possibly impractical, to standardise this process.

### **3.5. Bias and uncertainty in microplastic methodologies**

The findings of microplastic research are susceptible to biases and uncertainties associated with the methods used in their study. As a result, the collection, processing, and analysis of microplastic samples regularly preclude microplastic studies from presenting a comprehensive assessment of microplastic abundances in the sampled environment.

For example, in the aquatic environment most microplastic surveys choose to sample surface waters, and are thus unable to consider the prevalence of microplastic particles throughout the water column of the sampled system. The concentration of large volumes of water onto nets and sieves also precludes microplastic studies from considering particles smaller than the aperture of the mesh on which samples are concentrated. Bulk samples, whether aquatic, soil, sedimentary, or atmospheric, overcome this limitation but restrict the extent to which studies are representative of the environment sampled. In the processing of collected samples, density separation is only able to isolate plastic polymers less dense than the solution used. Beyond this, the largely arbitrary definition of microplastic particles as those smaller than 5 mm by Arthur (2009), presents a bias in its exclusion of particles larger than this from the concern of microplastic research.

Methodological uncertainties, particularly prevalent in the identification of microplastic particles, present a further challenge to the appropriate interpretation of microplastic research. The degree to which FTIR and Raman spectra of suspect particles match those of known polymers in spectral libraries is not consistent across microplastic studies, however, where lower thresholds are used the certainty of polymer identification decreases. The use of the fluorescent dye Nile red also introduces uncertainty as a result of the limited

scope of its testing. Stanton et al. (2019b) (Chapter 4) show that Nile red can both over- and underestimate microplastic abundance due to its staining of natural material even after H<sub>2</sub>O<sub>2</sub> digestion, and its inconsistent staining of microplastic particles, particularly those not white, translucent, or transparent. The visual characterisation of particles incorporates considerable uncertainty into microplastic quantification due to its subjectivity. However, it is a key step in microplastic quantification whether as a precursor to confirmatory analytical approaches, or as a standalone technique for microplastic identification. Training in microplastic identification, and particularly textile fibre characterisation (Stanton et al. 2019a) (Chapter 5), can increase the confidence that microplastic analysts can place in their visual identification, but it cannot overcome this uncertainty.

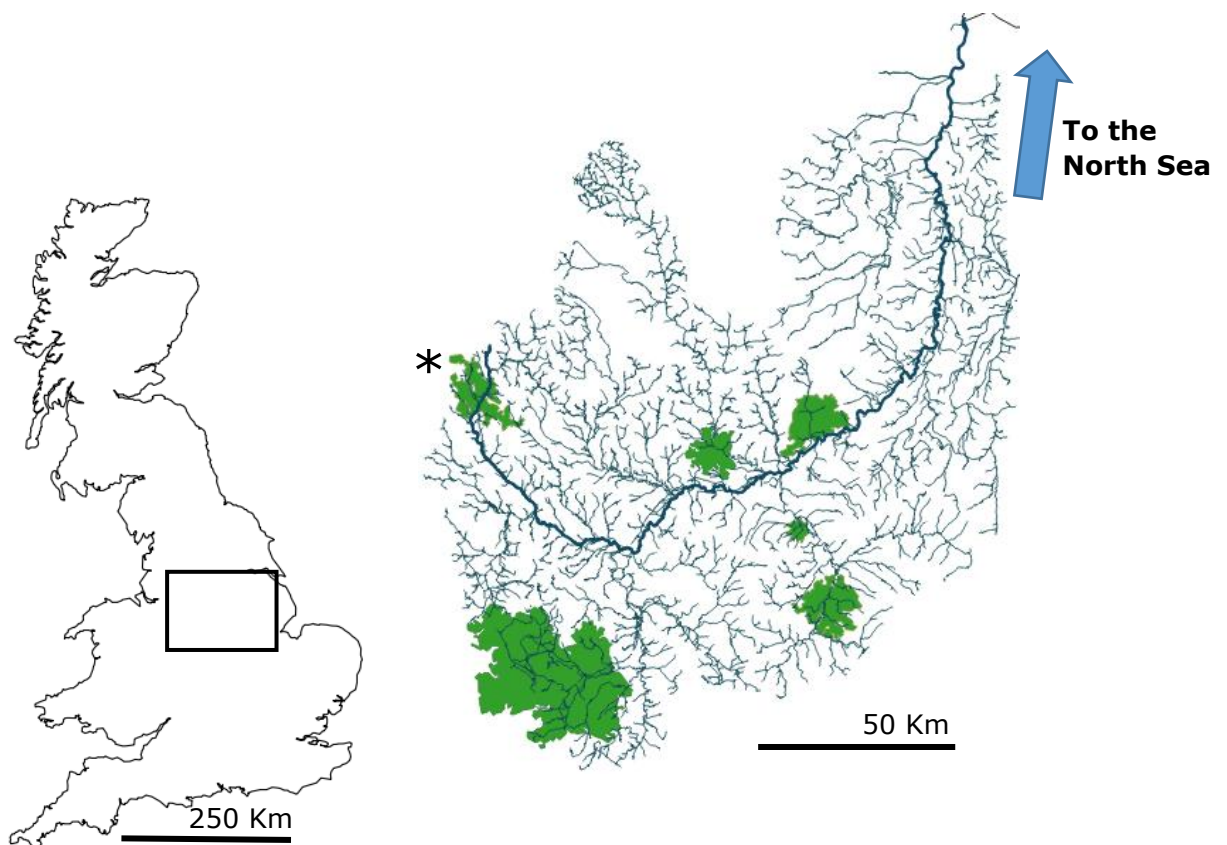
These biases and uncertainties may be unavoidable due to logistical and financial limitations of individual studies, and their prevalence in microplastic research should not undermine this field of study. However, interpretation of microplastic research must show an awareness of any implications of biases and uncertainties in order to ensure the objective and appropriate dissemination of findings.

### **3.6. Riverine and airborne microplastic pollution in the Trent catchment**

The River Trent is a 274 km river flowing from its source at Biddulph Moor to its confluence with the Humber Estuary (Figure 4.1) (Trent Rivers Trust, 2017). The third longest river in the United Kingdom, it has a catchment area of 10 440 km<sup>2</sup> (Trent Rivers Trust, 2017). Within the Trent catchment are multiple urban centres including Birmingham, Derby, Leicester, Nottingham, and Stoke-on-Trent. The total population of the Trent catchment exceeds 6 million people (Trent Rivers Trust, 2017).

The Trent catchment includes tributaries that do and do not receive WWTP effluent, and that flow through multiple urban areas in close proximity to one another. It therefore represents a suitable catchment for the comprehensive

spatiotemporal assessment of microplastic pollution across sites that enable critical consideration of microplastic ubiquity and sources. Additionally, within the Trent catchment are four campuses of the University of Nottingham that are located in residential suburban, recreational suburban, and rural areas. Buildings across these campuses offer a unique opportunity to assess variation in microplastic deposition across these different land uses.



**Figure 4.1: The River Trent catchment. The River Trent is denoted by the thick blue line. Green areas represent major urban areas which are, clockwise from the \*, Stoke-on-Trent, Derby, Nottingham, Loughborough, Leicester, and the conurbation of Birmingham and Wolverhampton.**

Despite the breadth of concern for microplastic pollution, methods to collect and quantify microplastic particles in natural environments are varied and unregulated. The results of microplastic studies are therefore not universally comparable, and are not always representative of the environments or organisms under investigation. Establishing an accurate understanding of the

distribution and threats that microplastic particles pose is therefore a particular challenge for environmental scientists. Within this there is a need for studies to describe spatial and temporal differences in microplastic pollution (Khan et al. 2018).

This thesis presents a thorough assessment of the efficacy of Nile red in microplastic analysis (chapter 4), and a spatially and temporally comprehensive assessment of textile fibre (chapter 5) and microplastic (chapter 6) concentrations in rivers and atmospheric deposition across the Trent catchment, UK. Its findings, both published (Stanton et al. 2019a) (Chapter 5) and in review, represent one of the most temporally comprehensive assessments of freshwater microplastic pollution. This temporal consideration has enabled this research to consider the extent to which microplastic pollution has previously been misrepresented, and place microplastic pollution within the broader context of anthropogenic particulates across the sampled systems.



## **4. Exploring the Efficacy of Nile Red in Microplastic Quantification: A Costaining Approach**

Thomas Stanton; Matthew Johnson; Paul Nathanail; Rachel L Gomes; Teresa Needham; Amanda Burson

### **4.1. Abstract**

The presence of microplastic particles (<5 mm) in the environment has generated considerable concern across public, political, and scientific platforms. However, the diversity of microplastics that persist in the environment poses complex analytical challenges for our understanding of their prevalence. The use of the dye Nile red to quantify microplastics is increasingly common. However, its use in microplastic analysis rarely accounts for its affinity with the breadth of particles that occur in environmental samples. Here, we examine Nile red's ability to stain a variety of microplastic particles and common natural and anthropogenic particles found in environmental samples. To better constrain microplastic estimates using Nile red, we test the coapplication of a second stain that binds to biological material, 4',6-diamidino-2-phenylindole (DAPI). We test the potential inflation of microplastic estimates using Nile red alone by applying this costaining approach to samples of drinking water and freshwater. The use of Nile red dye alone resulted in a maximum 100% overestimation of microplastic particles. These findings are of particular significance for the public dissemination of findings from an emotive field of study.

## 4.2. Introduction

Accurate quantification of microplastic particles in environmental samples is fundamental to our understanding of their environmental fate and prevalence. However, the identification of microplastic particles in environmental samples regularly adopts a time-consuming tiered approach of visual identification followed by confirmatory, often spectroscopic, particle characterisation using techniques including Fourier Transform Infrared (FTIR) spectroscopy (Harrison et al. 2012). Conducting confirmatory particle analysis on a subset of particles collected or, where FTIR or Raman imaging are available, on a just a subsample of a filter paper can decrease analysis time in microplastic studies. However, whilst this approach can increase the number of samples that a study is able to analyse, it is unable to account for the true extent of error in the visual identification of particles. Moreover, the visual preselection of particles is subject to size-dependent levels of error (Lenz et al. 2015), and spectroscopic particle characterisation requires expensive equipment. Both the visual and spectroscopic characterisation of microplastic particles also require a degree of specialist knowledge (Stanton et al. 2019a). A rapid, objective methodology for the identification of microplastic particles in samples should facilitate a more comprehensive assessment of microplastic particles in environmental samples, by enabling the analysis of large numbers of samples without the potential for particle misidentification.

The fluorescent dye Nile Red has been proposed as one such technique. A lipophilic stain, Nile Red is able to bind to the surfaces of microplastics due to the similar molecular structures of lipids and plastics. The stain binds to the surfaces of microplastic particles by van der Waals intermolecular forces (Maes et al. 2017), causing them to fluoresce under certain wavelengths of light. Its ability to stain microplastic particles has been repeatedly demonstrated (Cole, 2016; Shim et al. 2016; Maes et al. 2017; Erni-Cassola et al. 2017), and it has quantified microplastic particles in aquatic (Desforges et al. 2014; Fischer et al. 2016; Tamminga et al. 2018), sedimentary (Fischer et al. 2016; Hengstmann et al. 2018; Wang et al. 2018; Tiwari et al. 2019), and biological (Catarino et al.

2018) samples. Nile Red has also quantified microplastic particles in samples of bottled water (Mason et al. 2018), the findings of which are highly relevant to human health (Koellmans et al., 2019). Nile red offers microplastic analysts the opportunity to cheaply and rapidly analyse samples whilst removing the potential bias towards particles that stand out and are not too small (Wiggin and Holland, 2019). It has even been recognised as a suitable standalone technique for microplastic particle quantification (Renner et al. 2018).

The use of Nile red in microplastic analysis made global headlines following the publication of findings that used the technique to quantify the presence of microplastic particles <100 µm in bottled water sampled purchased across the world by Mason et al. (2018). This publication reportedly influenced a World Health Organisation review into the potential risk of microplastic pollution in drinking water (Readfern, 2018). Nile red was also used to identify microplastic particles in freshwater samples collected from across the UK by Friends of the Earth (FoE) (FoE, 2019; Carrington, 2019b) (Figure 5.1).

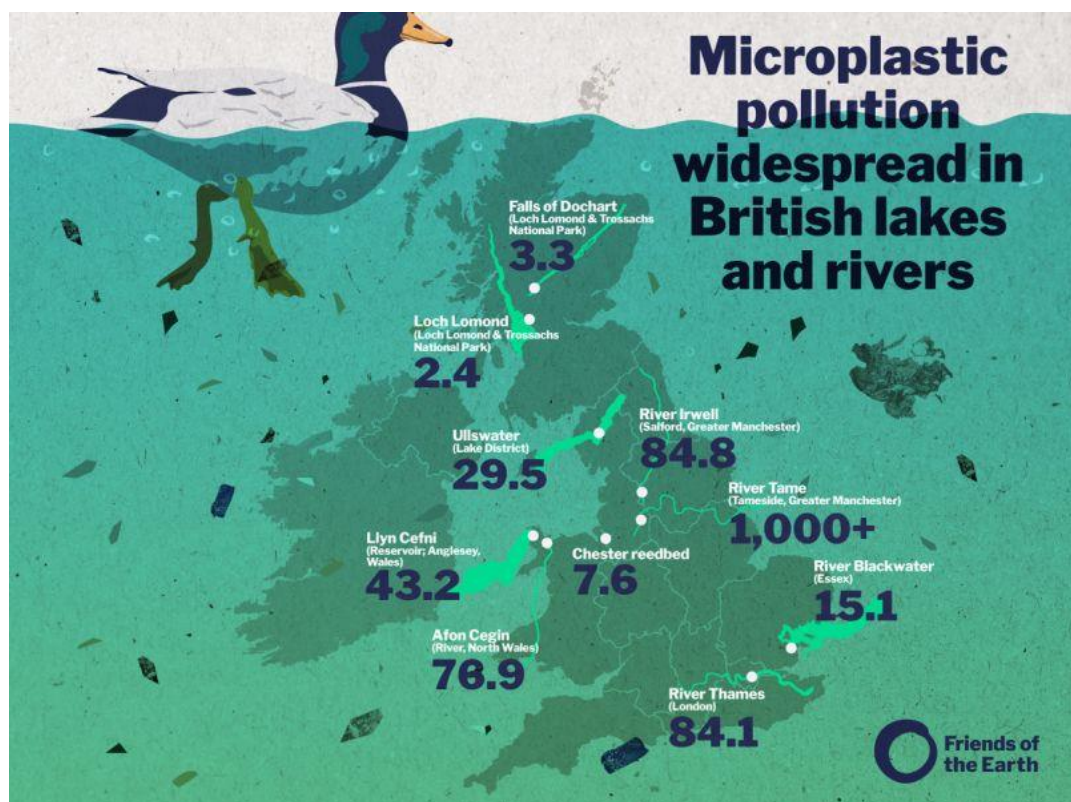


Figure 5.1: Friends of the Earth’s visualisation of microplastic pollution across UK freshwater systems, determined by Nile red analysis of samples, expressed as number of microplastic particles per litre.

However, though Nile red has been used to assess microplastic concentrations in high profile studies, and in samples that are of considerable importance to human health, its application to microplastic enumeration has not been comprehensively validated. Firstly, the colour of the particles previously used to assess its affinity with microplastics have predominantly been white, translucent, and transparent. It is therefore not known whether microplastic particles of different colours will be reliably stained with Nile red. Secondly, Nile red also has an affinity with any lipid containing biological material. This affinity has been found to decrease following treatment to remove biological material with Hydrogen Peroxide ( $H_2O_2$ ) (Erni-Cassola et al. 2017; Maes et al. 2017; Wiggin and Holland, 2019), however, the efficacy of  $H_2O_2$  digestion has been found to vary between these studies.

It is therefore possible that microplastic research that has applied this technique, including the publically disseminated works by Mason et al. (2018) and FoE (2019), have incorrectly reported microplastic particle concentrations in their samples. Communication of such research risks misleading stakeholders, compromising the trust that they place in the scientific community to inform their environmental behaviour, and potentially incorrectly alarming members of the public.

The comprehensive validation of a method for microplastic particle identification, that is not at least semi-quantitative (i.e. FTIR or Raman spectroscopy), must be able to account for the possibility of both false positives and false negatives. Nile red validation therefore requires consideration of microplastic particles of multiple colours and polymers, and the thorough assessment of its ability to stain non-plastic particles.

Fluorescent dyes are available that bind almost exclusively to biological materials (Porter and Feig, 1980). These dyes can aid the assessment of possible false positives in microplastic analysis from the Nile red staining of biological material. One such dye is 4',6-diamidino-2-phenylindole (DAPI), binding to adenine-thymine rich regions of DNA (Kapuscinski, 2009).

In order to thoroughly assess the efficacy of Nile red in microplastic identification, a study was devised that accounted for microplastic particles of different colours and polymers, and quantified false positives derived from the Nile red staining of natural textile fibres and biological particles. This study applied a costaining approach using both Nile red and DAPI to samples of drinking water, tap waters (tap, distilled, and deionised), and H<sub>2</sub>O<sub>2</sub> treated freshwater. It also assessed the autofluorescence of microplastic and non-plastic particles in the wavelengths of light used to observe Nile red fluorescence.

### **4.3. Materials and methods**

#### **Sample Preparation**

##### **Microplastic fragment and textile fibre production**

Microplastic particles were generated from common laboratory products using a utility knife, and were made from the following polymers: polypropylene (PP), high-density polyethylene (HDPE), expanded polystyrene (EPS), and polyvinyl chloride (PVC). Natural (cotton, wool, silk), regenerated (rayon), and microplastic (hereafter synthetic) (polyester, polyamide, acrylic) textile fibres were pulled from garments woven from 100% of each fibre type using tweezers. The colours of the materials studied are detailed in Table 5.1.

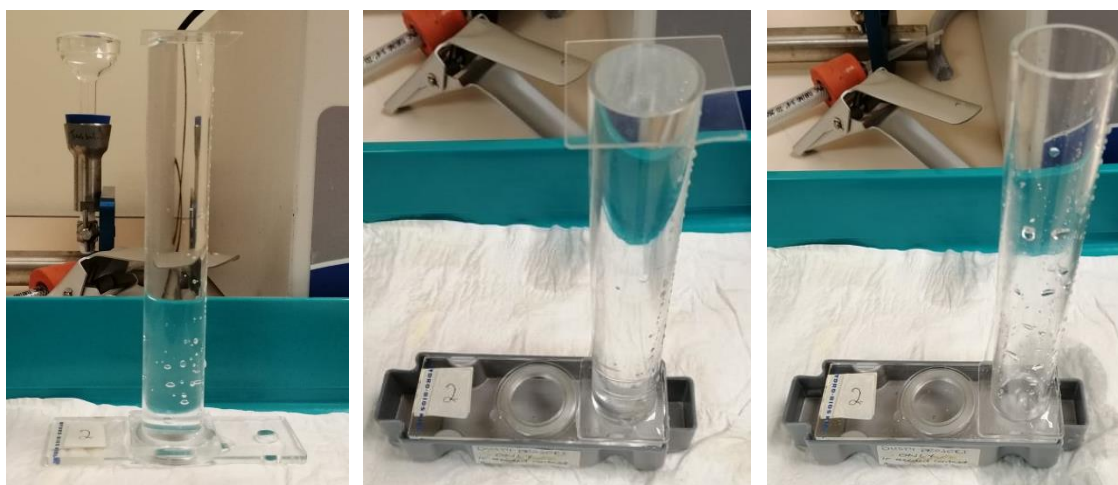
Erni-Cassola et al. (2017), treated polyethylene and polypropylene microplastic particles with a 7 hour 30% H<sub>2</sub>O<sub>2</sub> treatment at 100°C in their assessment of Nile red's efficacy. The particles prepared for this study were therefore placed in a 15 ml polypropylene centrifuge tube with 5 ml of distilled water, and 5 ml of 30% H<sub>2</sub>O<sub>2</sub>. Samples were heated to 80°C to avoid excessive thermal decomposition of the H<sub>2</sub>O<sub>2</sub>, and were left covered for 8 hours. No bubbles were observed following the addition of H<sub>2</sub>O<sub>2</sub>, indicating that these particles did not react with the H<sub>2</sub>O<sub>2</sub>.

##### **Freshwater samples**

In order to quantify the extent to which biological particles might be stained by Nile red in environmental samples, three samples of river water were collected from the River Soar, UK, in line with a previous microplastic pollution study (Stanton et al. 2019a) (Chapter 5). Briefly, 30 L of water was concentrated onto a metal sieve with a pore size of 63  $\mu\text{m}$ . Material retained on this sieve was transferred into a 200 ml glass sample bottle with an aluminium-lined plastic lid for transportation to the laboratory. In the laboratory, each sample was treated with 50 ml of 30%  $\text{H}_2\text{O}_2$  and was gradually heated to 75°C over 4–5 h. After being left to cool overnight, these samples were filtered onto 0.45  $\mu\text{m}$  mixed cellulose ester gridded filter papers (Whatman ME 25/41) using glass vacuum filtration apparatus.

To prevent sample contamination, the metal sieve and all glassware was thoroughly rinsed using distilled water prior to sample collection and filtration, and samples were covered with aluminium foil at all times except for during the transfer of samples to the vacuum filtration apparatus.

This method of sample collection was unable to account for Nile red's propensity to stain non-plastic particles smaller than the 63  $\mu\text{m}$  of the sieve mesh aperture used to concentrate river water samples. In order to assess this, samples of lake water were collected that were volume-reduced by sedimentation rather than concentration. For this, 500 ml of water was collected from Highfields Park Lake, Nottingham, UK. Three 60 ml subsamples of this lake water were transferred to a 100 ml Utermöhl sedimentation chamber (Figure 5.2). These chambers were then topped up to the brim with 40 ml of 30%  $\text{H}_2\text{O}_2$  to remove organic material from these samples. These samples were left to settle overnight. During this time the seal of one of the sedimentation chambers failed, and the sample was lost. In order to be processed alongside samples of known particles, the bottom 2 ml of the two successfully settled samples was transferred to a 15 ml centrifuge tube (Figure 5.2).



**Figure 5.2: Utermöhl sedimentation chambers used for the sedimentation of natural particles from lake water.**

The samples were then topped up to 10 ml with distilled water. The entire settling chamber apparatus was thoroughly rinsed in line with Stanton et al. (2019a (Chapter 5)) prior to the addition of samples to ensure the sedimentation chambers were not contaminated.

### **Drinking and tap water samples**

Five 500 ml samples of drinking water were purchased from a major UK supermarket (three single use plastic bottles of still water, one single use plastic bottle of sparkling water, and one can of still water). Tap water samples consisted of one 500 ml sample of safe to drink tap water, three 500 ml samples of deionised water, and three 500 ml samples of distilled water. Tap water samples were collected in 1 L glass beakers that were thoroughly cleaned in line with Stanton et al. (2019a) prior to the addition of each sample, and which were covered with aluminium foil until the sample had been filtered to minimise sample contamination. Each 500 ml sample was vacuum filtered onto 0.45  $\mu\text{m}$  mixed cellulose ester gridded filter papers (Whatman ME 25/41). The vacuum filtration apparatus was also covered with aluminium foil at all times except for during the transfer of samples to the vacuum filtration apparatus.

These samples were not treated with H<sub>2</sub>O<sub>2</sub> to allow for comparison with Mason et al. (2018), who did not treat samples of bottled water prior to Nile red analysis.

### **Staining procedure**

Nile red and DAPI stains were added to the 15 ml centrifuge tubes, containing known particles and lake water samples. Nile red dissolved in acetone, and DAPI dissolved in water, were pipetted into the centrifuge tubes to yield dye concentrations of 10 µg ml<sup>-1</sup> and 0.5 µg ml<sup>-1</sup> respectively. The samples were then left in the dark for 30 minutes before being vacuum filtered 0.8 µm Nuclepore black Track-Etch Membrane filter papers (Whatman 110659).

To the filtered river and drinking water samples, 10 ml of distilled water was added to the vacuum filtration apparatus after the samples had been filtered but before the filter papers were removed. To this, Nile red and DAPI were added to each sample to yield the same respective concentrations as stated above. The samples were then left in the dark for 30 minutes before filtering the remaining liquid.

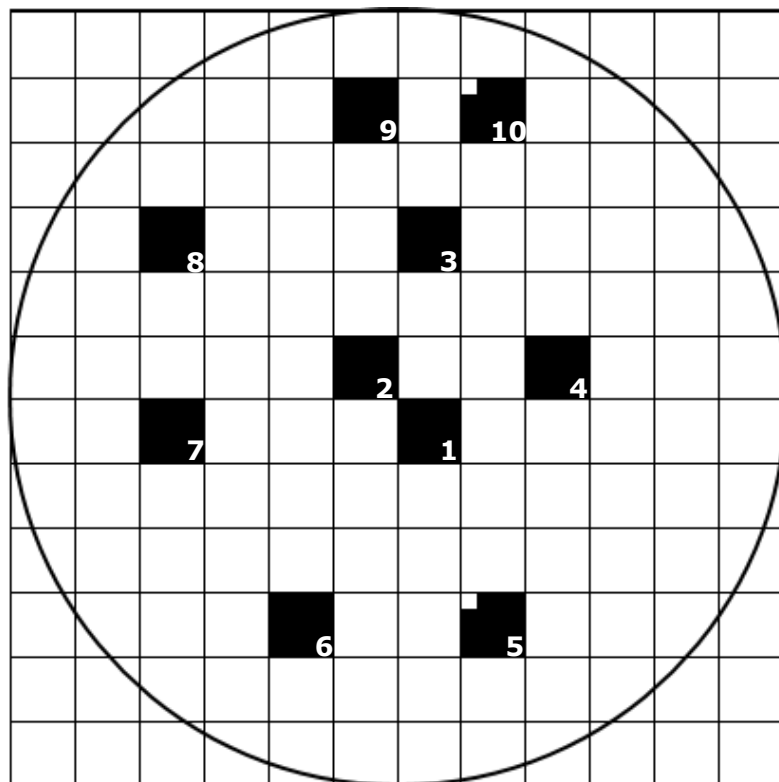
All filter papers were transferred to microscope slides and analysed immediately.

### **Visualisation**

Particle counts were conducted manually at 40x and 100x (total) magnification using a light microscope with a mercury vapour fluorescence illumination attachment (Euromex iScope, Euromex Microscopen B.V., Arnhem, The Netherlands). Nile red staining was observed in green fluorescence (excitation wavelength: 430-490 nm, emission wavelength: 510-560 nm) and DAPI staining was observed in blue fluorescence (excitation wavelength: 355-405 nm, emission wavelength: 420-480 nm).



For river and drinking water samples concentrated onto gridded filter papers, it was possible to standardize particle analysis across all samples. The same ten cells of the filter paper were analysed for each sample (Figure 5.3). All cells were analysed at 40x magnification, the lowest magnification possible given the configuration of the microscope. For each sample, two cells were also analysed at 100X magnification. Only fluorescent particles with a clearly defined edge were counted.



**Figure 5.3: The cells on the gridded filter papers that were observed at 40 x magnification in each sample. The two cells with the white square within them were also observed at 100x magnification.**

### **Autofluorescence**

Fluorescent dyes are not always the source of particle fluorescence. Some materials and organisms will fluoresce under certain wavelengths of light due to autofluorescence. The autofluorescence of a sample can be determined by observing it under the wavelengths of light specific to the dye being used without exposing samples to said dye. Autofluorescence in the wavelength

regions for Nile red (green) and DAPI (blue) was determined in this manner for all known plastic particles and natural textile fibres, as well as a replicate for each filtered sample of drinking water and the materials used for the caps and bodies of the drinking water samples.

#### **4.4. Results and Discussion**

##### **Nile Red staining of plastic fragments and fibres**

The validation of Nile Red as a technique for microplastic analysis has predominantly used white and translucent particles (Shim et al, 2016; Erni-Cassola et al.2017). Its ability to stain synthetic textile fibres has also been found to be limited (Wiggin and Holland, 2019) and it will not stain some microplastic particles, including tire rubber (Araujo et al. 2018; Hitchcock and Mitrovic, 2019). The autofluorescence of microplastic particles and natural textile fibres has not previously been reported in the validation of Nile red's application to microplastic research. In green fluorescence, only light blue wool and white PVC exhibited autofluorescence. In blue fluorescence, autofluorescence was noted in white cotton, light blue wool, red and grey polyamide, and polypropylene (Table 5.1).

**Table 5.1: Known plastic particles and natural and synthetic fibres, detailing their colour, autofluorescence at the green and blue wavelengths used to assess Nile red and DAPI staining respectively, and affinity with Nile Red and DAPI fluorescent stains where autofluorescence was not observed.**

Polymer	Colour	Autofluorescence		Nile red fluorescence?	DAPI fluorescence?
		Green	Blue		
PP	Transparent	No	No	Yes	No
	Orange	No	No	Yes	No
	Blue	No	Yes	Yes	-
	Red	No	No	Yes	No
	Black	No	No	Yes - at edges only	No
HDPE	Translucent	No	No	Yes	No
	Brown	No	No	Yes - at edges only	No
EPS	White	No	No	Yes	No
PVC	White	Yes	No	-	No
Polyester	Black	No	No	No	No
Polyamide	Red	No	Yes	No	-
	Grey	No	Yes	Yes	-
Acrylic	Blue	No	No	No	No
	Orange	No	No	Yes	No
Cotton	White	No	No	Yes	Yes
Wool	Light blue	Yes	Yes	-	-
	Dark blue	No	No	Yes	Yes
Silk	Blue	No	No	Yes	Yes
Rayon	Blue	No	No	Yes	Yes

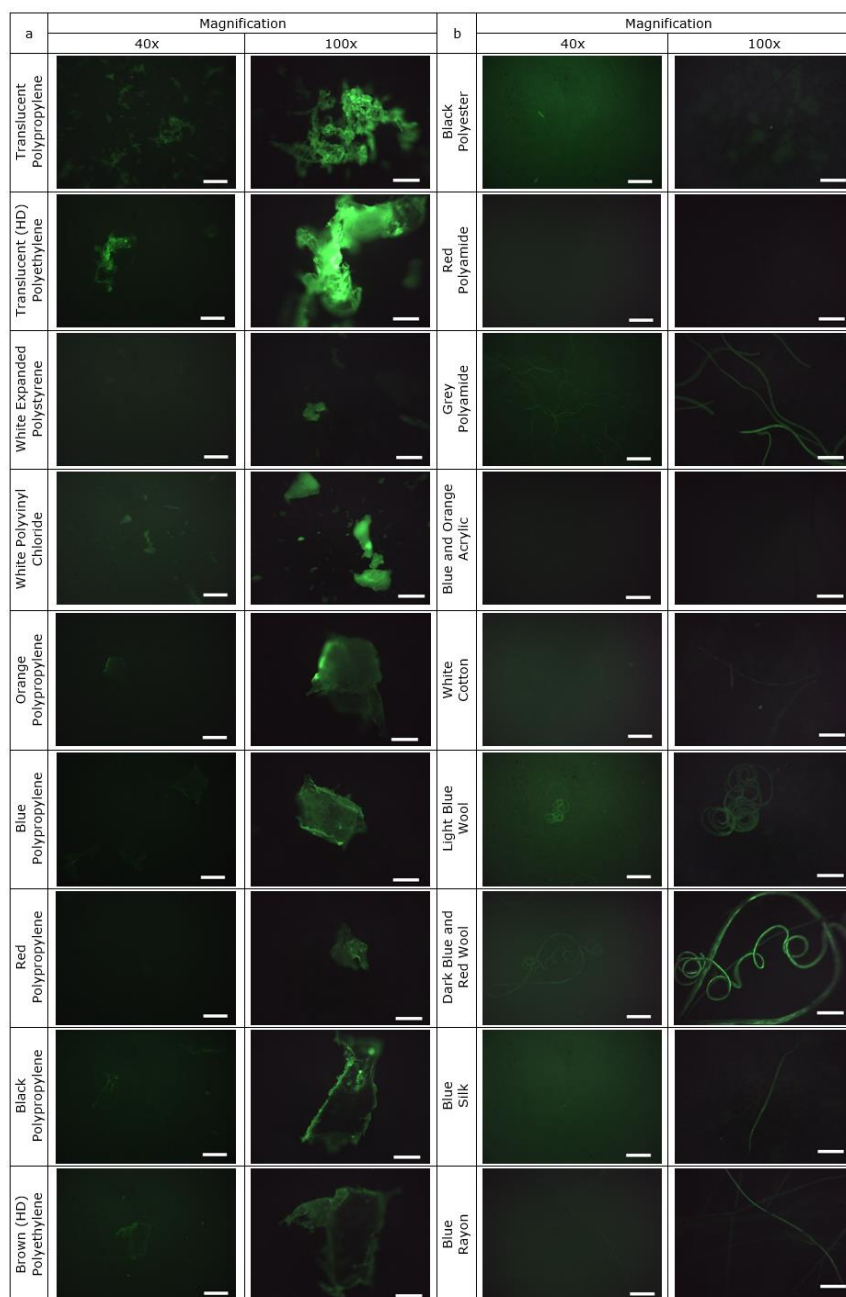
Following H<sub>2</sub>O<sub>2</sub> treatment, all white and transparent microplastic fragments were uniformly stained across their surface by Nile red (Figure 5.4). However, the staining of coloured fragments was not uniform. Brown HDPE and black PP were stained only around their edges and, though all coloured fragments fluoresced in some way at 100x magnification, their fluorescence at 40x magnification was less clear (Figure 5.4). Where staining is uneven, automated microplastic enumeration, using software such as ImageJ (Erni-Cassola et al. 2017), could lead to particle overestimation (Figure 5.4; Table 5.1).

The color of microplastic fragments identified by Nile red staining is not always reported (Fischer et al. 2016; Hengtsmann et al. 2018; Tamminga et al. 2018).

However, where it has been reported, proportions of white and colorless particles have been as high as 95% (Dobaradaran et al. 2018). These findings show that Nile red does not reliably stain all plastic particles, and that the presence of plastic dyes affects their affinity with Nile red. Numerous dyes can be used to stain plastics similar colors, and so it is possible that Nile red's affinity with particles of the same color and polymer will vary with the dye used. It is therefore not possible to speculate how much the use of Nile red has previously incorrectly quantified microplastics of different colors, it is possible that lighter colored particles have dominated previous microplastic studies that use Nile red alone.

Of the fibres assessed in the present study, grey polyamide fibres and some, but not all, orange acrylic fibres were stained by Nile red, however, Nile red did not stain black polyester, blue acrylic or red polyamide fibres (Figure 5.4). That no part of these synthetic textile fibres was stained indicates that the uneven staining of brown HDPE and black PP fragments may be due to the thinning, and therefore lightening, of fragment edges during their production.

Moreover, Erni-Cassola et al. (2017) state that following H<sub>2</sub>O<sub>2</sub> treatment natural particles do not fluoresce in green, and Wiggin and Holland (2019) report that natural and regenerated textile fibers are not stained by Nile red. However, here we show that even after H<sub>2</sub>O<sub>2</sub> treatment, cotton, wool, silk, and rayon, all exhibited varying levels of fluorescence (Figure 5.4). A basic understanding of textile fiber morphology can go some way to differentiating between natural and synthetic textile fibres (Stanton et al. 2019a), but this can be a time consuming exercise, negating one of the main benefits of Nile red in microplastic analysis.



**Figure 5.4: Nile red staining of H<sub>2</sub>O<sub>2</sub> treated microplastic fragments of known polymers, and known synthetic and non-synthetic textile fibers. Colors denote those of the particles in the field of view imaged. Scale bars represent 500  $\mu$ m and 200  $\mu$ m for 40x and 100x magnification respectively. Images of white PVC and light blue wool are a result of autofluorescence, not staining (Table 5.1).**

The fluorescence of wool and rayon fibres after Nile red staining is particularly limiting due to the morphological similarities they share with many synthetic

textile fibres, possessing largely uniform diameters similar to that of many synthetic textile fibres (Stanton et al. 2019a). Moreover, the smooth surface of rayon fibres, made from the extrusion of regenerated cellulose, bears a particularly close resemblance to synthetic textile fibres, which are also extruded (Greaves and Saville, 1995). These findings, question the efficacy of Nile red's application in the analysis of textile fibres all together. As expected, DAPI stained all natural fibres. Though plastic polymers of different colours are not stained by Nile red, it is beyond the scope of this study to quantify the extent to which particle counts will be underestimated given the heterogeneity of polymers and colours of microplastic particles that persist in the environment.

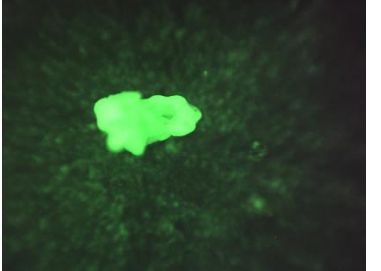
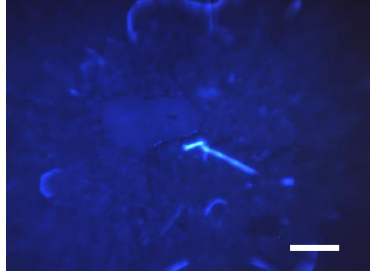
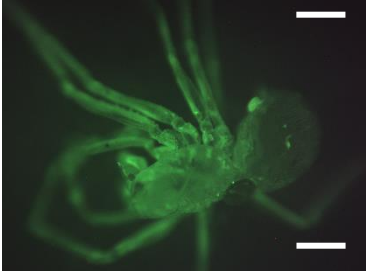
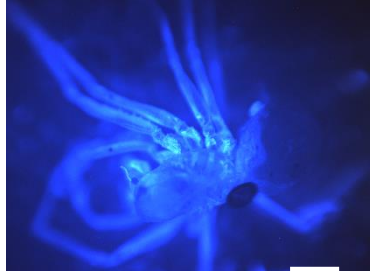
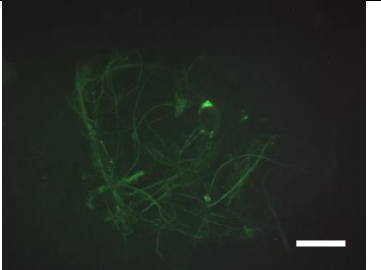
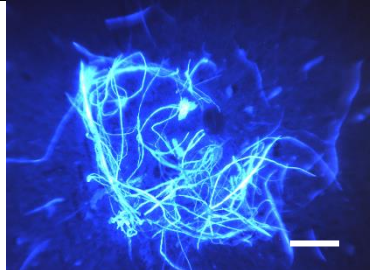
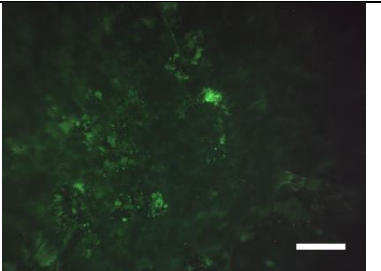
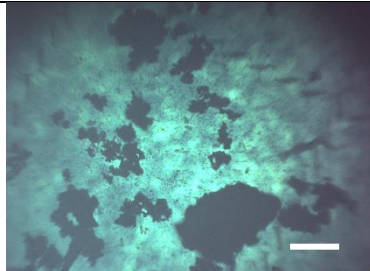
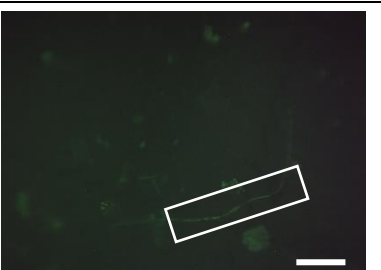
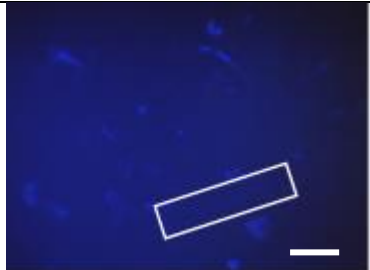
#### **Nile red and DAPI staining of natural particles in treated freshwater samples**

Though reportedly negligible (Maes et al. 2017), Nile red has been shown to stain natural, lipid containing, particles in environmental samples (Shim et al. 2016; Erni-Casolla et al. 2017). Even after H<sub>2</sub>O<sub>2</sub> treatment, material stained by both Nile red and DAPI was abundant in samples of river and lake water, and included diatoms and dinoflagellates (Figure 5.5). Because H<sub>2</sub>O<sub>2</sub> naturally occurs in aquatic environments, eukaryotic phytoplankton such as dinoflagellates produce peroxidase enzymes in order to counteract its damaging effects (Matthijs et al. 2012), which include cell lysis. They are therefore resistant to H<sub>2</sub>O<sub>2</sub> (Burson et al. 2014). These organisms can be identified by eye as with other biological indicators such as pollen, however, their presence in samples stained with Nile red could inflate particle abundance where analysts are not appropriately trained, or where automated particle counts are conducted.

Though plastics of some colours autofluoresced in the blue wavelengths of light used to observe DAPI fluorescence (Table 5.1), the majority did not. It was therefore possible to estimate the extent to which Nile red could overestimate microplastic counts using DAPI to identify particles of biological origin that are stained by Nile red.

Nile red significantly overestimated microplastic abundance in two of the three river water samples (Table 5.2). The median Nile red overestimation of microplastic abundance in river water was 48.4% (ranging from 10.8% to 66.67%) at 40x magnification. At 100x magnification this rose to 54.5% (ranging from 37.5% to 58.8%). Furthermore, 95% of fibers at 40x magnification, and 100% of fibers at 100x magnification fluoresced with both Nile red and DAPI. Particle counts for all samples are detailed in Table 5.2. Though particles stained by both Nile red and DAPI could have been autofluorescing (Table 5.1), it is unlikely that this accounted for all of the fluorescent particles. For example, though it autofluoresced under blue light, polyamide resins accounted for approximately just 2% of total plastic resin demand across the European Union in 2017 (Plastics Europe, 2018).

Across the river samples, multiple dark colored fragments exhibited inconsistent Nile red staining, and non-plastic particles were found to fluoresce (Figure 5.5). The results here cannot provide a universal estimate of false positive rates in environmental samples; however, they do illustrate considerable limitations to the use of a method of microplastic quantification that has not been appropriately validated.

Particle	Nile red image	DAPI or true colour image
Possible microplastic particle		
Invertebrate		
Bundle of fibres		
Black particles exhibiting uneven staining		
Natural textile fibre		

**Figure 5.5: Nile Red and DAPI images of natural and possible microplastic particles stained in samples of river water. All images taken at 40x magnification and the scale bar represents 500  $\mu\text{m}$ .**



**Table 5.2 (continued overleaf): Particle counts for Nile Red and DAPI fluorescence at x40 and x100 magnification across ten and two cells of the filter area respectively (Figure 5.3). Significant overestimations ( $p < 0.05$ ) were determined using a paired sample t-test for mean particle counts at x40 magnification, comparing counts for particles that fluoresced with Nile Red but not DAPI (i.e. suspected microplastics) and all particles that fluoresced with Nile red.**

Sample	Magnification	Particles that fluoresced with Nile Red	Particles that fluoresced with both Nile Red and DAPI	Suspected microplastics	Significance (p=)
River water 1	x40	33	22	11	0.003
River water 1	x100	17	10	7	
River water 2	x40	31	15	16	0.002
River water 2	x100	11	6	5	
River water 3	x40	74	8	68	0.087
River water 3	x100	16	6	10	
Bottled water 1	x40	14	12	2	0.005
Bottled water 1	x100	14	13	1	
Bottled water 2	x40	3	2	1	0.168
Bottled water 2	x100	5	3	2	
Bottled water 3	x40	9	9	0	0.019
Bottled water 3	x100	11	8	3	
Bottled water 4	x40	15	6	9	0.024
Bottled water 4	x100	18	10	8	
Canned water	x40	28	13	15	0.001

Canned water	x100	70	57	13	
Tap water	x40	24	18	6	0.001
Tap water	x100	20	12	8	

### **Microplastic particles in drinking water and tap water**

A small number of studies have identified microplastic particles in bottled water (Mason et al. 2018; Oßmann et al. 2018; Schymanski et al. 2018). Of these, Mason et al. (2018) relied solely on the use of Nile red in their analyses of particles <100 µm, reporting mean concentrations of 325 microplastic particles per liter of water. This finding influenced a World Health Organization review of bottled water (Readfearn, 2018) and garnered international media coverage. However, repeating this analysis using this co-staining approach highlighted considerable levels of error in the use of Nile red alone (Table 5.2).

In the six samples of drinking water to which Nile red and DAPI were added, Nile red significantly overestimated microplastic abundance in five samples (Table 5.2). Across these samples, Nile red's median microplastic overestimation was 66.7% (ranging from 40% to 100%) (Table 5.2), placing considerable doubt on the results reported by Mason et al. (2018) for particles <100 µm.

FTIR spectroscopy identified the materials used for the cap and bottle body of each plastic bottles, and the plastic clip used to reseal the aluminium can (Table 5.3). To determine whether or not any of the fluorescing particles in drinking water reported by Mason et al. (2018) and in the present study might have originated from the materials of the drinking water containers, each of these materials was also assessed for autofluorescence (Table 5.3). None of the cap materials exhibited autofluorescence. However, the translucent Polyethylene Terephthalate (PET) from which the bodies of all four plastic bottled drinking water samples were made did exhibit both green and blue autofluorescence. A second sample of each of the drinking water samples and the safe to drink tap water was therefore acquired and vacuum filtered in order to consider the possible contribution of autofluorescing PET particles to the Nile red particle counts from these samples. No dye was added to these samples. The presence of autofluorescent particles across the unstained filtered samples of drinking water was negligible (Table 5.4).

**Table 5.3: The material, colour, and autofluorescence of different parts of the containers of drinking water samples. The lid of bottled water 4 had two parts.**

Sample	Material	Colour	Autofluorescence		
			Green	Blue	
Bottled water 1	Lid	HDPE	Light blue	N	N
	Body	PET	Transparent	Y	Y
Bottled water 2	Lid	HDPE	Green	N	N
	Body	PET	Transparent	Y	Y
Bottled water 3	Lid	HDPE	Dark blue	N	N
	Body	PET	Transparent	Y	Y
Bottled water 4	Lid inner	HDPE	White	N	N
	Lid outer	PP	Dark blue	N	N
	Body	PET	Transparent	Y	Y
Canned water	Lid	Polypropylene	Black	N	N
	Body	Aluminium		N/A	

**Table 5.4: Abundance of autofluorescing particles across the six drinking water samples.**

Sample	Magnification	Autofluorescence	
		Green	Blue
Bottled water 1	x40	0	5
Bottled water 1	x100	0	1
Bottled water 2	x40	0	5
Bottled water 2	x100	0	0
Bottled water 3	x40	0	0
Bottled water 3	x100	0	0
Bottled water 4	x40	0	2
Bottled water 4	x100	0	2
Canned water	x40	2	4
Canned water	x100	1	2
Tap water	x40	0	8
Tap water	x100	0	3

In addition to bottled and canned water, Nile Red analysis of deionised and distilled water, identified particles that also fluoresced with Nile Red, and which might inflate microplastic particle abundances where Nile Red is used. In these samples, it was not possible to count the number of particles that fluoresced with both dyes at 100x magnification because they were so numerous. Therefore, these samples were analysed only at 40x magnification. The median overestimation of microplastic particles within deionised water samples using Nile Red alone was 38.5% (ranging from 25.3% to 47.8%). Of the three distilled water samples, one sample contained no particles that fluoresced with Nile Red. In the other two samples, Nile red overestimated microplastic abundance by 0% and 58.3%.

### **The future use of Nile red in microplastic analysis**

Accurate particle characterization underpins our understanding of the spatial and temporal distribution of microplastic pollution. This informs policy and influences both industry and public opinion. As Nile red's application to microplastic research has made global headlines, there is growing need for rigorous assessment of its application to microplastic quantification. Whilst Nile red's ability to mark certain types of microplastic particle has been repeatedly demonstrated, its validation has not accounted for the breadth of microplastic colors and polymers that are known to pollute the environment. Furthermore, the use of H<sub>2</sub>O<sub>2</sub> is shown here to not be effective in the removal of common biological material that can also be stained by Nile red. It is beyond the scope of this study to provide an exhaustive audit of Nile red's ability to identify different plastic types, and assess the breadth of natural particles that may give rise to false positives in different environmental matrices. Nevertheless, the extent of errors found in environmental and drinking water samples are indicative of the potential magnitude of such errors. As such, reliance on this approach in future studies should be discouraged without further development. This study has been published in Environmental Science and Technology Letters, and is cited as Stanton et al. (2019b) throughout this

thesis. A copy of this publication is appended in the 'Publications' section at the end of this thesis.

#### **4.5. Author Contributions**

T.S. conducted all field sampling, and sample preparation. T.S. processed and analysed all samples with the assistance of T.N. and A.B. All authors contributed to the interpretation of the presented data and the writing of the published manuscript.

## **5. Freshwater and Airborne Textile Fibre Populations are Dominated by ‘Natural’, not Microplastic, Fibres**

Thomas Stanton; Matthew Johnson; Paul Nathanail; William MacNaughtan;  
Rachel L Gomes

### **5.1. Abstract**

The potential role of natural textile fibres as environmental pollutants has been speculated upon by some environmental scientists, however, there is a general consensus that their biodegradability reduces their environmental threat. Whilst the risks that they pose remain poorly understood, their environmental prevalence has been noted in several recent microplastic pollution manuscripts. Here we highlight the extent to which natural textile fibres dominate fibre populations of upstream reaches of the River Trent, UK, as well as the atmospheric deposition within its catchment, over a twelvemonth microplastic sampling campaign. Across 223 samples, natural textile fibres represented 93.8% of the textile fibre population quantified. Moreover, though microplastic particles including synthetic fibres are known to be pervasive environmental pollutants, extruded textile fibres were absent from 82.8% of samples. Natural textile fibres were absent from just 9.7% of samples.

## 5.2. Introduction

Mismanaged plastic waste is known to exert a variety of pressures on the environment. As awareness of these pressures has grown, efforts have been made to reduce plastic consumption by industry, governments and the general public, including the increased use of plastic alternatives. However, the potential environmental impacts of plastic alternatives are seldom considered in an environmental discourse that is currently so concerned with plastic waste. In 2015, Ladewig et al. (2015) highlighted the potential environmental threat of one such alternative for plastic textile fibres: natural textile fibres. Natural textile fibres, such as cotton and wool, are the product of multiple environmentally hazardous anthropogenic processes and are, therefore, inherently unnatural. For example, the commercial production of cotton fibres requires large volumes of water, pesticides and herbicides (Suran, 2018). The wastewaters of the textile industry have also long been recognised as point sources of chemical pollutants (Correia et al. 1994).

Unlike microplastic textile fibres, natural textile fibres have received little environmental attention. Fibres have the potential to entangle the gut contents of organisms that ingest them (Lusher et al. 2013), and any chemical effects of fibres are exacerbated by the relatively large surface area to volume ratio that they possess. The propensity for organic pollutants to adsorb to the surface of microplastic particles has been previously reported (Bakir et al. 2014), however, the extent to which this is true of natural textile fibres is currently poorly understood. Nevertheless, the faster degradation of natural textile fibres in comparison to microplastic fibres is a potential route for the release of toxic compounds, including dyes, into the environment (Ladewig et al. 2015).

The prevalence of natural textile fibres alongside synthetic textile fibres and microplastic fragments in the gastrointestinal tract of terrestrial birds was reported by Zhao et al. (2016), and in invertebrates by Remy et al. (2015). Dris et al. also acknowledge the presence of natural and synthetic textile fibres in atmospheric deposition (Dris et al. 2016; 2017), as well as the River Seine and



one of its tributaries (Dris et al. 2018). However, since Ladewig et al. (2015), few other publications have acknowledged the potential environmental significance of natural textile fibres.

Though natural textile fibres are underrepresented in environmental literature, in the field of forensic science the relative proportions of textile fibres of different type and colour have been reported on a number of anthropogenic surfaces (Table 6.1). This work consistently records higher abundances of natural textile fibres in comparison to synthetic textile fibres. The findings of these studies are not necessarily representative of environmental matrices, but provide further evidence of the environmental prevalence of natural textile fibres.

**Table 6.1: Prevalence of natural textile fibres in some forensic science and microplastic publications. NS corresponds to information that is not stated.**

<b>Study</b>	<b>Field</b>	<b>Environment</b>	<b>Natural fibres (%)</b>	<b>Fibres Analysed</b>
Kelly and Griffin (1998)	Forensic Science	Public house seats	89.72	292
Cantrell et al. (2001)	Forensic Science	Cinema seats	84	3025
Cook et al. (1997)	Forensic Science	Human head hair	56.7	37
Palmer and Oliver (2004)	Forensic Science	Human head hair	72.3	>12 000
Watt et al. (2005)	Forensic Science	Washing machines	75	12 178
Dris et al. (2016)	Microplastics	Indoor air	67	NS
Dris et al. (2017)	Microplastics	Outdoor air	50	NS
Cai et al. (2017)	Microplastics	Outdoor air	73	NS
Zhao et al. (2016)	Microplastics	Digestive tracts of terrestrial birds from Shanghai	40.5	336

In the study of microplastic pollution, determining the proportion of a fibre population that is synthetic has been restricted by the methodologies used to analyse textile fibres. The visual identification of microplastic particles is a widely used and acceptable technique when supported by the chemical analysis of a subsample of particles (Lusher et al. 2017). However, within the study of microplastic pollution, the visual identification of synthetic textile fibres in particular has been criticised for its susceptibility to human error (Remy et al. 2015), despite being recognised as an important initial step in the classification of textile fibres to their main groups (Greaves and Saville, 1995; Nayak et al. 2012).

One common method of determining the chemical composition of microplastic particles, possessing the capability to conclusively identify synthetic polymers, is Fourier Transform Infrared (FTIR) spectroscopy. However, the difficulties of obtaining clear FTIR spectra from the small, often curved, surfaces of textile fibres is a limitation of FTIR spectroscopy that some have not been able to overcome. Microplastic surveys have, in the past, chosen to omit textile fibres from their study entirely (e.g. Foekema et al., 2013; Van Cauwenberghe et al., 2015), or to apply an analytical technique, such as FTIR spectroscopy, to only a small subsample of observed fibres and extrapolate from the identities of the fibres that could be chemically analysed (e.g. Dris et al. 2016).

Understanding the relative environmental concentrations of different types of textile fibre will facilitate a more critical consideration of the environmental impacts of textile fibres as a whole. Three broad categories of textile fibre are commonly used in the textile industry: 1. natural fibres derived from the processing of plant fibres, such as cotton, and animal fibres, such as wool; 2. regenerated fibres, such as rayon, which are reconstituted from the dissolved cellulose of plant materials and shaped into fibres by extrusion; and 3. synthetic fibres, formed by the extrusion of petrochemical based compounds. There are key visual distinctions, beyond those frequently used in the study of microplastic pollution, that differentiate between natural textile fibres and those formed by extrusion. The structures and formation of fibres in each of

these three categories, including the extrusion process, are described in detail in Greaves and Saville (1995) and Hearle (2009).

By exploiting these visual characteristics in the study of microplastic pollution it is possible to categorise textile fibres as either natural or extruded using simple stereomicroscopy. This more accurate preliminary characterisation of textile fibres will enable the consideration of textile fibres in microplastic studies where suitable analytical techniques are not available, and will reduce the number of fibres in need of subsequent spectroscopic identification where they are.

Synthetic textile fibres have received considerable negative press in the reporting of microplastic pollution. Though the potential role of natural textile fibres as environmental pollutants has been speculated upon by some environmental scientists, there is a general consensus that their biodegradability reduces their environmental threat. Whilst the risks that they pose remain poorly understood, their environmental prevalence has been noted in several recent microplastic pollution manuscripts. Therefore, though textile fibres represent only one type of fibre use, a thorough and accurate understanding of the threats that textile fibres of all types pose, which is not reliant on extensive extrapolation, is of great social and environmental importance. With the aim of placing synthetic textile fibre pollution into a broader environmental context of anthropogenic particulate pollutants, this study expands existing criteria for the visual categorisation of textile fibres used in microplastic quantification to quantify the textile fibre population of 223 samples of river water and atmospheric deposition from 14 sites across the River Trent Catchment, UK. These findings provide strong support for the concerns detailed by Ladewig et al. (2015).

### **5.3. Materials and Methods**

#### **Site descriptions**

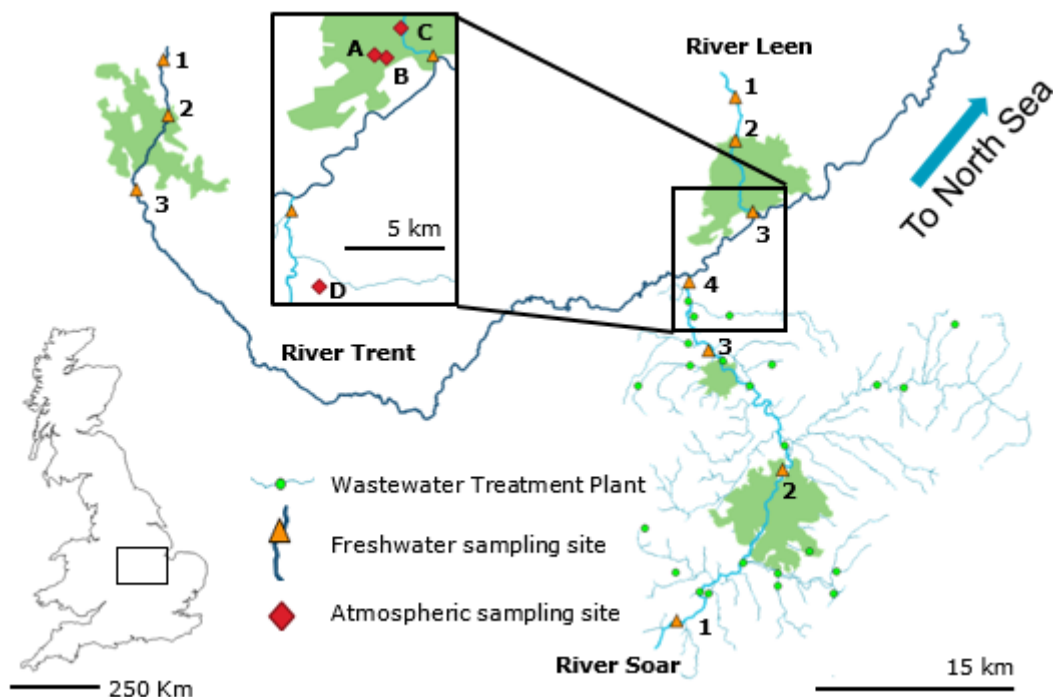
Surface water samples were collected from 10 sites in the Trent catchment; three on the River Trent (RT), three on the River Leen (RL) and four on the River

Soar (RS) (Figure 6.1). The location of these sites enabled the consideration of textile fibre concentrations near the sources of each river (RT1, RL1, RS1), immediately upstream (RT2, RL2) and immediately downstream (RT3, RL3, RS2, RS3) of urban population centres, and at sites that do (RS2-44) and do not (RT 1-3, RL 1-3, and RS1) receive wastewater treatment plant effluent, a known source of synthetic fibres (Leslie et al. 2017).

Atmospheric fallout was collected from the roofs of four buildings across the University of Nottingham's (UoN's) three UK teaching campuses (Figure 6.1). The UoN's University Park (UP) Campus is a 300 acre plot bordered on its south side by the approximately 50 acre Highfields Park and on its north by the approximately 500 acre Wollaton Hall Deer Park. Sites A and B are located on UP. Site A is located on the roof of one of University of Nottingham's main teaching buildings, which neighbours the central administrative building. It also spans a primary thoroughfare across the University Park Campus. The location of site B, on the roof of the University Of Nottingham's main gymnasium, is surrounded by student accommodation.

Approximately 650 m from UP, the UoN's 65 acre Jubilee Campus (JC) is surrounded by residential housing. Site C is located on JC. Site D is located on the UoN's 100 acre Sutton Bonington Campus. Approximately 12 km south of UP, Sutton Bonington lies close to the rural border of Nottinghamshire and Leicestershire.

Access to the roofs is restricted to maintenance staff only, and clear signage stressed the importance to maintenance staff of staying clear of sampling apparatus at each site.



**Figure 6.1: Locations of freshwater (numbered) and atmospheric (lettered) sampling sites within the Trent Catchment, UK. Green areas represent the urban areas of Stoke-on-Trent (River Trent), Nottingham (River Leen), Leicester (River Soar upstream) and Loughborough (River Soar downstream).**

### Sample collection

From the bank of the river at each freshwater sampling site, a 2 L paint kettle attached to a 5 m telescopic metal pole was used to retrieve 30 L of surface water. The water was concentrated in the field by passing it through a 63  $\mu\text{m}$  sieve, removing the suspended silt and clay fractions of the suspended solids within the sample. The residue retained on the sieve was washed into a 200 ml glass bottle using distilled water. As the lids of the bottles were plastic, each lid was lined with aluminium foil that was replaced for each sampling occasion. Samples were collected every four weeks over a 12 month period.

Samples of atmospheric fallout were collected fortnightly using an approach similar to that of Dris et al. (2016). The sampling apparatus consisted of a 2.5 L amber glass bottle, into which fallout was funnelled by a 12 cm diameter ( $0.0113 \text{ m}^2$ ) glass funnel. Each fortnight the glass funnel was thoroughly rinsed with distilled water, ensuring its entire surface was rinsed, before replacing the

amber glass bottle. Samples were collected over the same 12 month period as freshwater samples.

### **Sample processing**

All freshwater samples were treated with hydrogen peroxide ( $H_2O_2$ ) in order to digest organic material within the sample. The use of varying concentrations of  $H_2O_2$  in the digestion of organic matter is common in the study of microplastic pollution, including 15% (Zhao et al. 2016) 30% (Liebezeit and Dubaish, 2012; Mathalon and Hill, 2014; Tagg et al. 2015) and 35% (Mintenig et al. 2017), and its effect on the appearance of plastic particles has been documented by Nuelle et al. (2014). In this study, 30%  $H_2O_2$  was added to each aqueous sample. The sample was heated to 75°C for 4-5 hours. Initially, 100 ml of  $H_2O_2$  was added to each sample, however, during this stage of the third sampling occasion (week commencing 15/01/2018), the lids of the sample bottles perished exposing six of the samples to laboratory contamination. These six samples were therefore discarded. It is thought that the volume of  $H_2O_2$  used and the unusually high organic matter content of these samples, collected during a period of heavy rain, contributed to this. As a result, after sample occasion three the volume of  $H_2O_2$  added to each sample was reduced to 50 ml, and the lids of the glass jars were lined with two layers of aluminium foil. Moreover, just 15 L was collected during the fifth sampling occasion (week commencing 12/03/2018), falling during another period of heavy rain, to minimise the likelihood of sample bottles perishing.

Following  $H_2O_2$  digestion samples were, where necessary, once again passed through a 63  $\mu m$  sieve in order to remove any particles of silt and clay derived from the disaggregation of sedimentary agglomerations during the  $H_2O_2$  digestion. The retained residue was washed back into its respective sample bottle in the same manner as in the field. Millipore filtration apparatus was then used to vacuum filter samples through 0.45  $\mu m$  mixed cellulose ester gridded filter papers (Whatman ME 25/41) following the standard vacuum filtration procedures for  $H_2O_2$  of three distilled water washes. The sample bottle and the sides of the vacuum filtration glassware were then rinsed using

a distilled water wash bottle to ensure no particles remained adhered to the glassware, and the filter paper was immediately sealed in a plastic Petri dish.

In order to reduce the volume of collected rainfall, samples of atmospheric deposition were first shaken vigorously to ensure all particles were in suspension before being passed through a 38  $\mu\text{m}$  sieve (step one). 300 ml of distilled water was then added to the empty amber glass bottle, which was shaken vigorously to entrain any remaining particles, after which it was passed through the same 38  $\mu\text{m}$  sieve. This was done three times in succession (step two). The residue retained on the sieve from steps one and two was then washed into a 50 ml glass beaker using distilled water. Each sample was then vacuum filtered through the same mixed cellulose filter papers used for the freshwater samples, with the 50 ml beaker and sides of the vacuum filtration apparatus being rinsed using a distilled water wash bottle before the filter paper was sealed in a plastic petri dish.

The significance of the relative proportions of natural and synthetic textile fibres was assessed using a Wilcoxon test.

### **Contamination control**

At each freshwater site, prior to freshwater sample collection, the paint kettle was submerged and emptied three times. It was then used to reverse wash the 63  $\mu\text{m}$  sieve three times. The inside of the sieve was then rinsed with distilled water from a wash bottle ensuring the entire mesh and sides had been rinsed. On two occasions procedural blanks were collected for which this wash bottle rinse was collected and treated in the same manner as the environmental samples to assess the efficacy of these three steps.

A total of 8 amber glass bottles were used in the collection of atmospheric deposition, four of the bottles being rotated each fortnight. In addition to the three 300 ml distilled water washes that each sample received during sample processing, a further three distilled water washes were performed before a bottle was placed on the roof. To assess the efficacy of these steps, procedural blanks were collected for which the surfaces of the 38  $\mu\text{m}$  sieve were washed

into a glass beaker and processed in the same manner as the environmental samples. This was done in triplicate.

Unlike in the study of microplastics, wearing only natural textile fibred clothing was not a sufficient measure to limit contamination during sample collection. Instead, the type of fibre and colour of the garments worn during sample collection was recorded so that it could be considered during sample analysis. During sample collection tightly woven synthetic waterproof garments were worn. During sample processing a PVC apron was worn over a polyester / cotton blend laboratory coat to minimise the contamination of samples by fibres that had settled on the laboratory coat, and white / translucent fibres were excluded from analysis as these were assumed to have been sourced from the laboratory coat during sample processing.

Prior to sample processing, all laboratory surfaces were wiped down with dampened paper towels to remove surface dust. All glassware, including the freshwater sample bottles, and the sieves, were rinsed with distilled water prior to coming into contact with the samples. Sample bottles and beakers were triple washed with distilled water before being rinsed with distilled water using a wash bottle. All glassware components of the vacuum filtration apparatus, except for the conical flask into which the liquid fraction of the sample is sucked, were thoroughly rinsed with distilled water using a wash bottle, ensuring the entire surface was disturbed by the distilled water. The sieves were reverse rinsed with distilled water and the mesh and sides were then rinsed with distilled water using a wash bottle as done in the field. The filter papers were also rinsed with distilled water prior to being used.

During sample processing, the glassware and sieves were all covered with aluminium foil except for when the samples were being sieved, transferred between receptacles, or when the freshwater sample bottles, glass beakers containing atmospheric samples, or vacuum filtration apparatus were being rinsed. When the samples were not covered with aluminium foil, a dampened filter paper, placed in a petri dish, was exposed to the laboratory air to monitor the deposition of fibres over the same period of time that the samples were



exposed. One dampened filter paper was used for each batch of samples processed (1 batch = ten freshwater samples or four atmospheric samples) to measure the total deposition of airborne fibres within the laboratory during the processing of each batch. Furthermore, with only the sample processor was permitted within the laboratory whenever samples were being processed.

### **Textile fibre characterisation**

#### **FTIR spectroscopy of textiles**

In order to assess the suitability of FTIR spectroscopy in the analysis of environmental textile fibre populations, an FTIR library of seven common textile fibres was produced using a combination of Attenuated Total Reflectance (ATR) FTIR and reflectance FTIR spectroscopy. This library consisted of FTIR spectra for: acrylic, cotton, polyamide, polyester, polypropylene, silk and wool textiles.

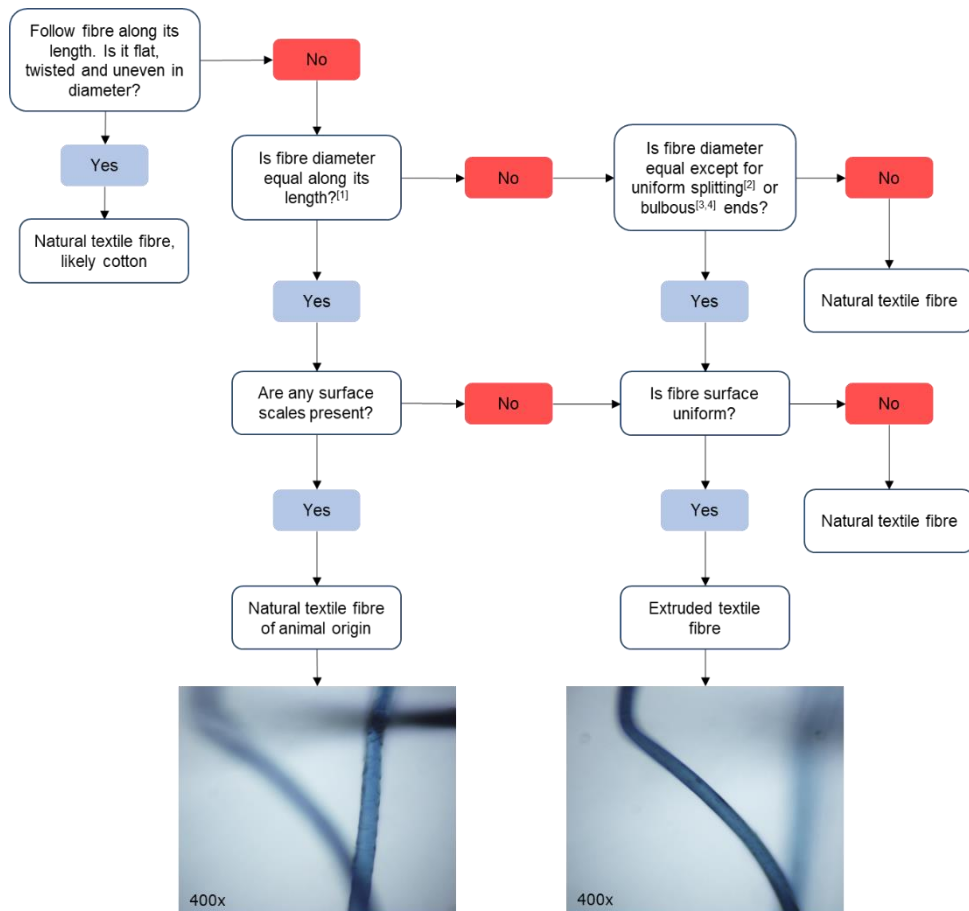
In the production of this library, garment patches were first analysed by ATR-FTIR spectroscopy using a Bruker Tensor 27 FTIR spectrometer (Bruker Optics, Coventry, UK, equipped with a Graseby-Specac Golden Gate ATR accessory (Orpington, UK). For each spectrum, 16 scans with a  $4\text{ cm}^{-1}$  resolution were co-added, providing FTIR spectra of the high fibre density samples. A pill of fibres was then pulled from each garment and analysed using the same technique, producing an ATR spectrum for a sample of lower fibre density.

Reflectance FTIR spectroscopy was then used to produce spectra of an individual fibre from each garment type using a Bruker Hyperion 2000 FTIR microscope (Bruker Optics, Coventry, UK). For each spectrum, 128 scans with a  $4\text{ cm}^{-1}$  resolution were co-added. These fibres were too small for analysis by ATR-FTIR spectroscopy.

#### **Visual analysis of textile fibres**

Samples were observed under a stereomicroscope (Medline Scientific CETI Varizoom-10, Chalgrove, UK) with a magnification range of 16-160 x and, where greater optical clarity was required, an optical microscope at 100 x magnification (Euromex Bioblue, Arnhem, The Netherlands). Every textile fibre

was categorised as either extruded or natural on the understanding that only synthetic textile fibres (e.g. polyester) and regenerated fibres (e.g. rayon) are manufactured by extrusion, whereby a molten polymer is forced through an aperture of fixed – and not necessarily circular – cross-section. The resultant individual fibres therefore have uniform diameter. Prior to sample analysis, the proficiency of the textile fibre analyst was developed through the extensive observation of textile fibres of known origin, aided by a literature-informed expansion of the criteria for visual textile fibre characterisation of the Royal Microscopical Society's *Microscopy of Textile Fibres* Handbook (Greaves and Saville, 1995) (Figure 6.2). These criteria were applied to the analysis of all environmental samples. Four textile fibres identified using the visual methodology outlined above were analysed by reflectance FTIR spectroscopy and compared to the FTIR library generated from known textile fibres.



**Figure 6.2: Flowchart used to characterise textile fibres as natural or extruded, with photographs highlighting the subtle differences between an animal (wool) and an extruded (polyester) fibre. Except where stated, these criteria have been adapted from the Royal Microscopical Society’s Microscopy of Textile Fibres handbook (Greaves and Saville, 1995).**

**[1] Norén (2007), [2] MERI (n.d.), [3] Wąs-Gubała and Krauß (2006), [4] Palenik et al. (2013).**

## **5.4. Results**

### **FTIR spectroscopy of textile patches, fibre pills and individual fibres**

FTIR spectroscopy of textile patches and fibre pills produced clear spectra (Figure 6.3) that, for the synthetic textile fibres analysed, could be identified by the available Bruker spectral library. However, the library available to this study was limited, being only a demonstration library, and was not able to identify the ATR-FTIR spectra generated from natural textile fibres. In contrast to the ATR-FTIR spectra, the spectra produced by reflectance FTIR spectroscopy were noisy (Figure 6.3). The identity of the four environmental fibres that were analysed by reflectance-FTIR spectra could not be ascertained from the spectra in Figure 6.3 due to this noise (Figure 6.4).

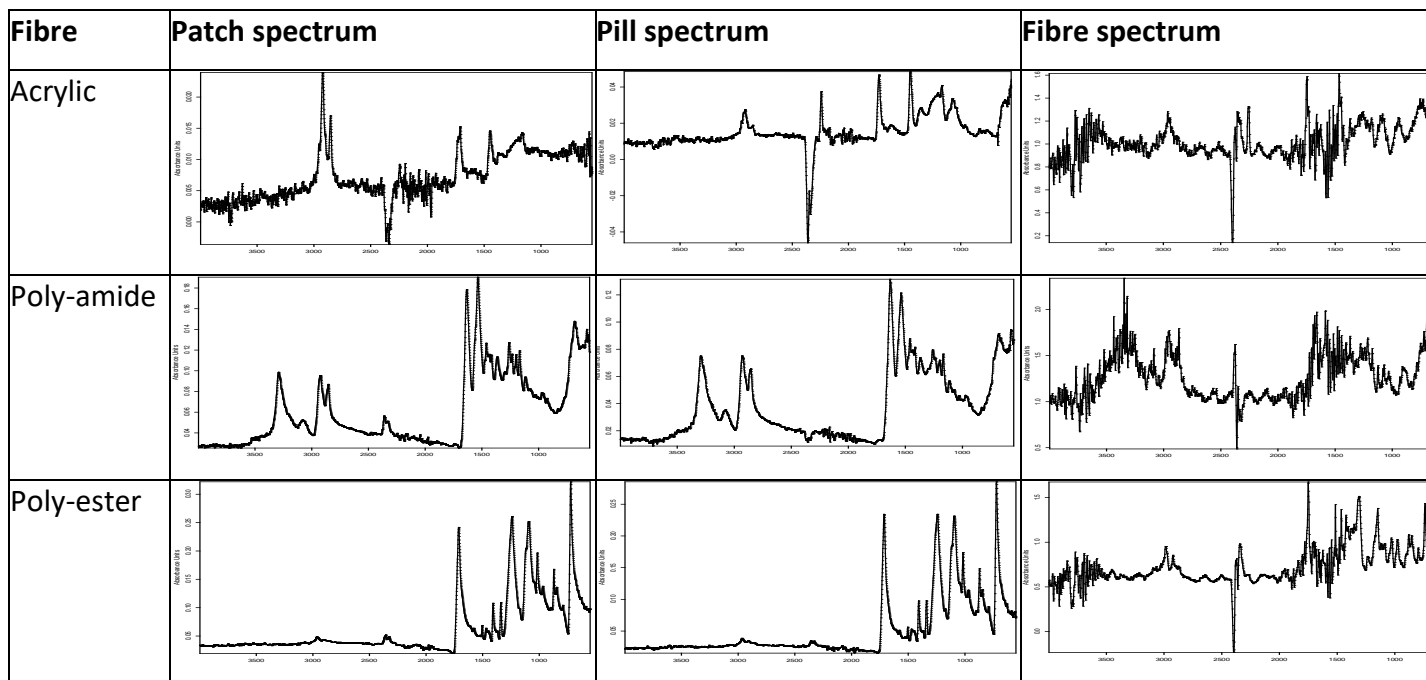
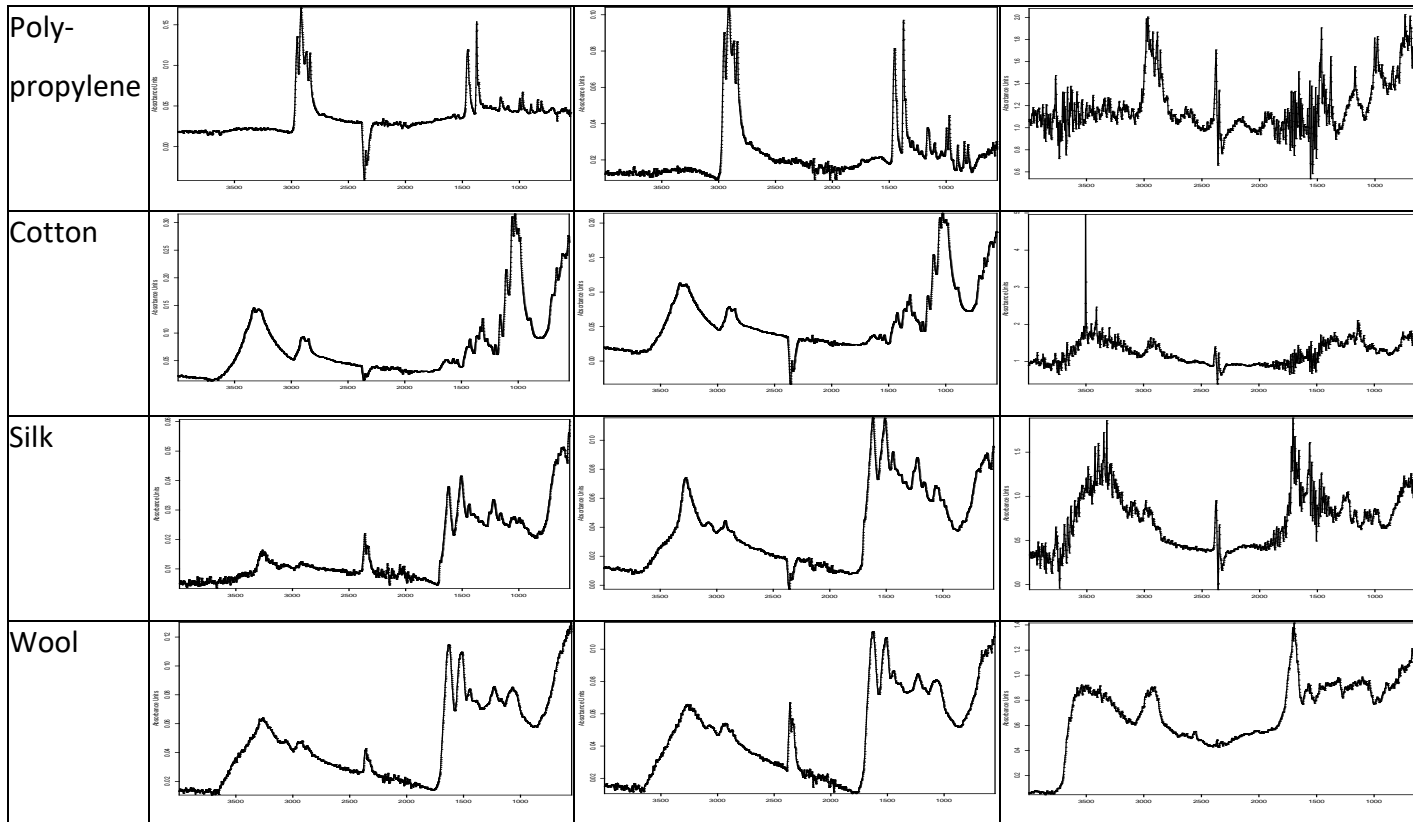
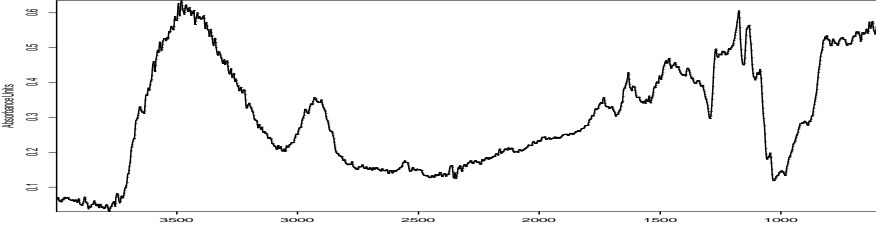
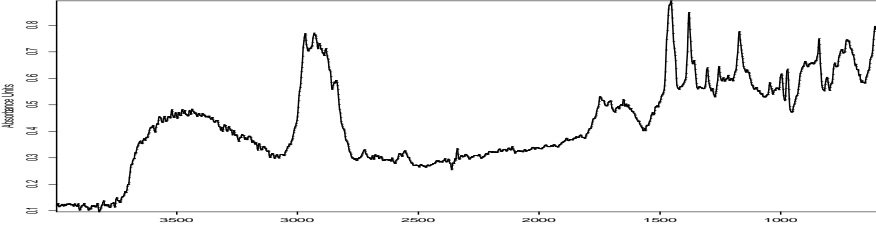
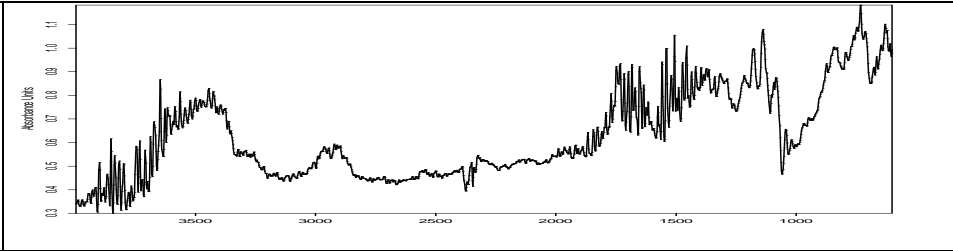
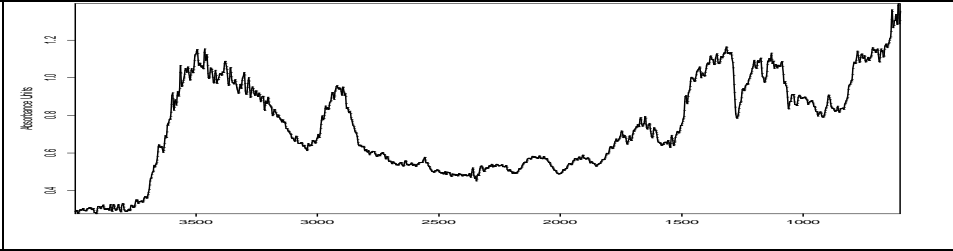


Figure 6.3 (continued overleaf): Library produced from the ATR-FTIR spectra of textile patches and pills and reflectance FTIR spectra of individual fibres from garments of known fibre composition. For each spectrum, the x axis represents the wavelength and the y axis represents absorbance.



Fibre ID	Visual categorisation	Reflectance spectrum
A	Natural	
B	Natural	

**Figure 6.4 (continued overleaf): Reflectance FTIR spectra of four fibres quantified from the 12 month sampling campaign. For each spectrum, the x axis represents the wavelength and the y axis represents absorbance.**

C	Extruded	
D	Extruded	



### **Freshwater and atmospheric textile fibre populations**

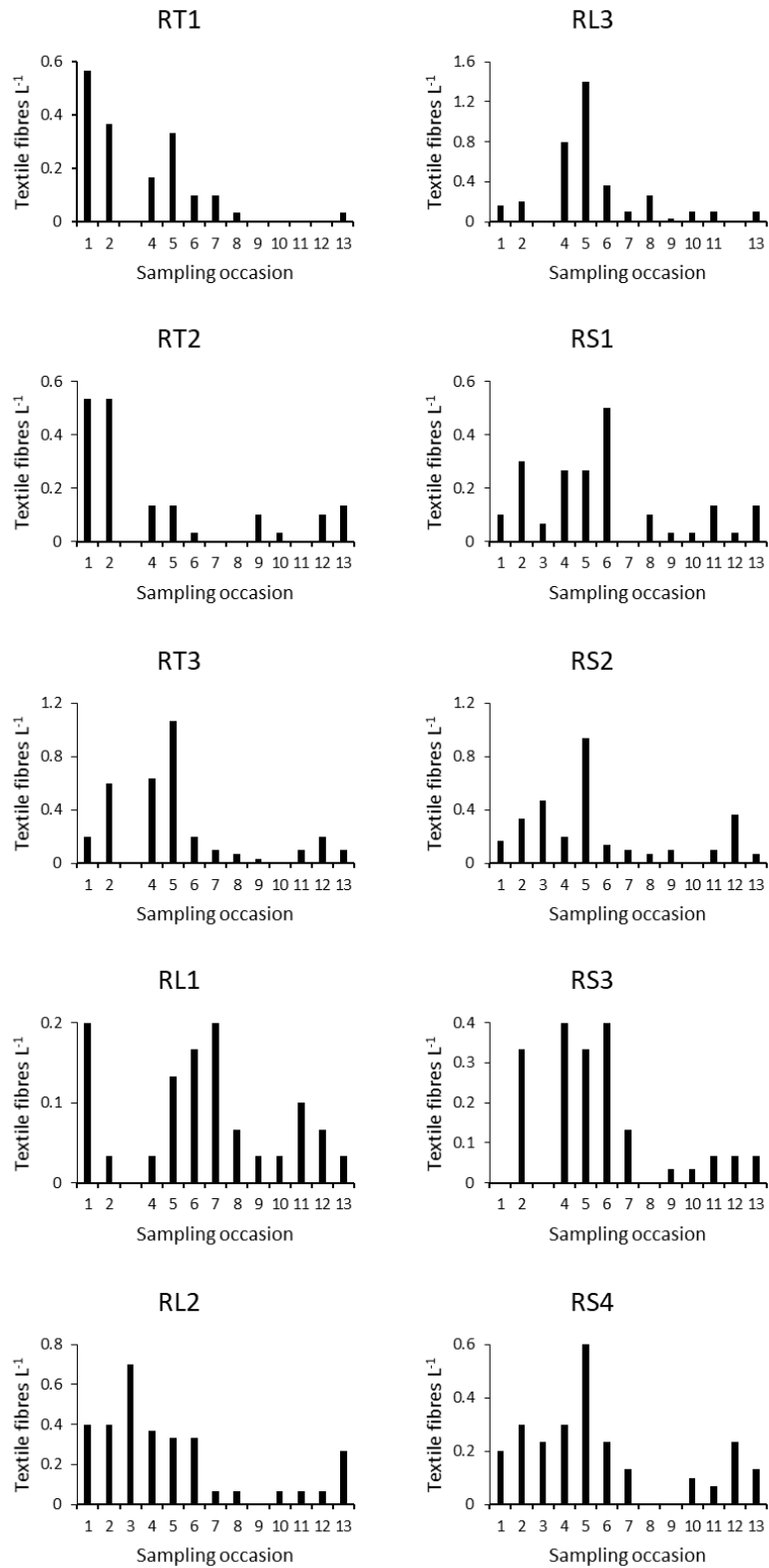
A total of 130 freshwater and 93 atmospheric samples were collected for microplastic and textile fibre analysis between 09/11/2017 and 31/07/2018. 720 fibres were categorised in the freshwater environment, 639 (87.3%) of which were identified as natural (Appendix 4.1). In atmospheric fallout, 1100 fibres were categorised of which 1075 (97.7%) were identified as natural (Appendix 4.2).

In the context of microplastic pollution, even if all of the extruded textile fibres were petrochemical-based, they made up just 6.2% of the total textile fibre population across all of the atmospheric and freshwater samples. The majority of textile fibres observed were either black/grey (47.09%, n=857) or blue (24.40%, n=444) in colour, as reported in multiple forensic textile fibre population surveys (e.g. Kelly and Griffin, 1998; Cook et al. 1997; Cantrell et al. 2001; Palmer and Oliver 2004; Watt et al. 2005). The absence of extruded textile fibres showing signs of degradation or bleaching support the findings of Nuelle et al. (2014), who demonstrate only limited effects of H<sub>2</sub>O<sub>2</sub> on plastic polymers including polyamide, from which nylon is derived, and Polyethylene Terephthalate, a common form of polyester. The effect of H<sub>2</sub>O<sub>2</sub> on natural textile fibres is expected to be limited. H<sub>2</sub>O<sub>2</sub> is a common bleaching agent used in the textile industry (Carmen and Daniela, 2012), and so the presence of natural textile fibres in such high abundance, of multiple types and in a variety of colours indicates that the H<sub>2</sub>O<sub>2</sub> concentrations used were too low to cause even the discolouration of textile fibres.

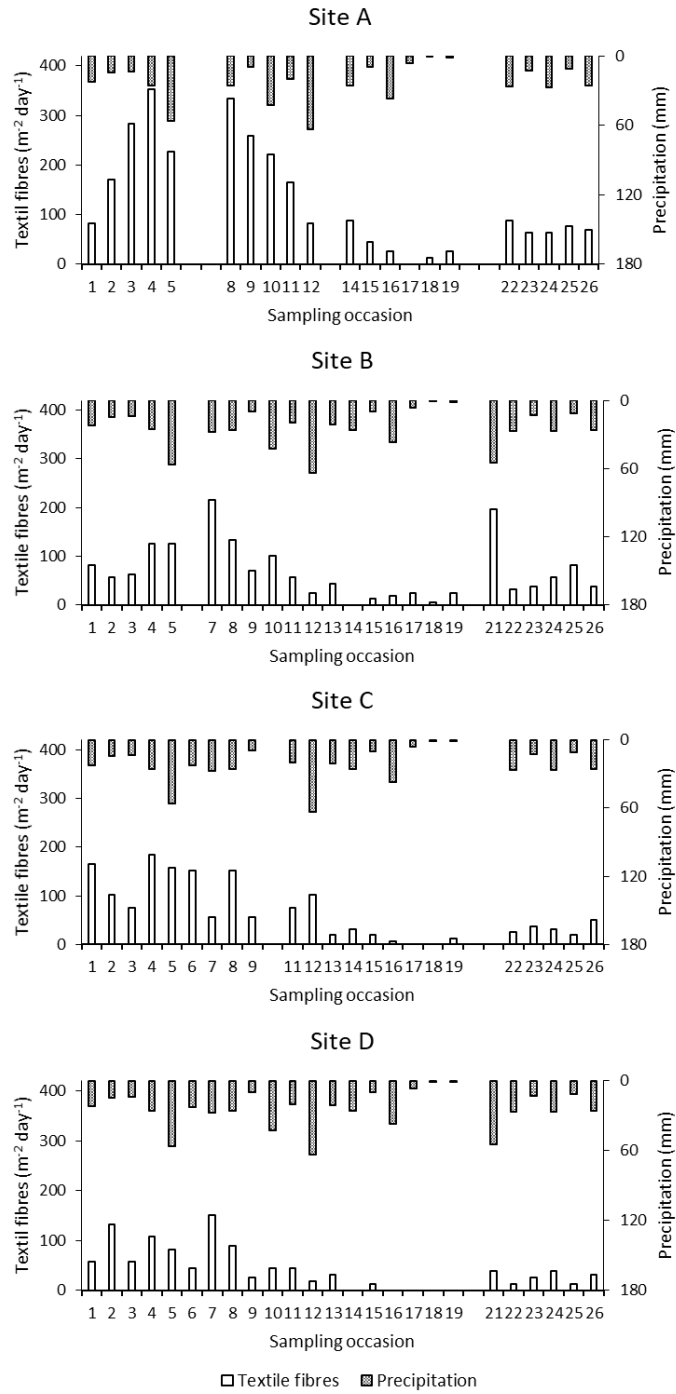
Textile fibre abundance varied through space and time in both freshwater (Figure 6.5) and atmospheric samples (Figure 6.6). Throughout the freshwater sampling campaign, site RL3 had the highest mean freshwater concentration of natural fibres ( $\bar{x} = 0.29$  fibres L<sup>-1</sup>) (Figure 6.7), whilst the highest mean extruded fibre concentration was observed at site RT3 ( $\bar{x} = 0.04$  fibres L<sup>-1</sup>) (Figure 6.8). Neither of these sites is in receipt of wastewater treatment plant effluent, but they are downstream of the cities of Nottingham and Stoke-on-Trent respectively. In atmospheric fallout, the highest mean natural textile

fibre deposition was observed at Site A ( $\bar{x} = 128.42$  fibres  $\text{m}^{-2}\text{day}^{-1}$ ), with the highest extruded textile fibre deposition observed at Site B ( $\bar{x} = 2.90$  fibres  $\text{m}^{-2}\text{day}^{-1}$ ) (Figure 6.9). In contrast to previous surveys of the atmospheric deposition of microplastics, the present study did not observe a correlation between fibre deposition and precipitation (Figure 6.10). In each of the rivers and across the sites of atmospheric deposition there were significantly more natural textile fibres than extruded fibres. Monte Carlo significance values for the Wilcoxon tests conducted in each of these datasets were all  $<0.001$ .

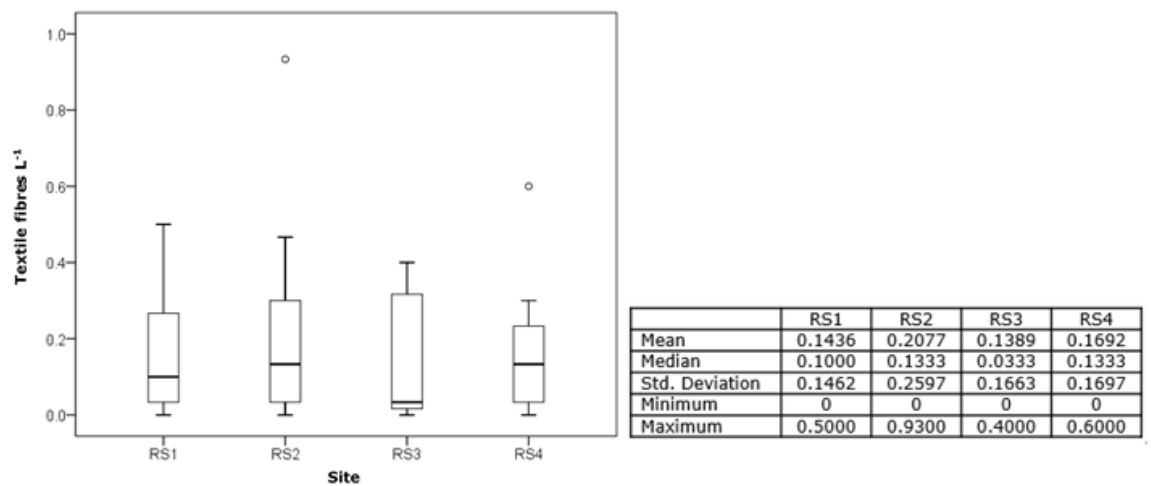
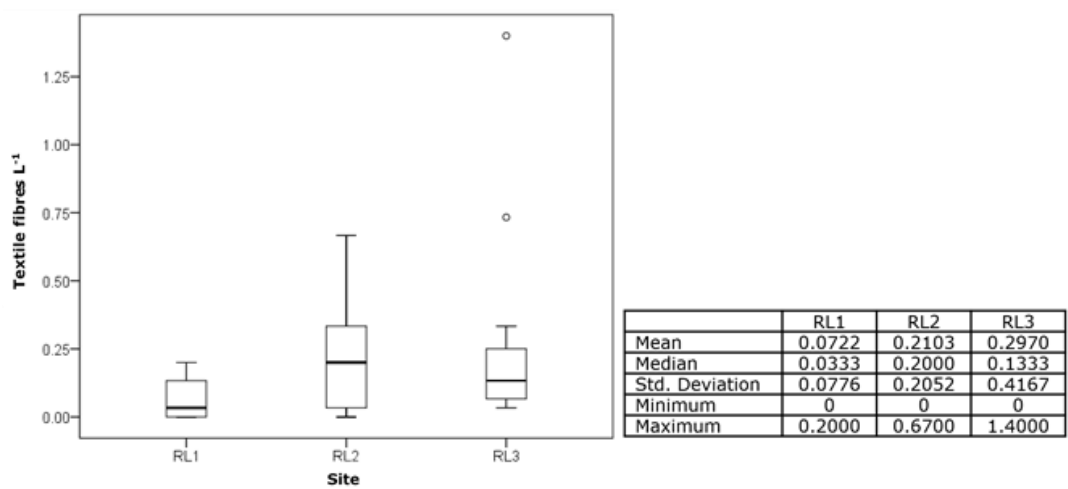
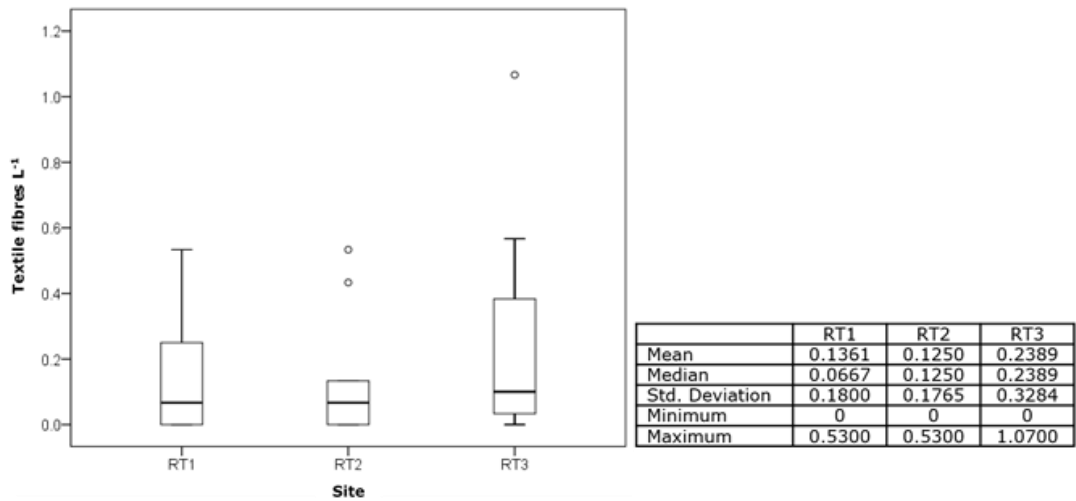
A total of 9 atmospheric and 15 freshwater samples contained no textile fibres at all. These samples in particular are testament to the efficacy of the contamination controls followed (Woodall et al. 2015; Taylor et al. 2016). The abundance of textile fibres from laboratory deposition is detailed in Tables 6.2 and 6.3, and across the procedural blanks in Table 6.4.



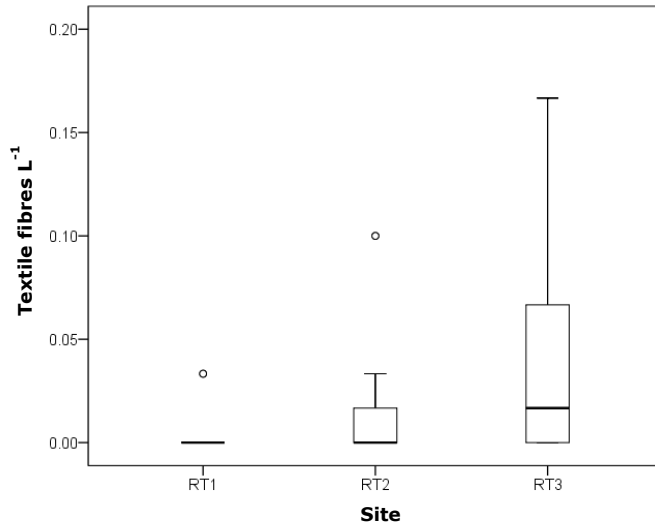
**Figure 6.5: Total fibre concentrations at each freshwater site over the 13 sampling occasions. Sampling occasion 1 was collected on 20/11/2017, and occasion 13 on 23/10/2018.**



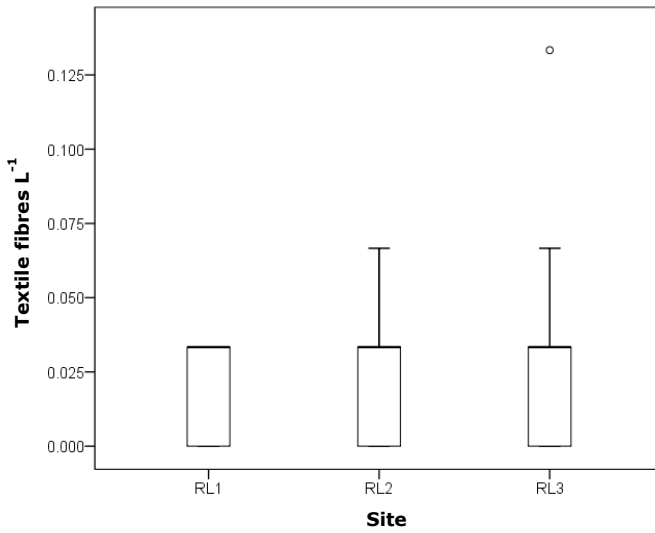
**Figure 6.6: Atmospheric deposition of natural and extruded textile fibres on each sampling occasion for each site. Daily precipitation data was collated from the Met Office’s HadUKP dataset for the Central England region (Alexander and Jones, 2001). Sampling occasion 1 was collected on 23/11/2017, and occasion 26 on 25/10/2018.**



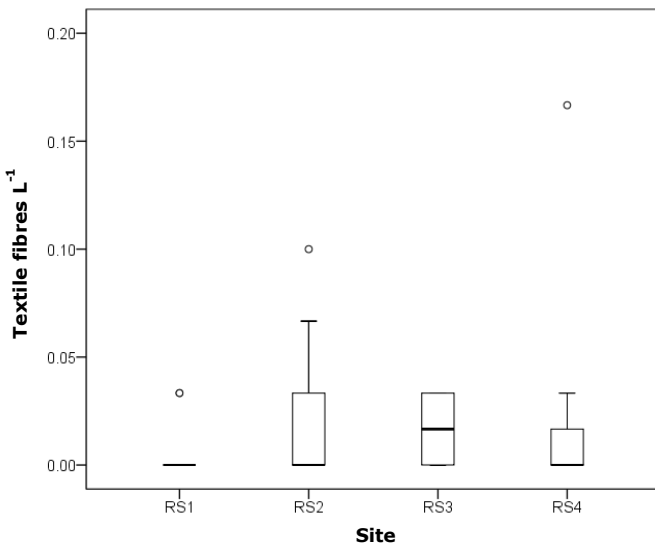
**Figure 6.7: Boxplots illustrating the median and range of natural textile fibre concentrations at each freshwater sampling site.**



	RT1	RT2	RT3
Mean	0.0056	0.0194	0.0361
Median	0	0	0.0361
Std. Deviation	0.0130	0.0388	0.0502
Minimum	0	0	0
Maximum	0.0300	0.1000	0.1700

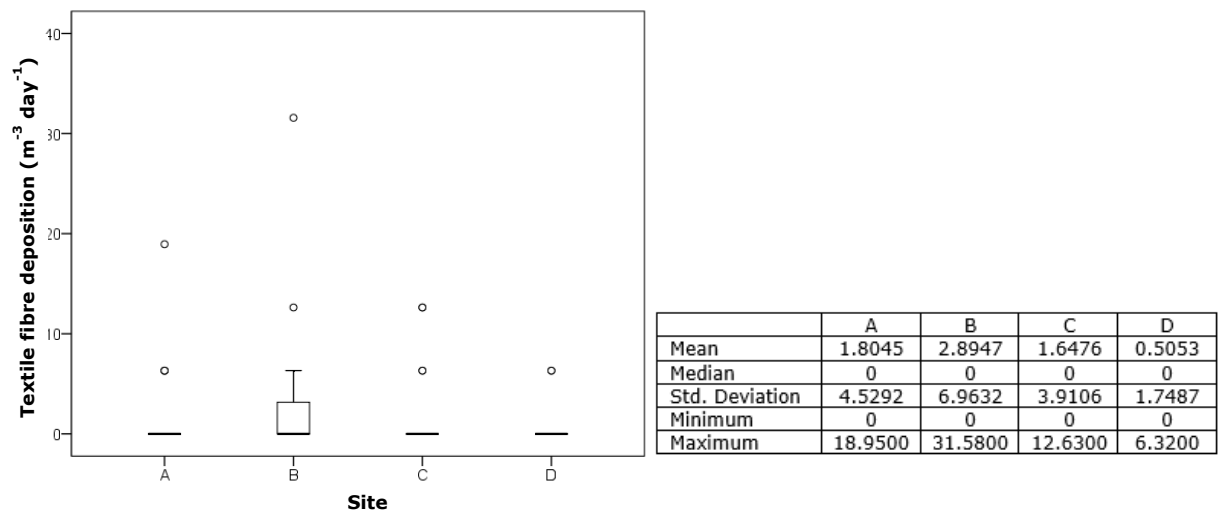
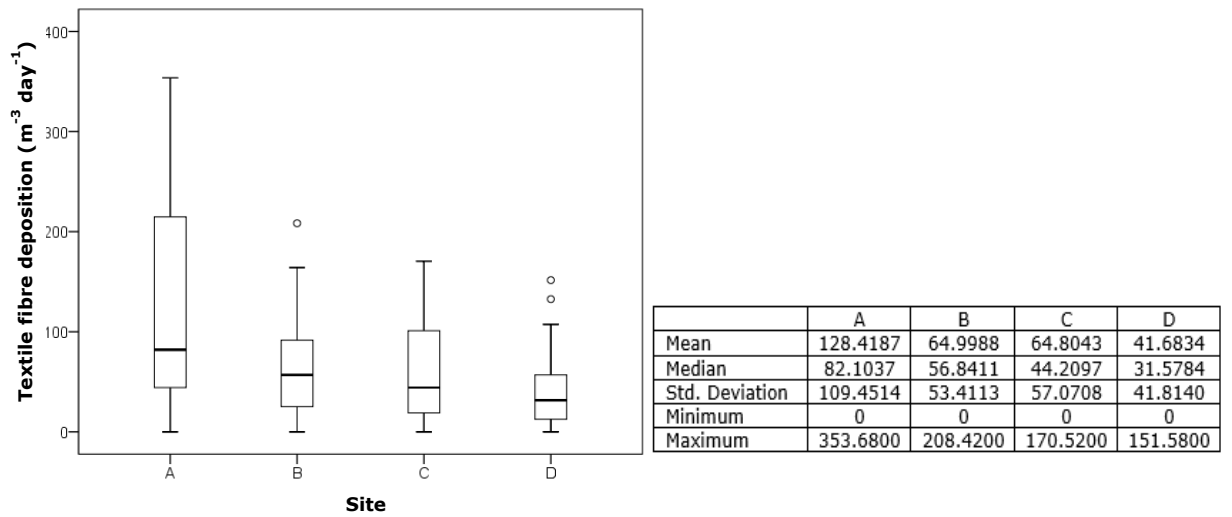


	RL1	RL2	RL3
Mean	0.0194	0.0308	0.0333
Median	0.0333	0.0333	0.0333
Std. Deviation	0.0172	0.0253	0.0394
Minimum	0	0	0
Maximum	0.0300	0.0700	0.1300

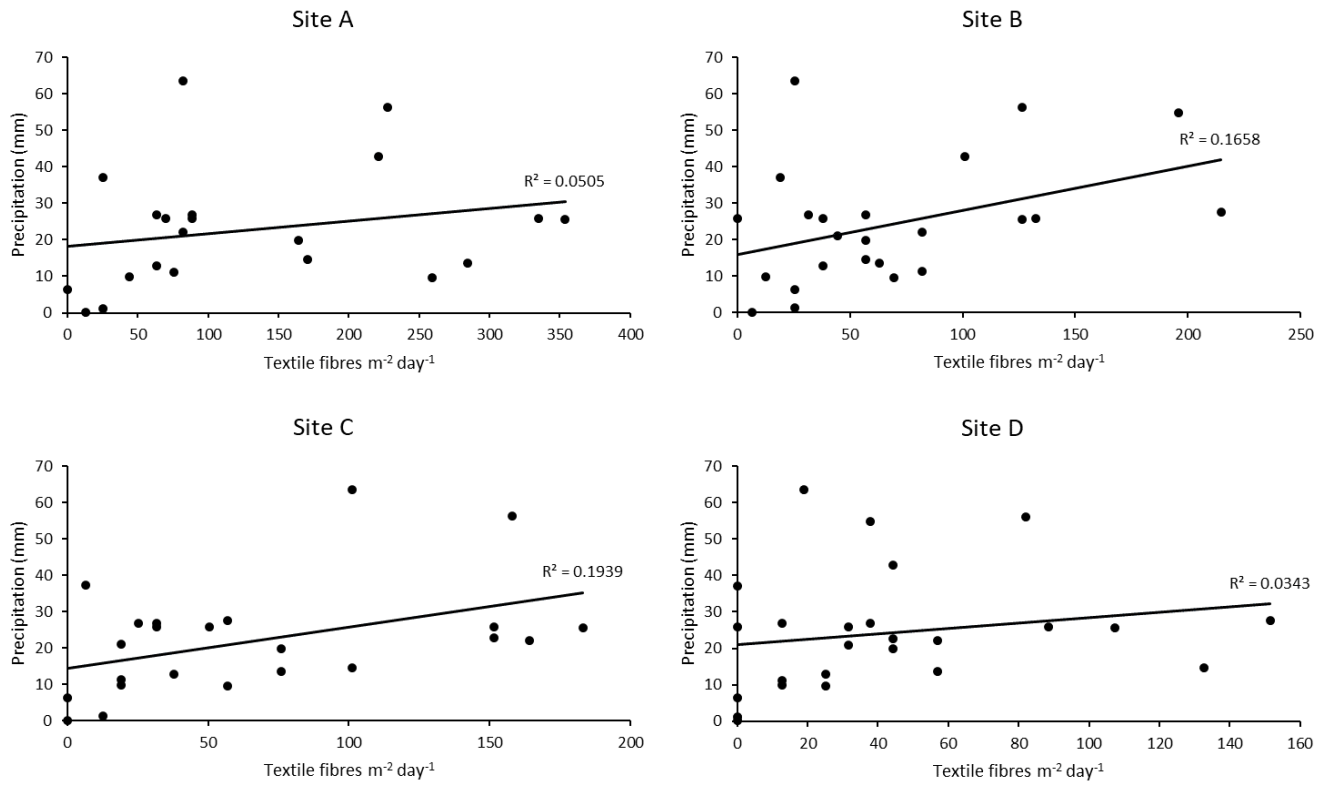


	RS1	RS2	RS3	RS4
Mean	0.0077	0.0256	0.0167	0.0256
Median	0	0	0.0167	0
Std. Deviation	0.0146	0.0338	0.0174	0.0512
Minimum	0	0	0	0
Maximum	0.0300	0.1000	0.0300	0.1700

**Figure 6.8: Boxplots illustrating the median and range of extruded textile fibre concentrations at each freshwater sampling site.**



**Figure 6.9: Boxplots illustrating the median and range of natural and extruded textile fibre concentrations at each atmospheric sampling site.**



**Figure 6.10: Correlation of number of fibres deposited and precipitation over each fortnightly sampling period.**



**Table 6.2: Textile fibres deposited during the processing of freshwater samples.**

<b>Sample occasion</b>	<b>Natural fibres</b>	<b>Extruded fibres</b>
1	0	0
2	0	0
3	0	0
4	0	0
5	1	0
6	3	0
7	0	0
8	1	0
9	1	0
10	0	0
11	1	0
12	0	0
13	0	0

**Table 6.3: Textile fibres deposited during the processing of Atmospheric samples.**

<b>Sample occasion</b>	<b>Natural fibres</b>	<b>Extruded fibres</b>
1	5	0
2	3	0
3	0	0
4	0	0
5	1	0
6	0	0
7	2	0
8	1	0
9	1	0
10	0	0
11	0	0
12	2	0
13	0	0
14	0	0
15	0	0
16	0	0
17	0	0
18	0	0
19	0	0
20	No samples	
21	0	0
22	0	0
23	1	0
24	1	0
25	0	0
26	0	0

**Table 6.4: Abundance of textile fibres across the 5 procedural blanks**

<b>Blank</b>	<b>Natural fibres</b>	<b>Extruded fibres</b>
Freshwater 1	1	0
Freshwater 2	0	0
Atmospheric 1	2	0
Atmospheric 2	0	0
Atmospheric 3	0	0

### Freshwater fluxes of textile fibres

The abundance of freshwater textile fibres at each site are presented here as particles per litre. Using UK National River Flow Archive (NRFA) gauging stations it is, however, possible to consider textile fibre fluxes at various points within the sampled reaches. NRFA gauging stations are located short distances downstream of site RT2, and upstream of sites RL3 and RS4 (Table 6.5). Flux calculations based on the mean discharges at each of these stations and the mean textile fibre concentrations quantified over this twelve month sampling campaign are detailed in Table 6.6.

**Table 6.5: Details of each of the UK National River Flow Archive gauging stations used to estimate microplastic fluxes.**

Gauged River	Nearest Sampling site	NRFA Gauging station name	Length of operation
River Trent	RT2	Trent at Stoke-On-Trent	01/1968-present
River Leen	RL3	Leen at Triumph Road Nottingham	01/1968-present
River Soar	RS4	Soar at Kegworth	12/1978-present

**Table 6.6: Textile fibre flux estimates at sites in close proximity to UK NRFA gauging stations presented to three significant figures. Mean flow data acquired 07/01/2019.**

Site	Mean flow (m <sup>3</sup> s <sup>-1</sup> )	Mean textile fibre flux (fibres/day)	Minimum textile fibre flux (fibres/day)	Maximum textile fibre flux (fibres/day)
RT2	0.626	7 810 000	0	28 800 000
RL3	0.685	19 500 000	1 970 000	82 900 000
RS4	11.727	197 000 000	0	608 000 000

## **5.5. Discussion**

### **FTIR characterisation of known and environmental textile fibres**

Whilst the use of ATR-FTIR is shown here to be an effective method of fibre identification for high fibre density samples such as garment patches and fibre pills, it is not one that can be easily applied to the analysis of individual textile fibres such as those from environmental samples, the majority of which are too small to be handled for ATR-FTIR analysis. Moreover, though the efficacy of reflectance FTIR spectroscopy has been demonstrated for larger (150  $\mu\text{m}$ ) microplastic particles (Harrison et al. 2012), its suitability in the analysis of textile fibres has not been assessed.

Of the four fibres from environmental samples analysed by reflectance FTIR spectroscopy (Figure 6.4), the two natural textile fibres were visually identified as unmercerised cotton. Cotton is the most common natural textile fibre (Ladewig et al. 2015), and in its unmercerised form is easily identifiable as a flat, twisted fibre of uneven diameter (Figure 6.2). As the identity of these environmental fibres was known, it was possible to make a direct comparison between the reflectance FTIR spectra of the two environmental cotton fibres (Figure 6.4) and that produced for the FTIR library (Figure 6.3). The variation between these three spectra not only provides further evidence of the limited value of reflectance FTIR spectroscopy in the analysis of textile fibres, but also highlights the degree of variation in reflectance spectra that can be produced from the analysis of fibres of the same type. It is, however, possible that this variation was influenced by the heterogeneous shape of unmercerised cotton, which will likely lead to a degree of variation in different reflectance FTIR spectra generated from multiple points of the same cotton fibre.

### **Visual categorisation of known and environmental textile fibres**

The approach applied in the present study cannot conclusively identify the origin of the unknown textile fibres. However, it successfully placed the abundance of extruded textile fibres in the context of environmental textile fibre populations. Categorising textile fibres as natural or extruded affords researchers greater consideration of textile fibres within the study of

microplastic pollution in the absence of suitable analytical techniques. Furthermore, where suitable analytical techniques are available, this approach reduces the sample size of textile fibres in need of chemical analysis from the total fibre population to extruded fibres only. Where available, a greater proportion, and therefore more representative subsample, of potentially synthetic textile fibres can then be chemically analysed by means such as FTIR spectroscopy.

### **Textile fibre populations in freshwater environments and the atmosphere**

The visual characterisation of textile fibres proved to be an effective technique in the analysis of environmental textile populations. Whilst the process outlined in Figure 6.2 clearly details the steps taken to analyse environmental textile fibres, the authors must stress the importance of fibre analysts developing their proficiency using known textile fibres prior to applying this technique to environmental samples.

The mean total textile fibre concentration observed across the freshwater sites sampled consistently exceeded that reported by Dris et al. (2018) in the Marne River, Paris, who recorded a maximum mean fibre concentration of 0.1 fibres L<sup>-1</sup>. A number of factors can explain this finding, including the finer mesh size and higher microscope magnification used in the present study and the dilution effect of the much greater volume of water flowing through the Marne River compared to the rivers sampled here.

The entrainment and transport of textile fibres in aquatic environments and the atmosphere might be expected to be influenced by their physical properties – including fibre morphology and density – as well as environmental conditions including rate of flow in rivers, precipitation and wind speed. Determining the extent to which this is the case is beyond the scope of the present study, and so it is not possible to comment on whether or not these factors will influence the environmental prevalence of fibres of different types in highly mobile fluid matrices. However, in relatively motionless fluid environments such as the settling tanks of WWTPs, common natural and extruded textile fibres, that are all denser than water (Table 6.7), might be

expected to settle. However, the emission of microplastic particles, and in particular synthetic textile fibres, from WWTPs has been quantified and is known to vary; across seven WWTPs, Leslie et al. (2017) report microplastic concentrations in WWTP effluent to range from 9-91 particles L<sup>-1</sup>.

**Table 6.7: Density of common textile fibres, as reported by Morton and Hearle (2008).**

<b>Fibre</b>	<b>Density (g/cm<sup>3</sup>)</b>
Cotton	1.55
Wool	1.30
Silk	1.34
Viscose Rayon	1.52
Polyester	1.39
Nylon 66, nylon 6	1.14
Acrylic	1.19

The sampling of freshwater environments was conducted at three sites that were in receipt of the effluent of wastewater treatment plants (WWTPs) (sites RS2-4) and seven that were not. There was no appreciable increase in textile fibre concentration at sites in receipt of WWTP effluent. This was true even of River Soar site 3, located approximately just 1.7 km downstream of the outflow of a WWTP serving a population equivalent of 72,500. However, whilst the concentration did not increase, the greater flow rates of sites RS2-RS4 will have increased textile fibre abundance. Instead, the highest cumulative abundance of natural and extruded textile fibres on each of the rivers sampled was recorded at sites immediately downstream of urban population centres (Sites RT3 and RL3).

Textile fibres were even observed at the most upstream sites on each of the rivers sampled. Though close to the sources of these rivers, sites RT1 and RL1 are popular recreational sites, whilst site RS1 neighbours a busy haulage yard. The observation of textile fibres at these sites demonstrates the role of localised anthropogenic activity on textile fibre abundance in the freshwater system. The prevalence of textile fibres in atmospheric fallout in both urban and rural sites highlights the role of atmospheric deposition in the transport of

textile fibres throughout the environment, including to relatively remote locations. It also raises questions regarding the role of wastewater treatment plants as sources of synthetic textile fibres in aquatic environments.

Wastewater treatment plants are partially open systems, with various stages of the wastewater treatment process exposed to the atmospheric deposition that has been recorded here and elsewhere (Cai et al. 2017; Dris et al. 2016; 2017). The extent to which this deposition contributes to the textile fibre concentrations of final effluent is yet to be quantified.

Though atmospheric deposition of fibres was comparable at sites B-D, the abundance of fibres observed at site A was noted to be much more variable (Figures 6.6 and 6.9) (Levene's test  $p$ -value  $<0.001$ ), despite its close proximity to sites B and C (Figure 6.1). Where previous records of atmospheric textile fibre deposition have extrapolated over large geographical areas from as few as two sample locations (e.g. Dris et al. 2016), the localised variation quantified here between sites that are fewer than 800 m apart, indicates that such extrapolations are likely to be inappropriate.

The recurrent observation of textile fibres at freshwater sites irrespective of rainfall prior to, or during, sample collection also suggests an atmospheric contribution of textile fibres to the freshwater system independent of precipitation and surface run off. Moreover, previous studies have assumed all, or at least the majority, of the fibres present in atmospheric samples were deposited during the rainfall event that prompted the sample collection (e.g. Dris et al. 2016). Here, the identification of textile fibres in atmospheric fallout during periods of no to low precipitation, where shows that such an assumption cannot be relied upon.

Though extruded textile fibres, a proportion of which may be microplastic, were present in both freshwater and atmospheric samples throughout this sampling campaign, the consistent dominance of natural textile fibres over extruded textile fibres provides strong support for the concerns raised by Ladewig et al (2015) and Zhao et al (2016).

### **Freshwater fluxes of textile fibres and difficulties in extrapolating fluxes**

The data presented in Table 6.6 goes some way to highlighting the potential fibre flux of even these smaller freshwater systems. However, the temporal nature of this study has also enabled consideration of the extent to which such extrapolations can vary. The range of fibre fluxes presented in Table 6.6 illustrates the importance of sample replication in order to account for seasonal variation and the influence of abnormal weather conditions. These extrapolations suggest approximate daily textile fibre fluxes of 19 500 000 and 197 000 000 from the Rivers Leen and Soar respectively, as well as an approximate daily textile fibre flux of 7 810 000 into the Stoke-on-Trent urban area. Despite flux extrapolations being frequently presented in microplastic literature, the large range in values recorded both between sites in a similar geographical area, and at individual sites through time, suggests that little confidence can be given to these values.

### **5.6. Conclusion**

The findings of the present study show that natural textile fibres constitute a significantly greater proportion of environmental textile fibre populations than extruded textile fibres in all three of the sampled rivers, as well as at all 4 sites of atmospheric deposition. It demonstrates a considerable limitation of the use of FTIR spectroscopy for the analysis of textile fibres in the study of microplastic pollution, and details the subtle differences between natural and extruded textile fibres, advancing the visual characterisation of particles that is still applied to the majority of microplastic pollution studies. Finally, textile fibre concentrations were found to vary greatly through both space and time. This has important implications for the legitimacy of previously extrapolated particle fluxes within the broader study of microplastic pollution, which do not sufficiently account for temporal and spatial variability.

A copy of this publication is appended in the 'Publications' section at the end of this thesis.



## **5.7. Author Contributions**

T.S. initiated the microplastics research and conducted all field sampling, and all laboratory processing and analysis. W.M. assisted in the FTIR spectroscopy. All authors contributed to the interpretation of the presented data and the writing of the published manuscript.

## 5.8. Appendices

### Appendix 4.1: Natural and extruded textile fibre concentrations at each freshwater sampling site for each of the 13 sample occasions

Sample occasion	Site	Natural textile fibres / litre	Extruded textile fibres / litre
1	RT1	0.53	0.03
	RT2	0.43	0.10
	RT3	0.20	0.00
	RL1	0.20	0.00
	RL2	0.37	0.03
	RL3	0.17	0.00
	RS1	0.10	0.00
	RS2	0.17	0.00
	RS3	0.00	0.00
RS4	0.20	0.00	
2	RT1	0.37	0.00
	RT2	0.53	0.00
	RT3	0.57	0.03
	RL1	0.03	0.00
	RL2	0.40	0.00
	RL3	0.17	0.03
	RS1	0.30	0.00
	RS2	0.30	0.03
	RS3	0.30	0.03
RS4	0.30	0.00	
3	RT1	Sample lost	
	RT2	Sample lost	
	RT3	Sample lost	
	RL1	Sample lost	
	RL2	0.67	0.03
	RL3	Sample lost	
	RS1	0.07	0.00
	RS2	0.47	0.00
	RS3	Sample lost	
RS4	0.23	0.00	
4	RT1	0.17	0.00
	RT2	0.13	0.00
	RT3	0.57	0.07
	RL1	0.00	0.03
	RL2	0.33	0.03
	RL3	0.73	0.07
	RS1	0.27	0.00
	RS2	0.20	0.00
	RS3	0.40	0.00
RS4	0.30	0.00	
5	RT1	0.33	0.00
	RT2	0.13	0.00
	RT3	1.07	0.00
	RL1	0.13	0.00
	RL2	0.33	0.00
	RL3	1.40	0.00
	RS1	0.27	0.00
	RS2	0.93	0.00
	RS3	0.33	0.00
RS4	0.60	0.00	
6	RT1	0.10	0.00
	RT2	0.03	0.00
	RT3	0.17	0.03
	RL1	0.13	0.03
	RL2	0.27	0.07
	RL3	0.33	0.03
	RS1	0.50	0.00
	RS2	0.13	0.00
	RS3	0.40	0.00
RS4	0.23	0.00	
7	RT1	0.10	0.00
	RT2	0.00	0.00
	RT3	0.10	0.00
	RL1	0.20	0.00
	RL2	0.03	0.03
	RL3	0.10	0.00
	RS1	0.00	0.00
	RS2	0.10	0.00
	RS3	0.10	0.03
RS4	0.13	0.00	

Sample occasion	Site	Natural textile fibres / litre	Extruded textile fibres / litre
8	RT1	0.00	0.03
	RT2	0.00	0.00
	RT3	0.00	0.07
	RL1	0.03	0.03
	RL2	0.03	0.03
	RL3	0.13	0.13
	RS1	0.07	0.03
	RS2	0.03	0.03
	RS3	0.00	0.00
RS4	0.00	0.00	
9	RT1	0.00	0.00
	RT2	0.10	0.00
	RT3	0.03	0.00
	RL1	0.00	0.03
	RL2	0.00	0.00
	RL3	0.03	0.00
	RS1	0.03	0.00
	RS2	0.00	0.10
	RS3	0.03	0.00
RS4	0.00	0.00	
10	RT1	0.00	0.00
	RT2	0.00	0.03
	RT3	0.00	0.00
	RL1	0.00	0.03
	RL2	0.03	0.03
	RL3	0.07	0.03
	RS1	0.03	0.00
	RS2	0.00	0.00
	RS3	0.00	0.03
RS4	0.07	0.03	
11	RT1	0.00	0.00
	RT2	0.00	0.00
	RT3	0.10	0.00
	RL1	0.10	0.00
	RL2	0.07	0.00
	RL3	0.07	0.03
	RS1	0.10	0.03
	RS2	0.07	0.03
	RS3	0.03	0.03
RS4	0.03	0.03	
12	RT1	0.00	0.00
	RT2	0.10	0.00
	RT3	0.03	0.17
	RL1	0.03	0.03
	RL2	0.00	0.07
	RL3	Sample lost	
	RS1	0.03	0.00
	RS2	0.30	0.07
	RS3	0.03	0.03
RS4	0.07	0.17	
13	RT1	0.03	0.00
	RT2	0.03	0.10
	RT3	0.03	0.07
	RL1	0.00	0.03
	RL2	0.20	0.07
	RL3	0.07	0.03
	RS1	0.10	0.03
	RS2	0.00	0.07
	RS3	0.03	0.03
RS4	0.03	0.10	

**Appendix 4.2: Natural and extruded textile fibre deposition at each atmospheric sampling site for each of the 26 sample occasions**

Sample occasion	Site	Natural textile fibres (fibres m <sup>2</sup> day <sup>-1</sup> )	Extruded textile fibres (fibres m <sup>2</sup> day <sup>-1</sup> )
1	A	75.79	6.32
	B	82.1	0
	C	151.58	12.63
	D	56.84	0
2	A	170.52	0
	B	56.84	0
	C	101.05	0
	D	132.63	0
3	A	284.21	0
	B	56.84	6.32
	C	75.79	0
	D	56.84	0
4	A	353.68	0
	B	120	6.32
	C	170.52	12.63
	D	107.37	0
5	A	227.36	0
	B	126.31	0
	C	157.89	0
	D	82.1	0
6	A	Sample lost	
	B	Sample lost	
	C	151.58	0
	D	44.21	0
7	A	Sample lost	
	B	208.42	6.32
	C	56.84	0
	D	151.58	0
8	A	334.73	0
	B	132.63	0
	C	151.58	0
	D	88.42	0
9	A	258.94	0
	B	69.47	0
	C	56.84	0
	D	25.26	0
10	A	214.73	6.32
	B	101.05	0
	C	Sample lost	
	D	44.21	0
11	A	164.21	0
	B	56.84	0
	C	75.79	0
	D	44.21	0
12	A	82.1	0
	B	25.26	0
	C	101.05	0
	D	18.95	0
13	A	Sample lost	
	B	44.21	0
	C	18.95	0
	D	31.58	0

Sample occasion	Site	Natural textile fibres (fibres m <sup>2</sup> day <sup>-1</sup> )	Extruded textile fibres (fibres m <sup>2</sup> day <sup>-1</sup> )
14	A	88.42	0
	B	0	0
	C	25.26	6.32
	D	0	0
15	A	44.21	0
	B	12.63	0
	C	18.95	0
	D	12.63	0
16	A	25.26	0
	B	18.95	0
	C	6.32	0
	D	0	0
17	A	0	0
	B	25.26	0
	C	0	0
	D	0	0
18	A	12.63	0
	B	6.32	0
	C	0	0
	D	0	0
19	A	25.26	0
	B	18.95	6.32
	C	12.63	0
	D	0	0
20	A	Sample lost	
	B	Sample lost	
	C	Sample lost	
	D	Sample lost	
21	A	Sample lost	
	B	164.21	31.58
	C	Sample lost	
	D	31.58	6.32
22	A	88.42	0
	B	31.58	0
	C	25.26	0
	D	6.32	6.32
23	A	44.21	18.95
	B	37.89	0
	C	37.89	0
	D	25.26	0
24	A	63.16	0
	B	56.84	0
	C	31.58	0
	D	37.89	0
25	A	69.47	6.32
	B	82.1	0
	C	18.95	0
	D	12.63	0
26	A	69.47	0
	B	25.26	12.63
	C	44.21	6.32
	D	31.58	0

## **6. Freshwater microplastic pollution varies through both space *and* time**

Thomas Stanton; Matthew Johnson; Paul Nathanail; William MacNaughtan; Rachel L Gomes

### **6.1. Abstract**

Plastic pollution represents one of the most salient indicators of society's impact on the environment. The microplastic component of this is ubiquitous, however, microplastic studies are seldom representative of the locations they sample. Over 12 months we explored spatiotemporal variation in microplastic prevalence across a freshwater system and in atmospheric deposition within its catchment, in one of the most temporally comprehensive study of freshwater microplastic. Microplastics were quantified in low concentrations (max 0.4 particles L<sup>-1</sup>) at all freshwater sites, including upstream of urban areas, and on rivers that do not receive wastewater treatment plant effluent. Extrapolated microplastic abundances at each site varied by up to 8 orders of magnitude, suggesting that microplastic surveys that do not account for temporal variability misrepresent microplastic prevalence. Whilst we do not wish to underplay the potential impacts of microplastic particles in the environment, we argue that microplastic pollution needs to be placed in a more critical context, including assessment of temporal variability, to appropriately inform legislators and consumers.

## 6.2. Introduction

Microplastic particles (<5 mm) are an environmental pollutant of substantial public and scientific concern. Functioning as pollutants in contemporary environmental systems, and demarking human activity for centuries to come as techno-fossils, microplastic particles are a widespread form of this plastic waste. Their prevalence in the marine environment has been reported since the early 1970s (Carpenter et al. 1972; Carpenter and Smith, 1972), and their presence in estuarine systems (Zhao et al. 2015; Gallagher et al, 2016; Gray et al. 2018) and freshwater environments (Zhang et al. 2015; Klein et al. 2015; Peng et al. 2018; Mani et al. 2019; Watkins et al. 2019) has also been documented. However, whilst microplastics are thought to be ubiquitous beyond these systems (Rochman, 2018), records of microplastic pollution are often reported at low spatial and temporal resolutions.

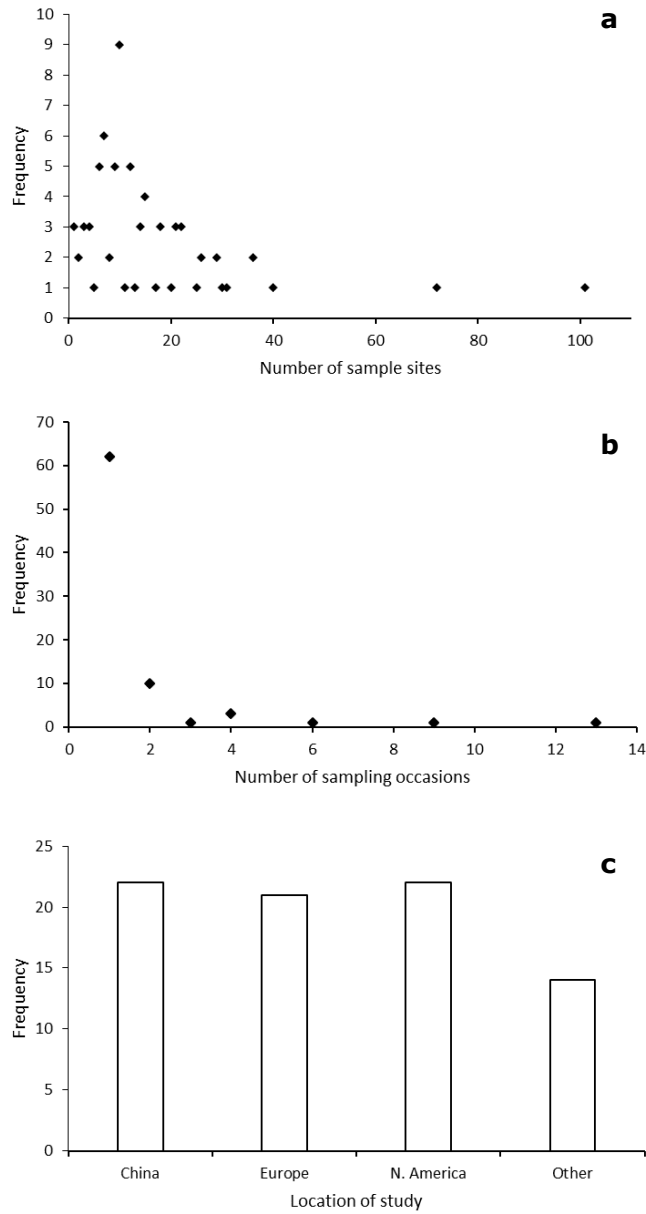
Freshwater catchments are a key pathway in the transport of microplastic debris, which accumulates in marine environments (Wagner et al. 2014). Sources of freshwater microplastic pollution are known to be varied, including wastewater treatment plants (WWTPs), urban centres and road runoff (Horton et al. 2017), industry (Lechner and Ramler, 2015), the atmosphere (Dris et al. 2016), and the degradation of larger items of plastic waste. However, the predominate focus of freshwater microplastic studies has been on downstream reaches of large, highly developed rivers in China, Europe, and North America (Figure 7.1). Understanding how microplastic concentrations change with distance downstream along rivers is lacking, yet it is critical to understanding this key source and pathway of microplastic particles.

Freshwater sampling campaigns also rarely account for temporal variability in microplastic concentrations (Figure 7.1) (Schmidt et al. 2017), limiting the extent to which measurements are representative of that site beyond the time of sample collection. Whilst studies that quantify microplastics in different freshwater environments are of great value, they are not able to further our understanding of microplastic sources and distributions without careful

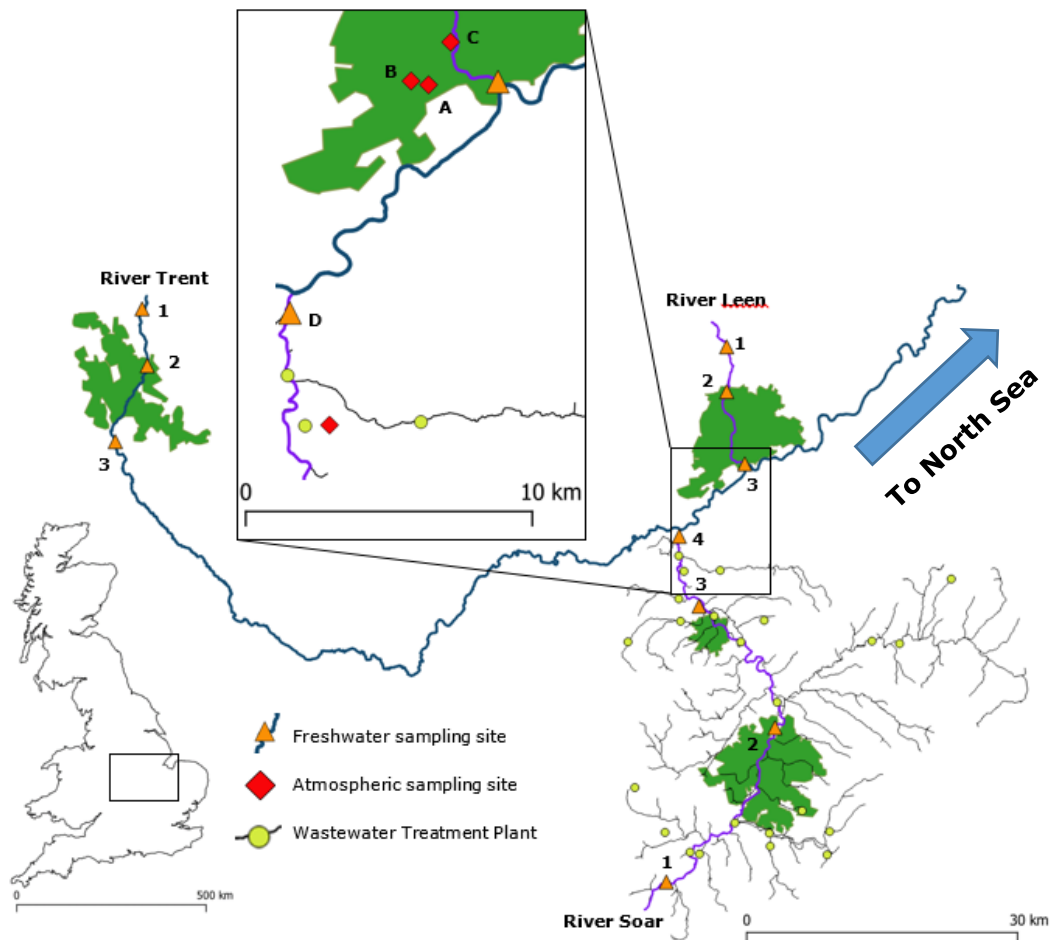
consideration of the intra-site variability over representative time periods (Prata et al. 2019).

A comprehensive understanding of the sources and vectors of freshwater microplastic pollution is further limited by a lack of consideration of atmospheric deposition to what is a largely open system. Atmospheric deposition is a known source of anthropogenic particles found in both the benthic and suspended sediments of freshwater systems, including Spheroidal Carbonaceous Particles and Inorganic Ash Spheres (Rose et al. 2012). However, the study of airborne microplastic particles is limited to a few records of their presence in urban (Dris et al. 2016; Cai et al. 2017; Bergman et al. 2019; Stanton et al. 2019a), and remote (Allen et al. 2019; Bergman et al. 2019) atmospheric deposition.

To address these research gaps, this study presents the findings of 12 months of freshwater and atmospheric sampling across the River Trent catchment, UK (Figure 7.2). We sampled the upstream reaches of River Trent (RT), and the entire length of two of its tributaries, the River Leen (RL), and River Soar (RS). By sampling sites upstream of urban centres and at points without WWTP inputs, we assess the contribution of these previously cited sources of microplastic pollution to freshwater microplastic loads, and highlight the importance of accounting for temporal variation when disseminating microplastic findings.



**Figure 7.1: The sampling frequency (a), number of sampling sites (b), and location (c) of 79 freshwater microplastic studies (see methods for Web of Knowledge search parameters used to identify relevant publications).**



**Figure 7.2: Locations of freshwater (numbered) and atmospheric (lettered) sampling sites within the Trent Catchment, UK. Green areas represent the urban areas of Stoke-on-Trent (River Trent), Nottingham (River Leen), Leicester (River Soar upstream) and Loughborough (River Soar downstream).**



## 6.3. Methods

### Literature search protocol for freshwater microplastic studies

Figure 7.1 was collated from the results of a Web of Knowledge publication search conducted on 24/07/2018 with the aim of collating the number of sampling sites, number of sampling occasions, and location of freshwater microplastic studies. This search was conducted using the following parameters:

Topic search for:

- Freshwater microplastic
- OR
- River Microplastic
- OR
- Lake Microplastic

Though these search terms are unlikely to have provided a complete coverage of all freshwater microplastic studies, they yielded 343 results from 2012 to 2019. All review articles and laboratory studies were excluded, leaving 93 studies (supplementary references), of which the authors had access to 79 that contained the necessary information to meet the above aim.

### Sample sites, and sample collection and processing

Sample site locations, and the procedure for contamination control, sample collection and processing are described in detail in Stanton et al. (2019a) (Chapter 5). In brief, 30 L freshwater samples were collected from 10 sites across three rivers within the Trent catchment (Figure 7.2). Samples were collected from the riverbank using a metal bucket on a telescopic pole. Each sample was concentrated onto a 63 µm sieve in the field, and the retained material was transferred into a glass sample bottle using distilled water. Freshwater samples were collected every four weeks for 12 months from 20/11/2017 to 23/10/2018.

Atmospheric samples were collected using a scaled-down adaptation of the methods used by Dris et al. (2016), in which atmospheric deposition was collected in 2.5 L amber glass bottles using a 12 cm diameter (0.011 m<sup>2</sup>) glass funnel. Atmospheric samples were collected fortnightly for 12 months from 23/11/2017 to 25/10/2018. To assess the potential influence of intra-site variation high spatial resolution sampling was also conducted at site D, in which five replicate samples were collected from the same rooftop between 04/12/2018 and 11/12/2018.

Freshwater samples were treated with 30% H<sub>2</sub>O<sub>2</sub> to rid them of organic matter before being vacuum filtered onto gridded cellulose nitrate filter papers with a 0.45 µm pore size (Whatman ME 25/41). The contents of the amber glass bottles used to collect atmospheric deposition was concentrated onto a 38 µm sieve in the laboratory, and the bottles triple rinsed with distilled water. The retained material was vacuum filtered onto the same gridded filter papers as the freshwater samples.

### **Microplastic identification**

Samples were visually inspected under a dissection microscope (Medline Scientific CETI Varizoom-10, Chalgrove, UK). Textile fibres were categorised according to Stanton et al. (2019a), and the grid reference for all suspected non-fibrous microplastic particles was recorded. This grid reference aided the subsequent FTIR spectroscopy of these particles. Analysed particles were subjected to one of the following FTIR spectroscopy techniques: Attenuated total reflectance (ATR) (Bruker Tensor 27 FTIR spectrometer [Bruker Optics, Coventry, UK] equipped with a Golden Gate ATR accessory [Specac, Orpington, UK]), reflectance FTIR microscopy (Bruker Hyperion 2000 microscope [Bruker Optics, Coventry, UK]). Spectra were identified using Bruker's demonstration library. Six particles were also analysed using an ATR-FTIR objective (Bruker Lumos microscope [Bruker Optics, Coventry, UK]).

## **Statistical analysis**

Non-parametric tests were carried out on the freshwater dataset. Kruskal-Wallis tests were performed to determine whether microplastic concentrations over the sampling period were significantly different between sites on each river. Mann-Whitney U tests were performed to determine whether microplastic concentrations were significantly different between any two sites on the same river.

## **6.4. Results and Discussion**

### **Microplastic particles in the River Trent and its tributaries**

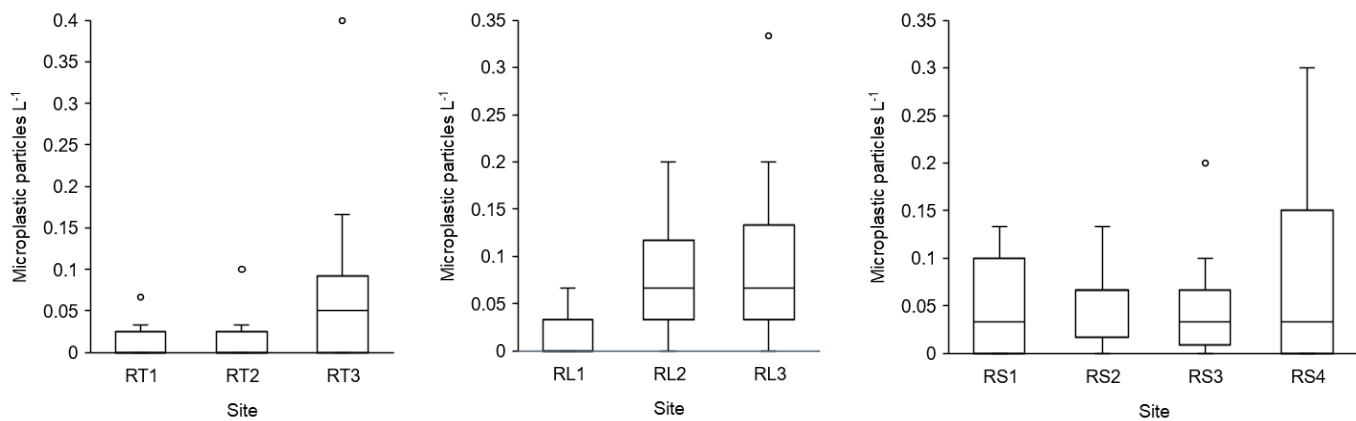
Throughout the 12 month freshwater sampling campaign (20/11/2017-23/10/2018) samples were collected from the riverbank at 10 sites every four weeks. Microplastic particles were identified at every site, including the most upstream sites. Identified microplastic particles included fragments, films and spherical beads, as well as extruded textile fibres. Extruded fibres include microplastic fibres (e.g. polyester) and regenerated cellulose fibres (e.g. rayon) (see Stanton et al. 2019a).

A total of 178 plastic particles were identified in the freshwater samples collected for this study. 79 particles were identified as extruded textile fibres. These fibres were present at sites not in receipt of WWTP effluent in concentrations similar to the three sites receiving WWTP effluent (sites RS2-4). This is significant because WWTPs are widely cited as a source of domestic microplastic particles, of which synthetic textile fibres form an infamous component (Napper and Thompson 2016; Ziajahromi et al. 2017).

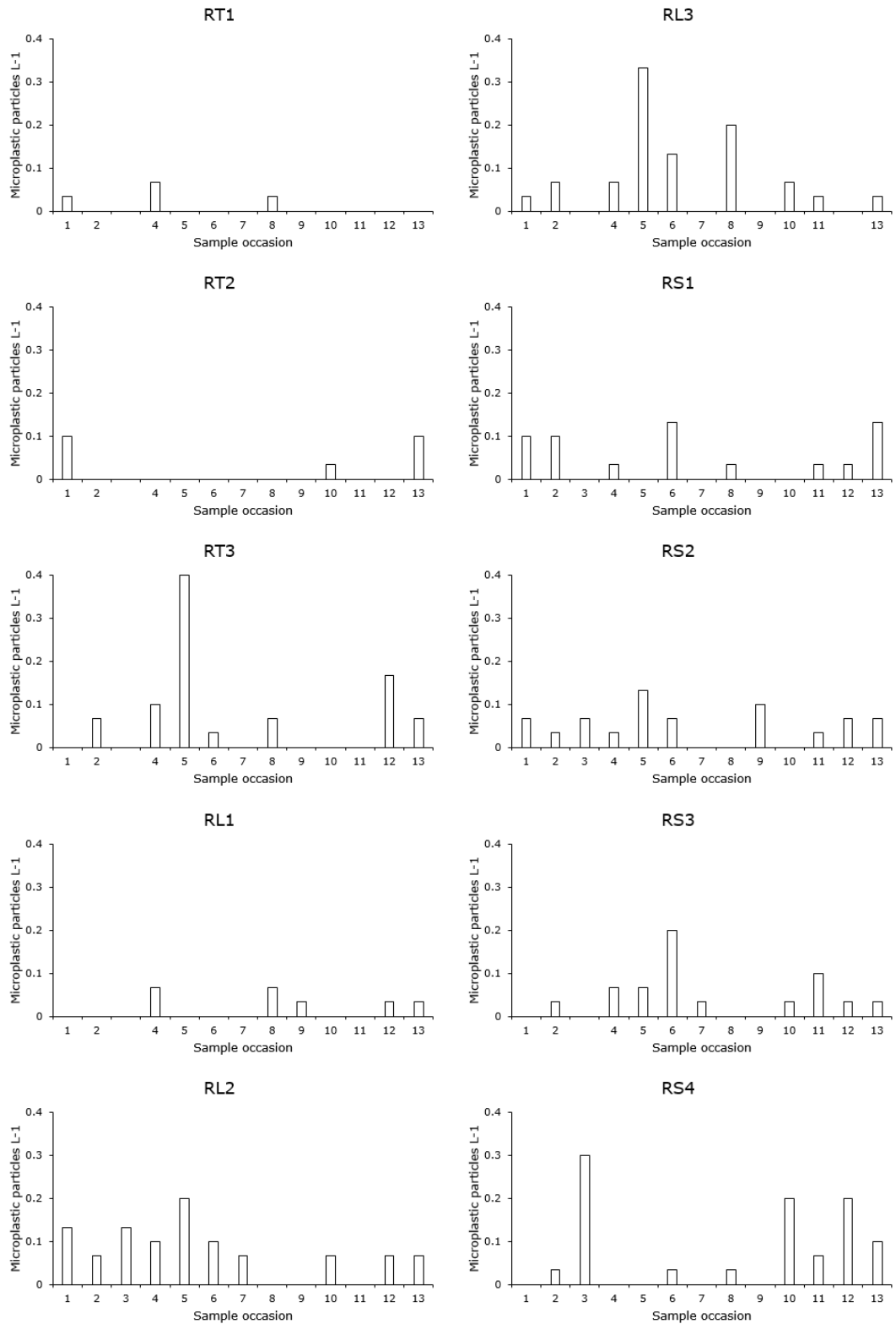
The remaining 99 plastic fragments included 95 microplastic particles and four plastic particles  $\geq 5$  mm, of which FTIR spectra were generated for 96. 19 (20%) of these spectra were confirmed as microplastic particles with  $>70\%$  match to the spectra of polymers in the available spectral libraries. Of these 19, 12 were polyethylene, three were polypropylene, two were polystyrene, and two were

polyvinyl acetate. The remaining 77 spectra were too noisy to be identified confidently.

Incidence of microplastic particles increased in both concentration and variability along the sampled reaches of the rivers Trent and Leen (Figures 7.3 and 7.4). The sampled reaches of these rivers do not receive WWTP effluent, but flow through the urban centres of Stoke-on-Trent and Nottingham, respectively.



**Figure 7.3: Boxplots illustrating the median and range of microplastic concentrations at each of the ten freshwater sites sampled.**



**Figure 7.4: Microplastic concentrations at each freshwater site and sampling occasion throughout the 12 month sampling campaign. Sampling occasion 1 was collected on 20/11/2017, and occasion 13 on 23/10/2018.**

Across the 12 month sampling campaign, Kruskal-Wallis tests showed that the concentration of microplastic particles between sites on the same river was not significantly different for the River Trent ( $p=0.88$ ) or the River Soar ( $p=0.936$ ). However, microplastic concentrations were significantly different between sites on the River Leen ( $p=0.015$ ). Levene's tests on each river showed that the variation between sites on each river was significant for the River Trent ( $p=0.027$ ), the River Leen ( $p=0.026$ ), and the River Soar ( $p=0.019$ ).

Mann-Whitney U tests were carried out to identify significant differences between any two sites on the same river (Table 7.1). Significant differences were only found between sites RT1 and RT3 ( $p=0.045$ ), RL1 and RL2 ( $p=0.007$ ), and RL1 and RL3 ( $p=0.022$ ). Therefore, the urban areas of Stoke-on-Trent, Nottingham, Leicester and Loughborough (Figure 7.2) did not significantly increase microplastic concentrations in the rivers that flow through them over this sampling period.

**Table 7.1: p values for Mann-Whitney U tests between sites on each river. Where  $p < 0.05$ , the difference between the compared sites is significant.**

A	Site number for respective river in column A		
	1	2	3
RT1		0.849	
RT3	0.045	0.106	
RL1		0.007	
RL3	0.022	0.699	
RS1		0.561	0.755
RS2			0.593
RS4	0.709	0.813	0.978

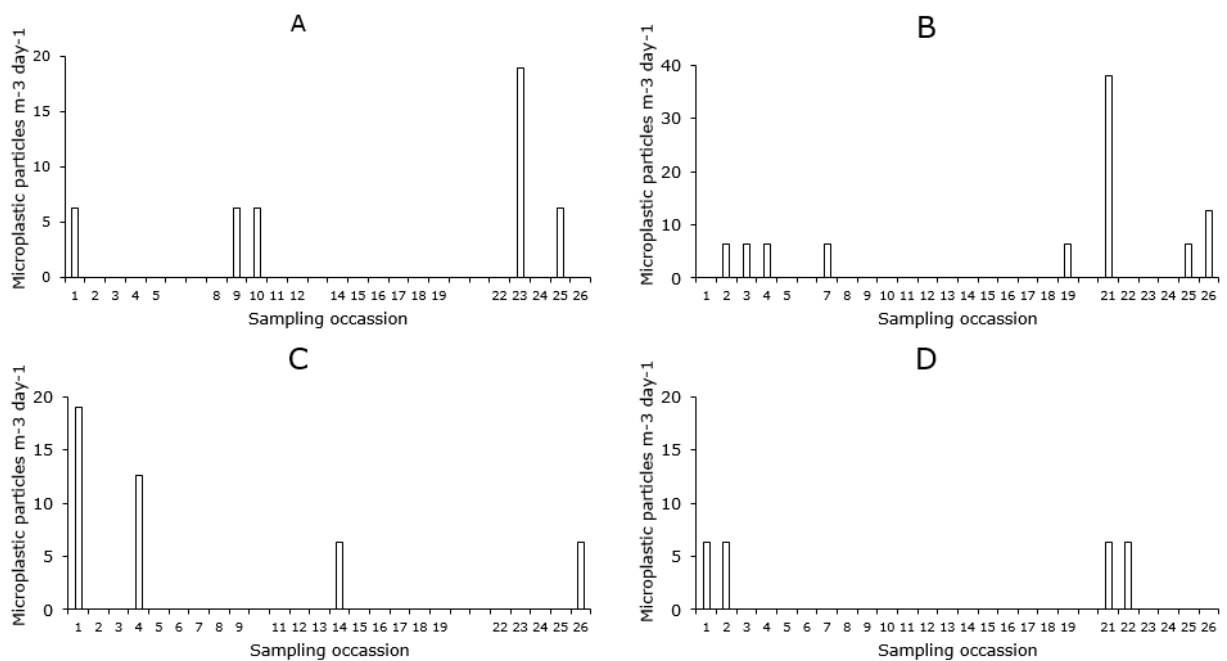
However, though not significant, mean microplastic concentrations ( $\pm$ SD) were almost four times greater downstream of Stoke-on-Trent at site RT3 ( $\bar{x}$  = 0.075  $\pm$ 0.11 particles L<sup>-1</sup>) than upstream of it at site RT2 ( $\bar{x}$  = 0.019  $\pm$ 0.04 particles L<sup>-1</sup>). The influence of the Nottingham urban area on the microplastic concentrations of the River Leen was less stark. The mean microplastic concentrations ( $\pm$ SD) were comparable at site RL2 ( $\bar{x}$  = 0.076  $\pm$ 0.06 particles L<sup>-1</sup>) and RL3 ( $\bar{x}$  = 0.083  $\pm$ 0.10 particles L<sup>-1</sup>). On the River Leen, the greatest increase in microplastic concentration was observed between site RL1 ( $\bar{x}$  = 0.019  $\pm$ 0.03 particles L<sup>-1</sup>) and site RL2 (Figure 7.2). Though located where the River Leen enters the urban area of Nottingham, anthropogenic activity near to site RL2 is extensive, highlighting the immediacy with which plastic debris associated with anthropogenic activity can enter the aquatic system.

Of the three rivers sampled in this study, the River Soar represented the largest system. There was no significant increase in microplastic concentration between any two sites sampled along the River Soar (Table 7.1). However, comparable microplastic concentrations between sites along the course of a river do not equate to comparable microplastic abundances, with water volume increasing along the river's course.

#### **Atmospheric deposition of microplastic particles**

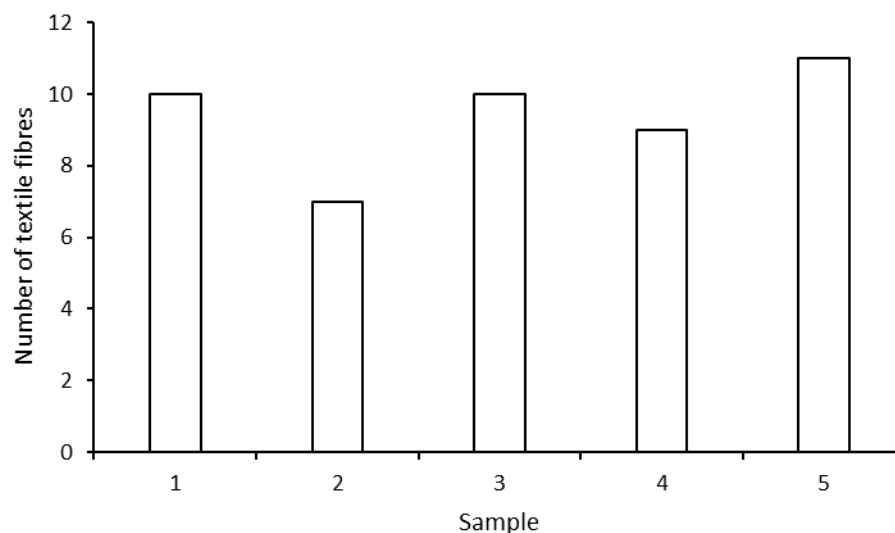
Throughout the 12 month atmospheric sampling campaign (23/11/2017-25/10/2018) samples were collected from rooftops at each site (Figure 7.2) on

a fortnightly basis. Microplastic deposition was sporadic and consistently low, with a total of 27 extruded textile fibres and eight microplastic fragments quantified across all four sites (Figure 7.5). Mean daily deposition ( $\pm$ SD) ranged from  $1.14 \pm 2.4$  to  $3.16 \pm 4.9$  particles  $m^{-2} day^{-1}$ , and the modal value for each site was 0 particles  $m^{-2} day^{-1}$ . Natural textile fibres were observed consistently across all sites throughout the sampling campaign (see Stanton et al. 2019a Chapter 5)). The high resolution sampling at site D showed little intra-site variation (Figure 7.6).



**Figure 7.5: Microplastic deposition across the four atmospheric sampling sites throughout the 12 month sampling campaign. Sampling occasion 1 was collected on 23/11/2017, and occasion 26 on 25/10/2018.**





**Figure 7.6: Textile fibre deposition in each of the five high-resolution atmospheric deposition samples collected from 04/12/2018 to 11/12/2018 at site D.**

The deposition of microplastic particles recorded in the present study is much lower than those reported previously. The sample sites in the present study were not as developed as those sampled by Dris et al. (2016), Cai et al. (2017), and some of those sampled by Bergmann et al. (2019), which is likely to contribute to the abundance of airborne particles. However, though the surface area of the atmospheric sampling device (0.011 m<sup>2</sup>) was similar to the largest device used by Allen et al. (2019) (0.014 m<sup>2</sup>), Allen et al. (2019) reported much higher mean microplastic concentrations ( $\pm$ SD) of 365  $\pm$ 69 particles m<sup>-2</sup> day<sup>-1</sup> at their remote sampling sites. Multiple environmental and methodological factors could influenced this discrepancy, including sampling height. The sampling reported here was undertaken on rooftops, as opposed to the sampling closer to ground level undertaken by Allen et al. (2019). Here we show that atmospheric deposition is a source of microplastic particles in both rural and urban reaches of the freshwater system. However, the negligible deposition recorded throughout this sampling campaign indicates that atmospheric deposition it is not a major contributor to microplastic pollution at the sites of deposition sampled for this study.

### **Temporal variation of freshwater and atmospheric microplastic particles**

Microplastic particles are known to be present in the freshwater system from source to sea (Miller et al. 2017). However, freshwater and atmospheric microplastic concentrations varied considerably throughout the sampling campaign, and were absent from 41% (51 of 123) of samples collected across the 12 month sampling campaign. The modal microplastic concentration was 0 particles L<sup>-1</sup> at six of the 10 freshwater sites samples (Figure 7.4).

Though recorded freshwater and atmospheric microplastic concentrations did vary through time, no seasonal variation in microplastic concentration was observed (Figures 7.4 and 7.5). During sample occasion five (12<sup>th</sup> and 13<sup>th</sup> March 2018) samples were collected during a storm event (Table 7.2) which saw suspended microplastic concentrations increase at some sites (Figure 7.4). This increase can be explained by the in wash of microplastic particles via surface runoff (Wagner et al. 2014; Li et al. 2018a), and the resuspension of sedimentary microplastic particles within the broader increase of the river's suspended solid loads during such an event (Hurley et al. 2018). However, the influence of this precipitation varied in the freshwater system, with microplastics concentrations at some sites also being present in similar, or lower, concentrations than their site average. Five of the 10 freshwater sites even recorded microplastic concentrations of 0 particles L<sup>-1</sup> during this sampling event. We therefore postulate that storm events can also dilute freshwater microplastic concentrations.

**Table 7.2: Variations in flux calculations, for each of the three sites in close proximity to UK NRFA gauging stations, throughout the sampling campaign. Results are presented to three significant figures. Numbers in brackets represent the codes for the NRFA gauging station used. Missing values are explained in Stanton et al. (2019a) with the exception of samples collected on 22<sup>nd</sup> and 23<sup>rd</sup> October 2018, for which no NRFA data was available at the time of publication. Samples were collected from sites RT2 and RL3 were collected on Mondays, and samples from site RS4 were collected on Tuesdays.**

Date	RT2 (28040)		RL3 (28035)		RS4 (28074)	
	$\bar{x}$ flow (m <sup>3</sup> s <sup>-1</sup> )	Microplastic flux (Particles/day)	$\bar{x}$ flow (m <sup>3</sup> s <sup>-1</sup> )	Microplastic flux (Particles/day)	$\bar{x}$ flow (m <sup>3</sup> s <sup>-1</sup> )	Microplastic flux (Particles/day)
Mon 20/11/17	0.295	2 550 000	0.323	930 000		
Tue 21/11/17					4.66	0
Mon 18/12/17	1.356	0	0.396	2 280 000		
Tue 19/12/17					10.9	31 400 000
Mon 15/01/18	1.266	-	1.13	-		
Tue 16/01/18					24.8	643 000 000
Mon 12/02/18	1.048	0	0.637	3 670 000		
Tue 13/02/18					15.4	0
Mon 12/03/18	1.8	0	3.07	88 400 000		
Tue 13/03/18					63.8	0
Mon 09/04/18	0.573	0	1.92	22 100 000		
Tue 10/04/18					56.1	162 000 000
Mon 07/05/18	0.245	0	0.656	0		
Tue 08/05/18					8.35	0
Mon 04/06/18	0.148	0	0.499	8 620 000		
Tue 05/06/18					5.48	15 800 000
Mon 02/07/18	0.086	0	0.319	0		
Tue 03/07/18					3.59	0
Mon 30/07/18	0.14	403 000	0.614	3 540 000		
Tue 31/07/18					5.2	89 900 000
Mon 27/08/18	0.159	0	0.38	1 090 000		
Tue 28/08/18					4.46	25 700 000
Mon 24/09/18	0.2	0	0.326	-		
Tue 25/09/18					3.54	61 200 000
Mon 22/10/18						
Tue 23/10/18						

This temporal variation and inconsistent relationship between particle concentration and flow can lead to considerable misrepresentation of findings when particle fluxes are calculated. Microplastic fluxes were extrapolated at

sites RT2, RL3, and RS4, which are located in close proximity to UK National River Flow Archive gauging stations. At site RS4 this flux extrapolation ranged from 0 to 643 000 000 particles depending on the sampling occasion (Table 7.3). These flux extrapolations are detailed for each sampling occasion throughout the sampling campaign in Table 7.2.

**Table 7.3: Microplastic flux estimates at sites in close proximity to UK NRFA gauging stations presented to three significant figures. Numbers in brackets represent the codes for the NRFA gauging station used. Mean flow for each station is as stated by the NRFA on 31/07/2019, and was used to calculate mean microplastic flux from the mean microplastic concentration quantified for each site in the present study. Maximum microplastic flux was calculated using the mean flow rate for the day of sampling, as detailed in Table 7.2.**

Site	Mean flow (m <sup>3</sup> s <sup>-1</sup> )	Mean microplastic flux (particles / day)	Minimum microplastic flux (particles / day)	Maximum microplastic flux (particles / day)
RT2 (28040)	0.624	1 050 000	0	2 550 000
RL3 (28035)	0.684	4 920 000	0	88 400 000
RS4 (28074)	11.729	69 900 000	0	643 000 000

### **Implications for our understanding of microplastic pollution**

Microplastic particles are ubiquitous in many environmental systems (Rochman, 2018). They are likely to be the most abundant form of plastic debris in the marine environment (Law and Thompson, 2014), with the freshwater system being a major source of marine plastic debris (Lebreton et al. 2017; Schmidt et al. 2017). However, the freshwater samples collected here are not representative of the four weeks that separated them and, therefore, this 12 month dataset is not to be interpreted as a representation of the annual variation in microplastic concentrations at the sites sampled. Moreover, whilst microplastic particles are identified at every site sampled here, the consistently low concentrations at some sites and the repeated dominance of non-plastic

anthropogenic particles in the form of natural fibres raises important questions about the relative risk that microplastics pose across some freshwater and atmospheric systems.

These systems are highly spatially and temporally variable, and by not considering this variability the findings of microplastic research risk being interpreted beyond the spatiotemporal context that they represent. Without such consideration, the subsequent public dissemination of such findings risks distracting attention from more pressing environmental concerns, including those whose harm has a stronger evidence base than that of microplastics.

## **6.5. Conclusion**

The freshwater system is an important pathway for microplastic pollution to marine and lacustrine environments and it is concerning that microplastics have been found in even the most remote environments (e.g. Bergman et al. 2019). However, whilst the presence of microplastic particles is widespread, their abundance in the environment is harder to quantify. Here we show a clear need to increase temporal consideration in sampling campaigns, and for complementary work to assess the similarity of this variability in sedimentary and biotic matrices. Extrapolation from few samples in space or time, is likely to lead to substantial errors in assessment. This research also raises important questions about sources of microplastics to environments given its observation of plastic particles, including fibres, upstream of both the urban areas and wastewater treatment processes that are often thought to represent major sources of such particles. To this end, the findings of this work bring the authors to recommend that future research into the impacts of microplastic pollution generate longer term, high temporal resolution, records of microplastics in the environment, and that they assess risk at environmentally representative concentrations.

## **6.6. Author contributions**

T.S. initiated the microplastics research and conducted all field sampling, and all laboratory processing and analysis. W.M. assisted in the FTIR spectroscopy.

All authors contributed to the interpretation of the presented data and the writing of the manuscript submitted for peer review.

## 6.7. Appendices

**Appendix 7.1: Reference list of the 93 studies collated from the Web of Science literature search used to produce figure 7.1. Bold citations indicate those that were excluded either because there was no access, or the necessary information was lacking or unclear.**

Alam, F.C., Sembiring, E., Muntalif, B.S. and Suendo, V., 2019. Microplastic distribution in surface water and sediment river around slum and industrial area (case study: Ciwalengke River, Majalaya district, Indonesia). *Chemosphere*, 224, 637-645.

Anderson, P.J., Warrack, S., Langen, V., Challis, J.K., Hanson, M.L. and Rennie, M.D., 2017. Microplastic contamination in Lake Winnipeg, Canada. *Environmental Pollution*, 225, 223-231.

Andrade, M.C., Winemiller, K.O., Barbosa, P.S., Fortunati, A., Chelazzi, D., Cincinelli, A. and Giarrizzo, T., 2019. First account of plastic pollution impacting freshwater fishes in the Amazon: Ingestion of plastic debris by piranhas and other serrasalmids with diverse feeding habits. *Environmental Pollution*, 244, 766-773.

Baldwin, A.K., Corsi, S.R. and Mason, S.A., 2016. Plastic debris in 29 Great Lakes tributaries: relations to watershed attributes and hydrology. *Environmental Science & Technology*, 50(19), 10377-10385.

**Ballent, A., Corcoran, P.L., Madden, O., Helm, P.A. and Longstaffe, F.J., 2016. Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. *Marine Pollution Bulletin*, 110(1), 383-395.**

Barrows, A.P., Christiansen, K.S., Bode, E.T. and Hoellein, T.J., 2018. A watershed-scale, citizen science approach to quantifying microplastic concentration in a mixed land-use river. *Water Research*, 147, 382-392.

Battulga, B., Kawahigashi, M. and Oyuntsetseg, B., 2019. Distribution and composition of plastic debris along the river shore in the Selenga River basin in

Mongolia. *Environmental Science and Pollution Research*, 26(14), 14059-14072.

**Biginagwa, F.J., Mayoma, B.S., Shashoua, Y., Syberg, K. and Khan, F.R., 2016. First evidence of microplastics in the African Great Lakes: recovery from Lake Victoria Nile perch and Nile tilapia. *Journal of Great Lakes Research*, 42(1), 146-149.**

Blair, R.M., Waldron, S., Phoenix, V.R. and Gauchotte-Lindsay, C., 2019. Microscopy and elemental analysis characterisation of microplastics in sediment of a freshwater urban river in Scotland, UK. *Environmental Science and Pollution Research*, 26(12), pp.12491-12504.

Bordós, G., Urbányi, B., Micsinai, A., Kriszt, B., Palotai, Z., Szabó, I., Hantosi, Z. and Szoboszlay, S., 2019. Identification of microplastics in fish ponds and natural freshwater environments of the Carpathian basin, Europe. *Chemosphere*, 216, 110-116.

**Boucher, J., Faure, F., Pompini, O., Plummer, Z., Wieser, O. and de Alencastro, L.F., 2018. (Micro) plastic fluxes in Lake Geneva basin. *TrAC Trends in Analytical Chemistry*, 112, 66-74.**

Campbell, S.H., Williamson, P.R. and Hall, B.D., 2017. Microplastics in the gastrointestinal tracts of fish and the water from an urban prairie creek. *Facets*, 2(1), 395-409.

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## 7. Thesis Summary

Plastic pollution is diverse in its sources, pathways and environmental impacts, and is an extremely emotive issue. Images of household products such as plastic bags and straws inflicting harm on organisms evoke a sense of responsibility that cannot be achieved with less tangible anthropogenic pressures such as greenhouse gas emissions and heavy metal pollution. However, though the saliency of plastic pollution has helped promote anti-plastic discourses and encouraged environmental action, these dialogues do not always acknowledge the full complexity of this pollutant. And despite extensive environmental concern, plastic is integral to much of modern day life.

In response to public and scientific concern, political and industrial stakeholders have resolved to minimise future emissions of plastic pollution, which has seen positive results (Maes et al. 2018). However, effecting and monitoring similar change specific to microplastic pollution is complicated. Attributing sources of secondary microplastics, which dominate this form of pollution, is not always possible, and the methodologies used to study microplastic pollution are inconsistent and not always reliable.

The research conducted in this thesis aimed to produce an objective spatiotemporal record of microplastic pollution in a freshwater system and in atmospheric deposition across its catchment. This required a systematic sampling campaign and a thorough assessment of the available methodologies for sample collection and particle characterisation. In meeting this aim, this research has highlighted important considerations for the future study of microplastic pollution, particularly in the freshwater system. These are:

1. The need for extensive validation of the methods used to quantify microplastic pollution that is able to account for both false positives and false negatives, and is validated using a variety of plastic and non-plastic particles found in the environment.

2. Recognition of the relative prevalence and environmental importance of natural textile fibres alongside microplastic textile fibres.
3. The need for temporal consideration of microplastic pollution.
4. Recognition of the potential role of atmospheric deposition in microplastic studies of freshwater systems.

The first of these considerations is highlighted in chapter 4, which comprehensively assessed the use of Nile red in microplastic analysis. Previously validated on a limited number of polymers and particle colours, the use of Nile red in microplastic analysis is susceptible to large degrees of uncertainty. Chapter 4 exposes the potential impact of failing to acknowledge these uncertainties, particularly when Nile red is used as a standalone technique for microplastic identification. It details the first study to highlight the limited affinity of Nile red with microplastic particles of different colours, and it provides contradictory evidence to previous publications that suggest that H<sub>2</sub>O<sub>2</sub> effectively removes biological material that might otherwise lead to false positives in microplastic counts. Nile red has quantified microplastic particles in bottled water (Mason et al. 2018), and in studies with large spatial coverage (FoE, 2019) that have received extensive media coverage.

Since the publication of Stanton et al. (2019b) (the manuscript associated with chapter 4), Prata et al. (2019b) claim to further validate the use of Nile red in microplastic identification. They assess the affinity of Nile red with natural textile fibres, identifying similar limitations to those detailed in chapter 4, with inconsistent staining of both natural and synthetic textile fibres, and that 'fluorescence was also observed in white textiles, but not attributed to staining with Nile red' (autofluorescence). However, unlike the systematic assessment of colour performed in this research, Prata et al. (2019b) do not consider the effect of the colour of the tested material on its affinity with Nile red.

Nile red has also been used in the quantification of atmospheric microplastic pollution since the publication of Stanton et al. (2019b). Klein and Fischer (2019) used Nile red to assist the identification of particles before the confirmatory analysis of a subset of particles using Raman spectroscopy.

Chapter 5 (Stanton et al. 2019a) addresses the second of these research considerations, highlighting the prevalence of natural textile fibres over their microplastic analogues in freshwater environments and the atmosphere. In this work natural textile fibres constituted 93.8% of the sampled textile fibre population. This research was the first to comprehensively quantify natural textile fibres in the context of microplastic pollution, and placed its findings alongside forensic science literature that has documented the dominance of non-plastic fibres in environmental textile fibre populations for over two decades. Not only do the findings of Stanton et al. (2019a) question the extent to which previous microplastic research has incorrectly identified natural textile fibres as microplastic fibres, but it also highlights the need for caution when promoting plastic alternatives in the context of solutions to plastic pollution.

The research detailed in chapter 5 was only possible following the development of a visual identification procedure informed by forensic knowledge of fibre morphology. This approach has since been recognised as an 'easy' step in the analysis of textile fibres for microplastic research that can complement efforts to identify fibres using Raman spectroscopy and density separation by Zhu et al. (2019). As such, it has reduced the uncertainty associated with the characterisation of textile fibres in microplastic research.

The third and fourth considerations detailed above are outlined in the findings presented in chapter 6. This chapter presents one of the most temporally comprehensive accounts of freshwater microplastic research published to date. The results of this microplastic study indicate that surface microplastic concentrations across the sampled freshwater reaches are relatively low, not once exceeding 0.4 particles per litre of water collected during the 12 month sampling campaign. By applying a systematic sampling campaign to the study of microplastic pollution, this work was able to demonstrate the importance of temporal sampling when presenting freshwater flux estimations of microplastic particles.

Chapters 5 and 6 could not avoid logistical and financial biases that limit the extent to which their findings can be deemed representative of the sampled systems. These include the sampling frequency; the volume of freshwater samples and area of the funnel used to collect atmospheric deposition; the focus on surface waters in the freshwater sampling campaign; and the reliance FTIR spectroscopy techniques that could not provide a confident identification of all of the particles that were visually identified as microplastics. The findings presented in these chapters are therefore conservative in their extrapolation beyond the sampled times and locations. However, the flux calculations detailed in these chapters do highlight the unquantified variability, and subsequent bias, of sampling campaigns that report microplastic fluxes based on temporally limited sampling campaigns.

As a single body of work, the findings of this thesis stress the need for careful consideration in the interpretation and dissemination of microplastic research. Given the limitations in previous work that this thesis identifies, the dissemination of its own findings must also be carefully considered so as not to generate unwarranted public mistrust of the scientific research that informs consumer choices. Furthermore, misrepresentation of the low levels of microplastic pollution found in this study, and the identification of potential environmental problems from plastic alternatives, risk undermining the environmental good-will that awareness of plastic pollution has fostered.

Whilst this thesis has furthered current understanding of microplastic pollution, particularly in freshwater environments, its findings have also identified knowledge gaps that should inform future research. These include:

- The importance of temporally representative sampling in freshwater microplastic research is clearly shown in chapters 5 and 6 of this thesis. Without future consideration of this, microplastic research will not be able to report microplastic fluxes from rivers accurately. To address this knowledge gap, future research must also identify the ideal spatial and temporal sampling resolutions needed in order to quantify freshwater

microplastic concentrations reliably. This could be achieved through spatially and temporally nested sampling campaigns, and should assess microplastic concentrations throughout the water column, not just in the surface waters and sediments that currently dominate freshwater microplastic research.

- Microplastic research must also not dismiss the impacts of microplastic pollution relative to other environmental pressures. In particular, there is an urgent need to assess the impact and prevalence of natural textile fibres, where current work has dismissed their potential threat informed primarily by speculation.
- As efforts to identify microplastic particles simply and cheaply continue, it is important that identification techniques be tested on a breadth of microplastic polymers, colours, and shapes, as well as a diversity of natural particulates. Failure to do so risks the widespread adoption of techniques that risk misrepresenting the prevalence of microplastic pollution.

Without adhering to these recommendations, microplastic researchers risk misleading the public, the media, industry, legislators, and others working in this field of study. In turn, this risks undermining more pressing threats to the environment such as chemical pollution, biodiversity loss, and climate change. The scientific community has a duty to inform decision makers objectively and as accurately as possible. Current scientific practices in microplastic research are not able to do this.

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## 9. Publications

On the pages that follow are the published manuscripts associated with chapters 4 and 5 of this thesis. They are:

**Stanton, T.**, Johnson, M., Nathanail, P., MacNaughtan, W., and Gomes, R.L. 2019a. Freshwater and airborne textile fiber populations are dominated by 'natural', not microplastic, fibers. *Science of The Total Environment*, 666, 377-389.

**Stanton, T.**, Johnson, M., Nathanail, P., Gomes, R.L., Needham, T. and Burson, A., 2019b. Exploring the Efficacy of Nile Red in Microplastic Quantification: A Costaining Approach. *Environmental Science & Technology Letters*, 6(10), 606-611