Microplastic, Anthropogenic Fibre, and Plasticizer Retention by Integrated Constructed Wetlands

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Abstract

Microplastic, anthropogenic fibre and plasticizer pollution from wastewater treatment plant (WWTP) effluent is an emerging environmental issue, as conventional sewage treatments typically cannot remove these contaminants. Integrated constructed wetlands (ICWs) are gaining increased interest as a promising technology for sustainable wastewater treatment, and previous research has shown that they effectively remove nutrients, but there is little known about their performance for treating emerging contaminants. Addressing this deficiency, an improved laboratory methodology for detecting microplastics and anthropogenic fibres in organic-rich wastewater and sediment samples was developed. Then, to assess the anthropogenic fibre removal performance of ICWs, a 12-month field campaign (May 2022–May 2023) was conducted involving collection of water samples at approximately monthly intervals from two ICWs (Northrepps and Ingoldisthorpe) of different ages (constructed in 2014 and 2018, respectively) in Norfolk, UK, which received sewage effluent with contrasting levels of prior treatment. Additionally, sediment samples were collected across the wetland cells to reveal storage areas within ICWs. This was supported by high frequency monitoring (hourly) over a 12-hour period to assess temporal dynamics in removal performance at the Northrepps ICW. The results revealed that ICWs can receive highly variable loads of anthropogenic fibres and microplastics, depending on the level of WWTP treatment and the time of sampling, although over 99 % were consistently retained by both ICWs, with the majority stored within the first cell. A further field campaign was conducted over six months (January–June 2024) to assess phthalate removal performance of the ICWs. The results indicated some potential for phthalate removal by adsorption to sediment and plant uptake, but further research is required. This thesis advances understanding of ICWs by demonstrating their ability to retain significant loads of microplastics and anthropogenic fibres, with implications for future wetland design and maintenance to minimize long-term leakage of these pollutants.

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Abbreviations

ABS – Acrylonitrile Butadiene Styrene

BBP – Benzyl Butyl Phthalate

BDP – Butyl Decyl Phthalate

BOP – Butyl Octyl Phthalate

BPA – Bisphenol A

BPS – Bisphenol S

DBP – Dibutyl Phthalate

DCHP – Dicyclohexyl Phthalate

DCP – Dicapryl Phthalate

DEHP – Di(2-ethylhexyl) Phthalate

DEP – Diethyl Phthalate

DIDP – Diisodecyl Phthalate

DINP – Diisononyl Phthalate

DIOP – Diisooctyl phthalate

DIUP – Diisoundecyl Phthalate

DMP – Dimethyl Phthalate

DNOP – Di-n-octyl Phthalate

DPP – Diphenyl Phthalate

HDPE – High-Density Polyethylene

LDPE – Low-Density Polyethylene

ODP – Octyl Decyl Phthalate

PBT – Polybutylene Terephthalate

PC – Polycarbonate

PET – Polyethylene Terephthalate

PFAS – Per- and Polyfluoroalkyl Substances

PLA – Polylactic Acid

PP – Polypropylene

PS – Polystyrene

PU – Polyurethane

PVC – Polyvinyl Chloride

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Chapter 1

Introduction

1.1 Introduction to plastics

Plastics are man-made, synthetic, polymeric materials that can be classified as thermosets (insoluble and infusible in their final shape) or thermoplastics (repeatedly softened by heating and re-hardened on cooling). A polymer is a large molecule with a high molecular weight consisting of numerous similar units bonded together (monomers). Monomers are generally simple organic molecules containing a double bond or a minimum of two functional groups (small groups of atoms that exhibit a characteristic reactivity).

Because of their large molecular size, polymers possess unique chemical and physical properties. For example, plastics are highly resistant to many chemicals (including acids, bases and solvents), lightweight, durable, flexible (designed to be either rigid or flexible), possess good thermal insulation properties, are electrical insulators, are easily mouldable into complex shapes, and can be easily coloured or transparent (Ehrenstein and Theriault, 2001). Plastics have significant advantages over alternative materials, they are lightweight, easy to manufacture, inexpensive, abundant, durable, resistant to corrosion and moisture, low maintenance, and hygienic. It is for these reasons that they have replaced alternative materials such as wood, metal, rubber, concrete, and ceramics. Plastics have therefore provided significant benefits to society in their application (Andrady and Neal, 2009) and have thus become embedded in our everyday lives.

The first plastic, named Parkesine, was produced in 1862 by Alexander Parkes. Parkesine was a cellulose nitrate material, produced by dissolving cotton fibres in nitric and sulphuric acids, then mixing with vegetable oil (Rasmussen, 2021). Parkesine was a cheap and colourful alternative to tortoiseshell or ivory, for which demand was high as industrialization increased. Plastic production has been rising exponentially since mass production began in the 1940s (Figure 1.1), spurred on by the Second World War when production went from <100,000 tonnes in 1939 to 365,000 tonnes in 1945 (ENL, 2022). During this period there was a need to preserve natural resources that were essential to the war effort, and plastic therefore replaced many consumer products. For example, Japan's conquest of southeast Asia reduced the supply of natural rubber to the United States, encouraging a synthetic alternative to be developed. Plastics were also used in military equipment, for example nylon, a durable material, was used in ropes, parachutes, helmet liners and body armour, artificial glass was used as an alternative in aircraft windows, polyethylene was used to insulate radar, and acrylic sheets were moulded into noses for bombers. Fast forward to modern times and, in 2019, global plastic production was almost 460 million tonnes (Our World in Data, 2023), with seven polymers making up most of this (Figure 1.2). Today the

most common use of plastic is in packaging, accounting for 44 % of total consumption (Figure 1.3).

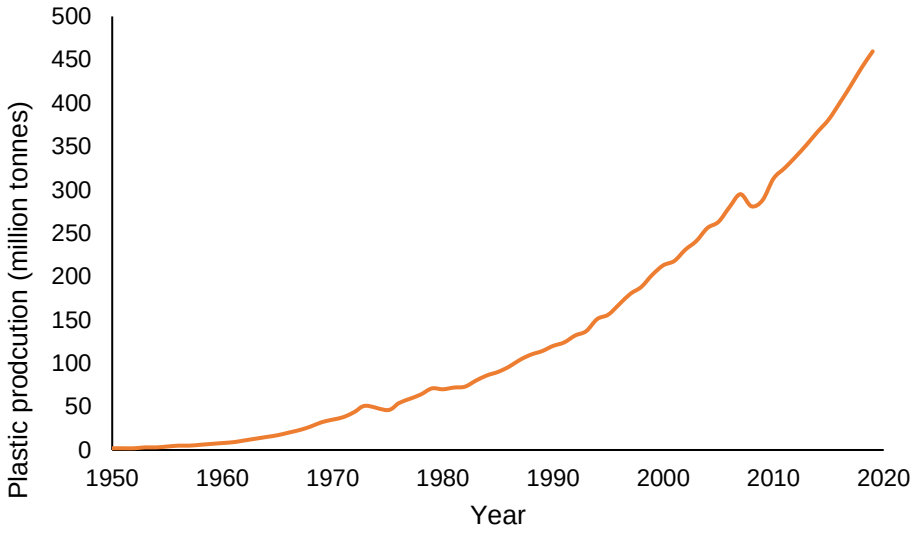


Figure 1.1 Global annual plastic production between 1950 and 2019. Data from Our World in Data (2024).

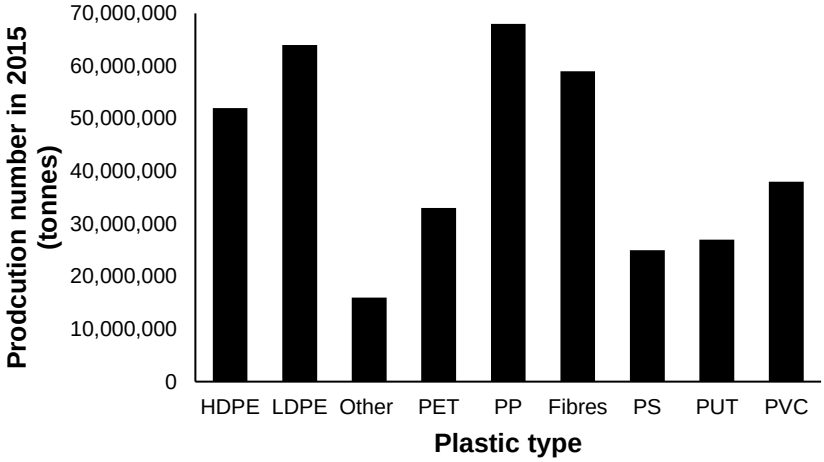


Figure 1.2 Global primary plastic production by polymer type. HDPE (High-density polyethylene); LDPE (Low-density polyethylene); other polymer types; PET (Polyethylene terephthalate); PP (Polypropylene); fibres (including polyester, polyamide, and acrylic), PS (Polystyrene); PUT (Polyurethane); and PVC (Polyvinyl chloride). Data from Geyer, Jambeck and Law (2017) referenced in (Our World in Data, 2023b).

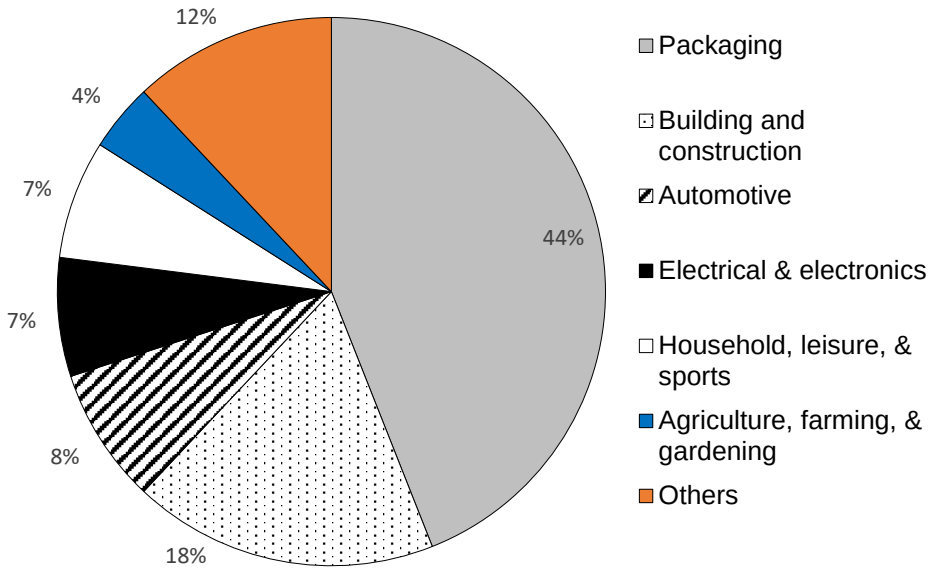


Figure 1.3 Global plastic consumption share 2021, by application. Data from Statista (2024).

The average lifespan of plastic products is approximately ten years, although plastic packaging, the main use of plastic, has an average product lifetime of just 0.5 years (Statista, 2024). Consequently, packaging accounted for 40.2 % (141.96 million tonnes) of the plastic waste generation in 2019 (353 million tonnes) (Statista, 2023a). In 2019, 82 million tonnes of plastic waste were mismanaged and littered (Figure 1.4). China and India accounted for the largest share of globally mismanaged plastic waste in 2019 (20 % and 21 %, respectively) (Statista, 2023a), although exports of plastic waste by developed countries to these regions contributes to the mismanagement (Barnes, 2019).

Accordingly, plastic contamination in aquatic environments has risen, with an estimated 19 to 23 million tons year^{-1} entering aquatic ecosystems in 2016 (Borrelle *et al*., 2020), much of which originates from packaging (Bergmann *et al*., 2015).

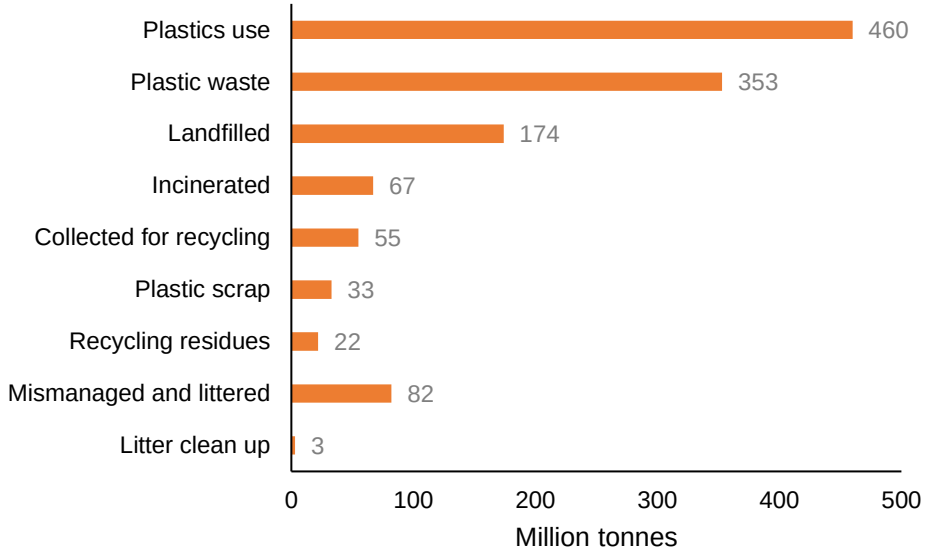


Figure 1.4 Lifecycle of plastic waste worldwide in 2019. Data from Statista (2023a).

1.2 Introduction to microplastics

Microplastics in the environment originate from either manufactured primary microplastics, or from the mechanical degradation of macro plastics (termed secondary microplastics). There is little agreement on the upper and lower size limits of microplastics, although generally the upper is 5 mm and lower 20 µm (Frias and Nash, 2019). Below this size, microplastic visual identification and chemical verification is often prohibitively difficult with common microplastic laboratory equipment. Microplastic sources are numerous, partly because they are a highly diverse contaminant class (Rochman *et al*., 2019), with >10,000 substances used in various plastics (Wiesinger *et al*., 2021). Microplastics can be broadly divided into primary and secondary categories. Primary microplastics are those that have been manufactured to a size of < 5 mm, such as plastic pellets and cosmetic beads. Secondary microplastics are produced from the mechanical, biological, and chemical degradation of larger plastics. For example, degradation of PP films (like those commonly used in packaging) in air produced sub-mm particles between 9-months and 3.2 years, with higher fragmentation rates in high temperatures and higher total solar radiation (Huber *et al*., 2022). Since the COVID-19 pandemic personal protective equipment has become a significant source of secondary microplastics, for example a single face mask can release approximately 173,000 anthropogenic fibres per day under ambient conditions (Saliu *et al*., 2021), while the proportion of masks in litter increased by >80-fold during the pandemic (Roberts *et al*., 2021).

1.3 Potential environmental impacts of microplastics in aquatic environments

Microplastics are a contaminant of emerging concern primarily because they are very slow to degrade in the environment, meaning negative impacts will be exemplified with continued deposition. Polymer degradation is the change of polymer properties caused by physical, biological, or chemical reactions (Figure 1.5). The most important degradation processes are (Singh and Sharma, 2008):

- Physical degradation (abrasive, thermal)
- Photodegradation (normally near-UV radiations 290-400 nm)
- Chemical degradation (hydrolysis or oxidation)
- Biodegradation

Plastics are generally slower to degrade in aquatic environments compared to terrestrial because exposure to UV light is lower, thus photooxidation rates are less (Duan *et al*., 2021). Plastics can therefore remain in the aquatic environment for decades or hundreds of years (Wang *et al*., 2016).

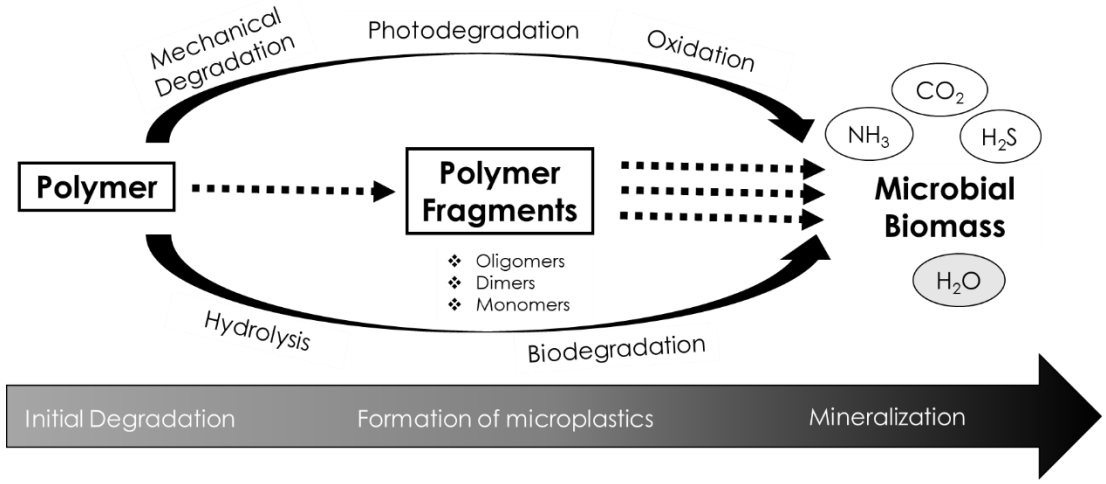


Figure 1.5 Processes involved in polymer degradation in aquatic environments. Based on (Wagner and Lambert, 2018, p.61).

The small size and large number mean microplastics can be easily ingested by a broad range of animals, presenting toxic hazards. The effects of ingestion include starvation caused by gut obstruction, false satiation, changes in behaviour, reduced fitness, and altered reproduction and growth (Gall and Thompson, 2015). Additionally, compared to natural particles, microplastic particles have been shown to have some negative effects on aquatic invertebrates (Doyle, Sundh and Almroth, 2022). However, the experimental designs of microplastic particle toxicity studies are frequently flawed because they do not properly compare effects to those by natural particles, rendering many studies uninformative (Ogonowski, Gerdes and Gorokhova, 2018). Ingestion of plastic has been

observed by a wide variety of organisms, including fish (McIlwraith *et al*., 2021), birds (Clark *et al*., 2023), invertebrates (Scherer *et al*., 2017), reptiles (Clause *et al*., 2021), and mammals (Thrift *et al*., 2022), although population level impacts of plastic ingestion have not been established (Gall and Thompson, 2015). Identifying the harmful impacts of microplastic consumption is a developing field of study, although a significant concern is of toxic substances associated with microplastics. Microplastics can adsorb heavy metals and persistent organic pollutants from the aquatic environment, impacting their distribution and potentially having toxic impacts on aquatic life (Guo and Wang, 2021, Rochman *et al*., 2013). However, the abundance of microplastic is lower than suspended sediments/other media, that can also adsorb similar contaminants, meaning the significance of microplastics as a vector for contaminants may be low (Koelmans *et al*., 2016). Plastic additives can also be released from microplastic fragments, such as phthalates and BPA, potentially having deleterious impacts on organisms (Godfray *et al*., 2019).

Although risk assessment for microplastics is complicated (Koelmans *et al*., 2023) and consensus has not been achieved, it remains important that actions are taken in the present to reduce the contamination of freshwaters by microplastics (Horton, 2022).

1.4 Sources of microplastics in rivers

Microplastics have been found in surface water (Woodward *et al*., 2021), river sediment (Horton *et al*., 2017) and in freshwater macroinvertebrates (McGoran *et al*., 2017). There are a number of ways that microplastics can arrive in waterbodies, for example microplastics can be deposited from the atmosphere (Napper *et al*., 2023), enter via direct road and land runoff (Horton *et al*., 2017), and accidental and deliberate littering (Vaughan, Turner and Rose, 2017). A major, consistent, source of microplastics to rivers is from wastewater treatment plant (WWTP) and combined sewer overflow (CSO) effluent. England has a wastewater system that combines rainwater and domestic sewage in the same pipes, meaning that during heavy rainfall the capacity of these pipes can be exceeded, potentially causing backup and flooding in properties (Chaplin, 2020). As a result, CSO's frequently discharge during heavy rainfall events to prevent damage to infrastructure and homes. In 2021, 372,533 spills from CSOs were recorded in the UK, for a combined duration of 2.7 million hours (Giakoumis and Voulvoulis, 2023). A CSO on the River Tame, UK, was estimated to be capable of releasing up to 3.2 ± 0.3 million microplastic particles per day (Woodward *et al*., 2021), although there is a large plastic industry within the catchment, so these values are likely higher than for an average UK CSO.

Wastewater treatment processes remove a large proportion of incoming microplastics, up to 99.9 %, although some are dismissed in effluent, even at plants with tertiary treatment (Carr *et al*., 2016). A review of 21 published studies by Iyare *et al*. (2020) found that the average microplastic removal was 72 % during preliminary and primary treatment, 88 %

after secondary treatment and 94 % after tertiary treatment. Microplastic concentrations ranged from 1–7216 microplastics L^{-1} entering WWTPs and from 0.0007–54 microplastics L^{-1} in WWTP effluent (Iyare *et al*., 2020). A large part of this variability is likely explained by the different sampling techniques and lower size limits for microplastic identification used in these studies because WWTPs are generally better at removing microplastics >100 µm (Talvitie *et al*., 2017). Given the high removal rates at WWTPs, sewage sludge is rich in microplastics, meaning WWTPs contaminate agricultural land when sewage sludge is spread as fertiliser. Consequently, between 31,000 and 42,000 tonnes of microplastics (1–5 mm) are applied to agricultural soils in Europe per year (Lofty *et al*., 2022).

WWTPs discharge large volumes of effluent, meaning they can be a significant, continuous, source of microplastics. A high proportion of microplastics in WWTPs are in the form of fibres (Figure 1.6), largely deriving from laundry items and the breakdown of synthetic bathroom wipes (Briain *et al*., 2020, Browne *et al*., 2011). Fibres can represent up to 100 % of microplastics in riverbed sediment samples (Figure 1.7), and are easily flushed downstream (Woodward *et al*., 2021). Following installation of washing machine micro fibre filters in 10 % of homes serving a WWTP, micro-fibre concentration has been observed to decline by 41 % in WWTP effluent, highlighting the importance of laundry fibres as a microplastic source (Erdle *et al*., 2021). Technologies do exist to reduce fibre loads from washing machines (McIlwraith *et al*., 2019), although their uptake has not been widespread. Some authors suggest that there is little value in further microplastic removal efficiency studies in WWTPs because it is known that removal rates are high and that there are few technological solutions that are not prohibitively expensive (Frehland *et al*., 2020).

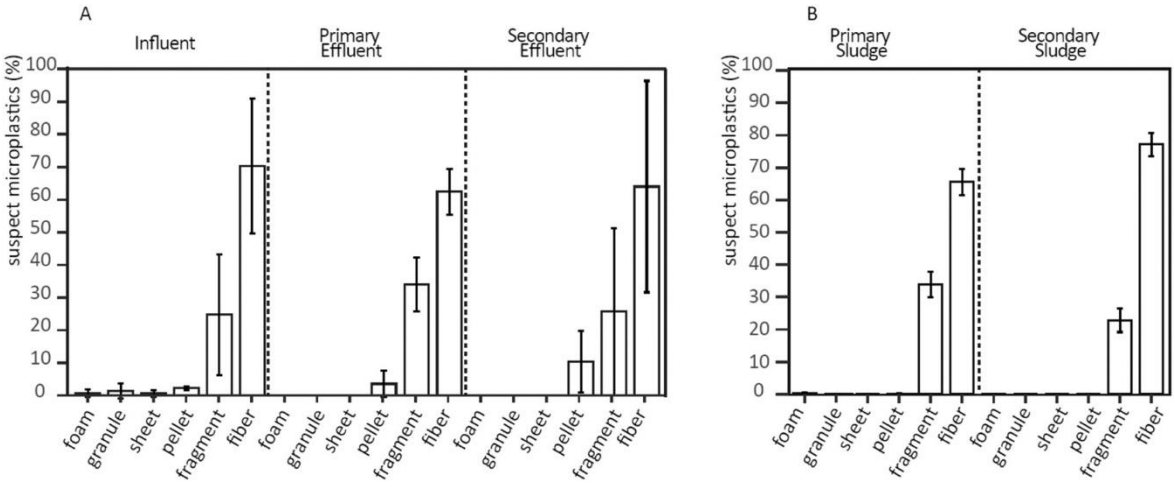


Figure 1.6 Microplastic type (foam, granule, sheet, pellet, fragment, and fibre) from liquid (A) and solid (B) wastewater treatment plant samples from a large WWTP (serving 1.3 million people) in Vancouver, Canada (Gies *et al*., 2018).

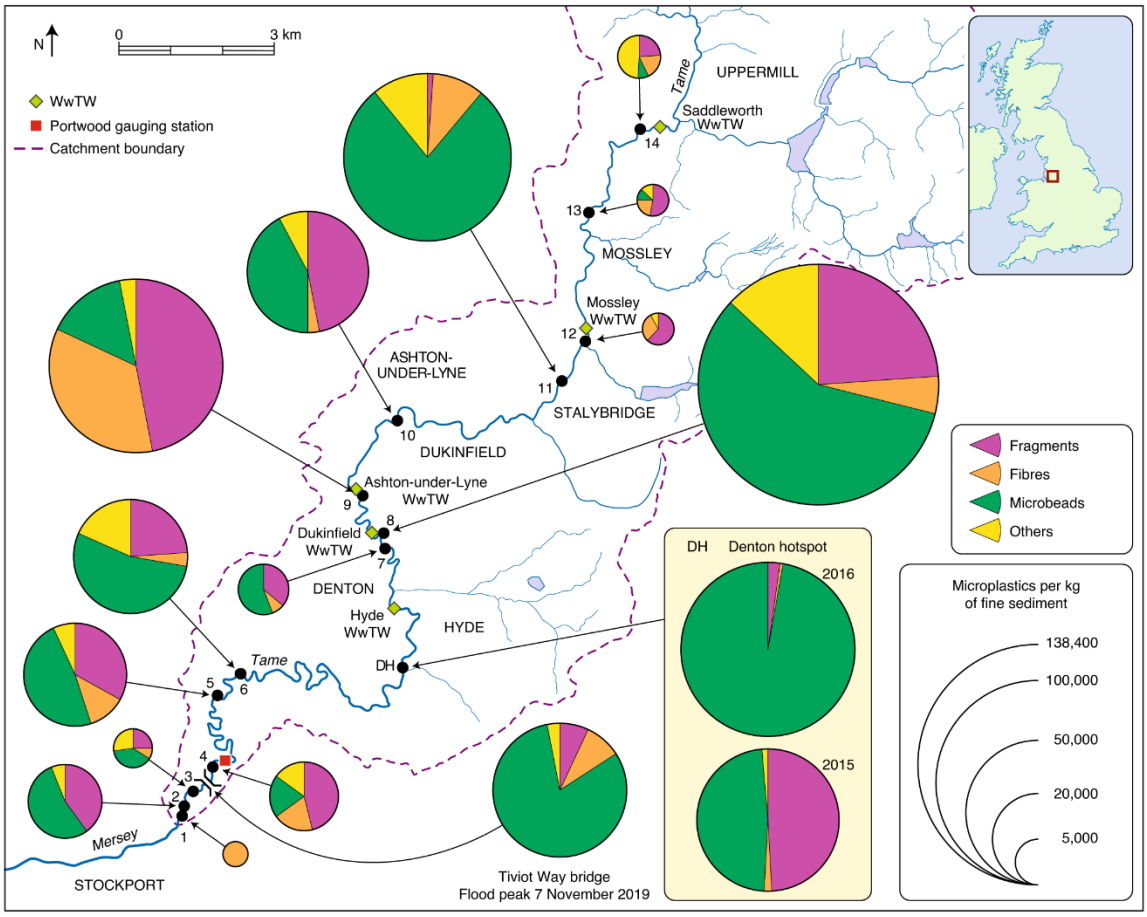


Figure 1.7 Concentrations and types (fragments, fibres, microbeads, and other) of microplastics found in fine bed sediment samples in the River Tame, UK (Woodward *et al*., 2021). A large plastic industry exists within the catchment of this River, contributing to the high microbead counts.

1.5 Differentiating anthropogenic fibres and microplastics

In chapter four and five of this thesis anthropogenic fibres and microplastics are identified separately in samples. This approach was chosen to avoid overlooking non-plastic fibres that may present similar environmental concerns as plastic counterparts. Anthropogenic fibres are an extensive environmental contaminant originating predominately from textiles, as well as cigarette filters and personal care products (e.g., wet wipes, face masks). Clothing items can shed a large number of fibres during washing (up to 700,000 per load) (Napper and Thompson, 2016), a large amount of which are transported to WWTPs, unless fibre capture devices are used (McIlwraith *et al*., 2019). As a result, fibres are often the most widely reported microplastic type in WWTP effluent. The worldwide textile fibre production in 2021 was: 54 % polyester, 22 % cotton, 12 % other cellulosic, 5 % polyamide, 5 % other synthetics, 2 % animal fibres (Statista, 2023). If only plastic fibres are included in analysis, a significant portion of anthropogenic fibre emissions from WWTP effluent are likely to be

missed. Non-plastic fibres are quicker to degrade than plastic ones, for example 76 % of cotton yarns were biodegraded after 243 days in simulated aquatic environments, compared to just 4 % for polyester yarns (Zambrano *et al*., 2019). However, non-synthetic fibres are still relatively persistent in the environment and present similar impacts on organisms in terms of ingestion (Walkinshaw *et al*., 2023) and leaching of chemical additives (Carney Almroth *et al*., 2021). Chemical treatments are applied to non-synthetic fibres, for example cotton is naturally flammable, easily wrinkled, and prone to microbial degradation, so chemicals are applied to change these properties (Lam, Kan and Yuen, 2012). Flame retardants are added in the form of halogenated compounds such as chlorinated paraffins or poly-decabromodiphenyl esters which have been linked to neurotoxic effects (Yang *et al*., 2012). To create resistance and improve dye fastness of fabrics, silicone-based softeners and formaldehyde-based resins are added to fabrics which have been shown to bioaccumulate and be carcinogenic (Ji *et al*., 2024). Azo dyes are among the most commonly used to dye natural and synthetic fabrics, despite the environmental and health risks associated. On break down, aromatic amines are produced which can be carcinogenic. Sorensen *et al*. (2021) even found higher concentrations of additives in wool fibres than polyester, including phthalates. Moreover, the precise composition, concentration, and chemical cocktail of the additives used within clothing production (and such released microfibres) are unknown. This lack of transparency complicates the risks to aquatic and terrestrial ecosystems associated with the release of chemically laden microfibres. As a result, there is increasing pressure for non-synthetic fibres to not be classified as 'natural' due to the extensive chemical processing during manufacturing (Stanton *et al*., 2024). Non-synthetic fibres are therefore important to identify in environmental samples (Athey and Erdle, 2022).

1.6 Microplastics in drinking water

The World Health Organisation (WHO) does not recommend routine monitoring of microplastics in drinking water, principally because there is no evidence that it is a human health concern. Conventional drinking water treatment should be effective at removing small particles, including microplastics, from source water (Na *et al*., 2021). An important consideration is that drinking water treatment may not be effective in other less developed countries (World Health Organization, 2023), leading to potentially higher microplastic contamination in drinking water. In the UK context, most drinking water microplastic contamination likely originates from the distribution system. For example, plastics are used within the water treatment and delivery system, including polyamide as coagulant aids, plastic membrane filters, polyethylene and polyvinylchloride water distribution pipes, and polyurethane for relining pipes. Drinking water bottles are also mostly made from PET, while bottle lids are often polypropylene or polyethylene. In a review study across 34 countries, Sun *et al*. (2024) found that microplastics were detected in 87 % of 1148 drinking water

samples, and concentrations stretched seven orders of magnitude, with the cumulative concentrations at 5th, 50th, and 95th percentiles of 0.028, 4.491, and 728.105 microplastics L^{-1}, respectively (Sun *et al*., 2024). They also found that the concentrations of microplastics in tap waters were very close to those of bottled water, while particles below 50 µm were the dominant size fraction globally (Sun *et al*., 2024).

Various analytical techniques (with varying degrees of reliability) have been used to quantify microplastics in drinking water, consequently making comparisons between studies is difficult, if not impossible (Oßmann, 2021). This also complicates comparing the intake of microplastics by drinking water with other routes (such as food), meaning the importance of microplastic concentrations in drinking water are unclear. As a result, there have been calls for the harmonization of analysis methods for detecting microplastics in drinking water (Primpke *et al*., 2020), although in practice this remains problematic.

1.7 Public perceptions of microplastic

Public perceptions of microplastic pollution have evolved, largely driven by media coverage, scientific research, and advocacy efforts. These perceptions have, in turn, influenced policy responses aimed at mitigating the impact of microplastics.

One of the earliest studies reporting microplastic contamination was Carpenter and Smith (1972), who found 3500 microplastic km^{-2} in the western Sargasso Sea and stated that “plastics could be a source of some of the polychlorinated biphenyls recently observed in oceanic organisms”. The awareness of microplastic pollution began to rise in the early 2000s, following research that highlighted the presence of these particles in marine environments (Moore *et al*., 2001; Thompson, 2004; Browne, Galloway and Thompson, 2007; Betts, 2008; Fendall and Sewell, 2009). Media coverage played a crucial role in bringing these scientific findings to the public’s attention. Documentaries, news articles, and social media campaigns have significantly contributed to increasing public awareness and concern about microplastic pollution. Documentaries like "A Plastic Ocean" (Plasticoceans, 2018) and widespread news coverage of incidents like the discovery of microplastics in remote locations (National Geographic, 2019), in human testicles (Euronews, 2024) and blood clots (ScienceAlert, 2024) have been particularly impactful. Environmental NGOs have been at the forefront of raising awareness and advocating for action on microplastic pollution. Campaigns by organizations such as Greenpeace and the Plastic Pollution Coalition have mobilized public support and pressured policymakers to address the issue.

The visibility of plastic pollution on beaches and in oceans helps stimulate public concern (Hartley *et al*., 2018). Images of wildlife affected by plastic waste, such as turtles entangled in plastic bags, resonate strongly with the public. Potential health risks associated with microplastics, such as their presence in drinking water and seafood, are significant drivers of public concern (Forleo and Romagnoli, 2021). There are also common misconceptions

about the sources and solutions to microplastic pollution. For instance, while banning single-use plastics is important, it is not a comprehensive solution, as microplastics also originate from sources like synthetic clothing and tyre wear.

Public perception has been a critical factor in shaping policy responses to microplastic pollution. Policymakers are responsive to public concern, which can drive legislative and regulatory actions. For example, the European Union has implemented the Single-Use Plastics Directive, which bans certain plastic products and promotes the use of sustainable alternatives (Kiessling *et al*., 2023). In response to public campaigns and scientific evidence, the UK government implemented a ban on microbeads in rinse-off cosmetics in 2018 (Meier, 2018). Similarly, the Microbead-Free Waters Act in the United States bans the manufacture and sale of rinse-off cosmetics containing plastic microbeads (McDevitt *et al*., 2017).

Public demand for action has also pressured corporations to take responsibility for their plastic waste (Landon-Lane, 2018). Many companies have committed to reducing plastic packaging, using recycled materials, and supporting cleanup efforts. This corporate shift is often driven by consumer expectations and the desire to maintain a positive public image.

Despite progress, several challenges remain in addressing microplastic pollution through policy. While public concern about microplastics is high, scientific understanding of their health impacts is still evolving. Policymakers must navigate this uncertainty and balance precautionary measures with evidence-based actions. Microplastic pollution is also a global issue that requires coordinated international action. Ensuring that policies are harmonized across countries and that developing nations are supported in their efforts is crucial. Continued public engagement is essential for sustaining momentum. This includes not only raising awareness but also educating the public about effective actions and addressing misconceptions.

1.8 Integrated Constructed Wetlands

Since WWTPs are a source of microplastics and expensive technological installations are required to retain 100 % of microplastics, a cheaper solution is required. Here integrated constructed wetlands (ICWs) may be applicable. ICWs are plant-soil-water systems designed to enhance water quality. ICWs generally consist of a series of connected pools containing shallow water year-round. Water flows freely on the surface through the stands of planted emergent vegetation. The ICW concept integrates open water ponds with shallow, vegetated marshes to mimic a natural wetland, while replicating the simultaneous physical, chemical, and biological treatment processes that occur in such environments. ICWs balance ecological aims of wetland restoration and engineering targets for economically and consistently enhancing water quality. The emphasis in ICWs, compared to other constructed wetland layouts, is on integration with the surrounding environment,

thereby increasing both natural and social capital, which are key objectives in the UK government's 25-year Environment Plan (DEFRA, 2018), and offering advantages beyond conventional 'grey' infrastructure solutions (Stantec, 2021). Other types of constructed wetland include horizontal subsurface flow, where water flows horizontally through a filtration layer (Vymazal, 2005), and vertical flow constructed wetlands, where water flows vertically through filtration layers (Torrens *et al*., 2009) (Figure 1.8).

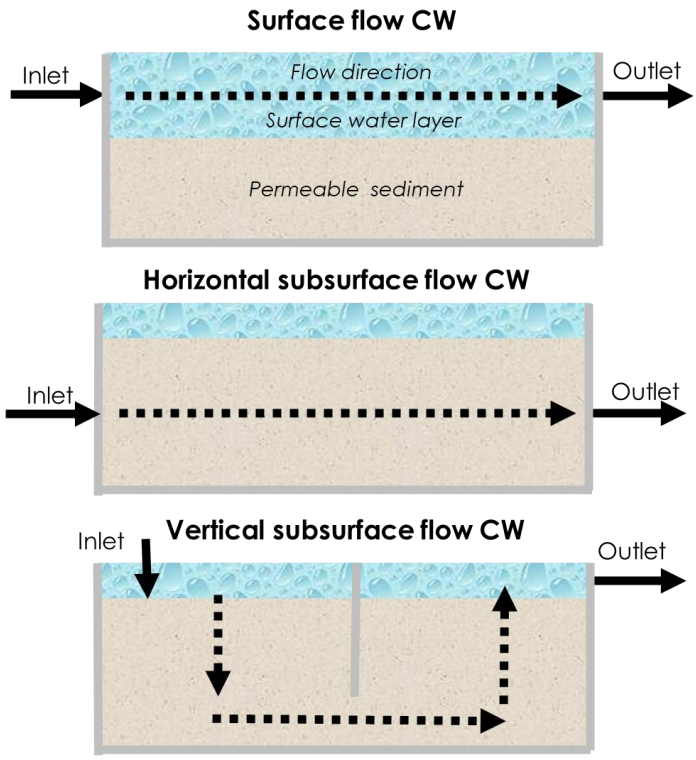


Figure 1.8 Basic diagrams showing common constructed wetland design types.

ICWs are designed to have as a low as flow velocity as possible to maximise time available for pollutant removal by plants (Scholz *et al.,* 2007). In practice, hydraulic retention times of 5–30 days are recommended for surface flow constructed wetlands receiving wastewater effluent (Wu *et al*., 2015). The low water velocities present in ICWs promote sedimentation of suspended particulates, including microplastics and anthropogenic fibres (Figure 1.9).

Because of their low density and slow settling velocity, it is generally assumed that microplastics behave most like fine grained sediments (e.g., silt, clay) and organic material (Helcoski *et al.,* 2020). Suspended sediments can settle on submerged vegetation, increasing the suspended sediment removal efficiency by up to 33 % compared to unvegetated areas (Elliott, 2000). Horppila and Nurminen (2005) found that submerged plants reduce average sediment resuspension by 67 % compared to unvegetated areas of a shallow lake. Water velocities >20 mm s^{-1} can cause sediment particles to dislodge from the vegetation and re-suspend (Elliott, 2000). However, the particle shape, density, and size

(which are highly variable for microplastics) will impact particle deposition and transport (Waldschläger and Schüttrumpf, 2019). Microplastic dynamics in freshwater environments may therefore be dissimilar to natural sediments (Mendrik *et al.,* 2023).

The settling velocity of microplastics is enhanced by biofilm growth on microplastics due to an increase in particle specific density and size. Compared to clean ones, Mendrik *et al*. (2023) found that bio-fouled plastics had on average a 40 % higher settling velocity. Total phosphorus and total nitrogen are positively associated with the growth rate of biofilms on microplastics (Wang, Guo and Xue, 2021), thus enhanced removal due to biofilm formation is expected in nutrient rich ICW waters. The epibiont and biofilm coverage on submerged vegetation may also enhance microplastic trapping in densely vegetated constructed wetlands. Heterotrophic epibiont species can catch suspended microplastics and surround them with organic matter (Goss *et al*., 2018). Bacteria are known to colonise submerged aquatic vegetation in densities commonly ranging 10^5 to 10^7 cm^{-2} (Baker and Orr, 1986, Korner, 1999). Submerged plant epiphytic bacterial community composition changes depending on whether plants are living or decomposing (Han *et al*., 2019), potentially impacting microplastic adhesion. Furthermore, reduced water flow rates have been shown to increase the surface roughness of submerged plant epiphyte communities (Han *et al*., 2018), potentially increasing microplastic attachment. Biofilms can also attach microplastics to vegetation, even on epiphyte free vegetation (Goss *et al*., 2018).

Preferential flow pathways around flow resistant vegetation stands in ICWs may hinder the microplastic settling rates on submerged vegetation. Helcoski *et al*. (2020) found that microplastics were most abundant on the edges of dense emergent vegetation stands in tidal mudflats. This occurs because the submerged vegetation traps suspended microplastics before they can reach the interior of the vegetation stand. Most ICWs will be designed to minimise preferential flow pathways because this is also beneficial from a nutrient removal perspective.

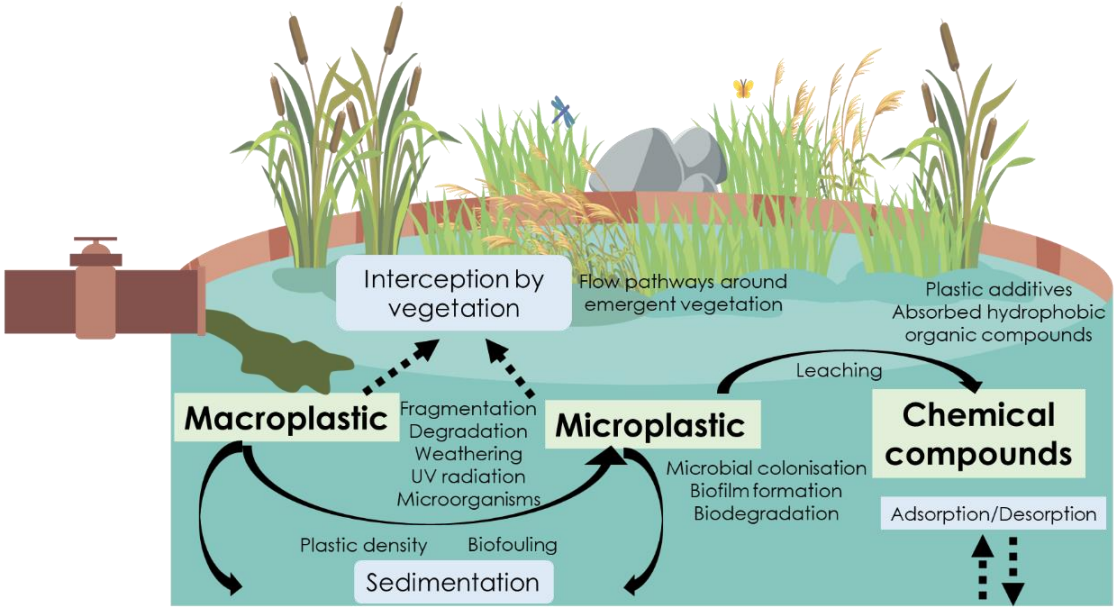


Figure 1.9 Plastic cycling in constructed wetlands.

1.9 Current evidence of microplastic retention by constructed wetlands

Current evidence shows that surface flow constructed wetlands can retain microplastics to a relatively high degree. However, there is a literature gap, being that no studies have assessed retention efficiencies during winter months.

In a surface flow CW in Northern China, the Lingang Ecological Wetland Park, average microplastic removal rates were 29.4 % (Zhou *et al*., 2022). In a nearby surface flow CW, Konggang, microplastic removal rates were 43.65 % (Zhou *et al*., 2022). These removal rates are reported based on the numbers of microplastics down to a reported size of 20 µm. In the Lingang SFCW, larger particles were better retained than smaller particles. Fibres were most well retained, and fragments least well retained in both the Lingang and Konggang SFCW. All data were collected from September to October 2020 (Zhou *et al*., 2022).

In a horizontal subsurface flow CW in Aalbeke, Belgium, average microplastic concentration declined from 6.45 particles L^{-1} in wetland influent to 0.77 particles L^{-1} in effluent, corresponding to an 88 % removal efficiency (Wang *et al*., 2020). The microplastic shape distribution in the CW influent was 54 % fibres, 43 % particles, and 4 % films, and in the CW effluent was 71 % fibres, 28 % particles, and 1 % films. Films and particles are thus better retained than fibres by this wetland. The areal removal rate was calculated at 3120 MPs m^{-2} d^{-1} (Wang *et al*., 2020). This study assessed a horizontal subsurface flow CW, meaning effluent from the wetland comes only from within the soil layer, not from surface flowing water.

The study identified in the literature that is most like the present study is that by Bydalek *et al*. (2023). Their study aimed to assess microplastic fate in a free water surface constructed wetland: the Cromhall ICW, Gloucestershire, UK. Here the loading rate is 1400 m^3 per day from a secondary treatment WWTP serving 2000 people. The Cromhall ICW surface area is approximately 8000 m^2 with 12 cells (Figure 1.10). At the Cromhall ICW, the first cell has 10 % surface plant cover, acting as a settling pond, cells 2-4 have over 90 % cover, and cells 5-12 have between 30-80 % cover. Vegetation here is dominated by *Schoenoplectus lacustris* and *Typha angustifolia*.

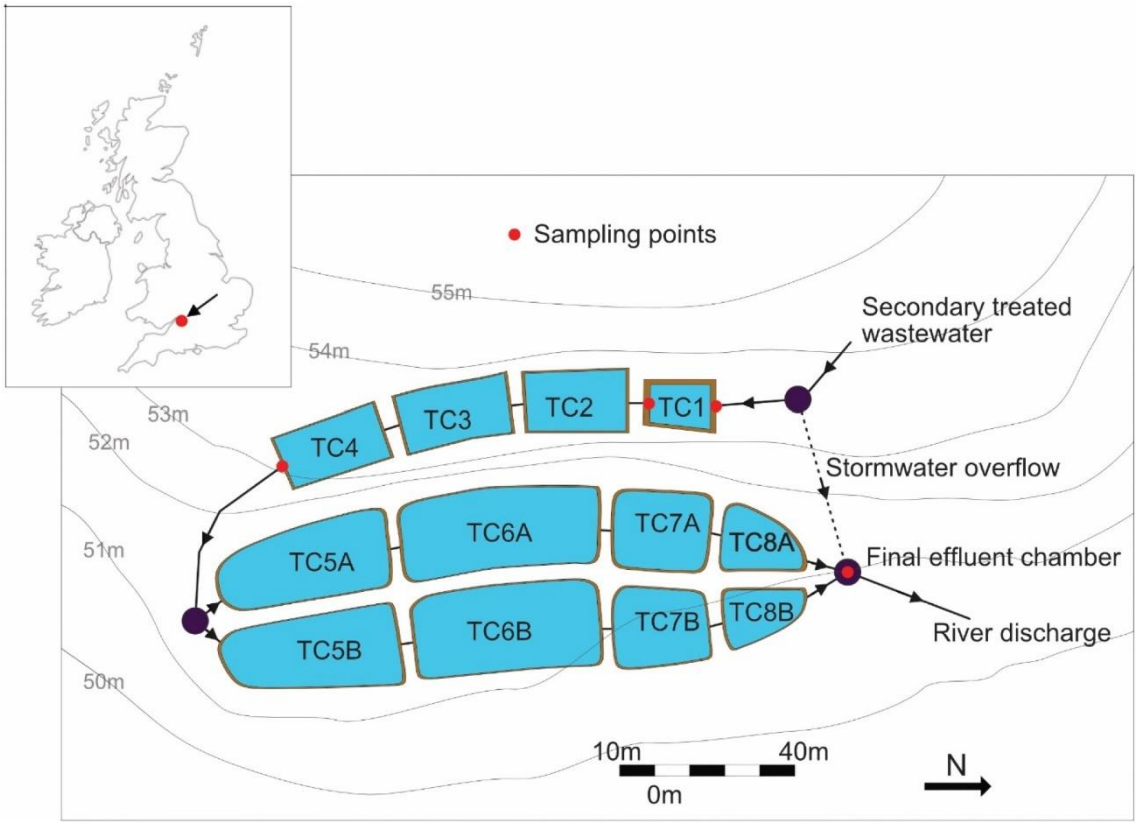


Figure 1.10 Schematic of the Cromhall ICW design (Bydalek *et al*., 2023).

The Cromhall ICW received an average of 6 microplastics L^{-1}, equating to loading rates of over 5 million microplastics day^{-1}, most of which (55 %) were fibres (Bydalek *et al*., 2023). Microplastic concentrations in outlet water samples were 0.3 microplastics L^{-1}, equating to a removal efficiency of 95 % (Bydalek *et al*., 2023).

An analysis of data quality was performed in the studies assessing microplastics in constructed wetlands. These studies were graded against criteria developed by Koelmans *et al*. (2019) that attempts to assess the quality of microplastic analysis. Nine criteria are employed that aim to maximise the reproducibility of microplastic analysis methods. Koelmans *et al*. (2019) recommend the assignment of a value of 2 (reliable), 1 (reliable to a limited extent), or 0 (unreliable) to each criterion. No 'zero' values should be scored for any study to be deemed adequately reliable. Table 1.1 demonstrates that there are

undesirable features of these studies. All studies scored a zero under the ‘sample processing and storage’ criteria because sieves were rinsed into glass containers on site and re-used for the next sample. Ideally, sieves would be sealed and transported back to the laboratory where they can be thoroughly rinsed under a laminar flow cabinet (Koelmans *et al*., 2019). Some studies also failed to run positive controls, meaning there is no indication as to the recovery rate of the microplastic analysis methods. Importantly, all studies had inadequate particle verification rates using FTIR, meaning the accuracy of reported microplastic numbers could be low.

Table 1.1 Criteria used for the quantitative evaluation of the quality of microplastic concentration data, adapted from Koelmans *et al*. (2019), with scores applied to studies investigating microplastics in ICWs.

Criteria	Score criteria			Study score		
	2	**1**	**0**	**Wang *et al*. (2020)**	**Zhou *et al*. (2022)**	**Bydalek *et al*. (2023)**
Sampling methods	**Surface & Ground water:** - Pump - Location - Materials used - Date - Depth of sampling **WWTP:** - Location - Treatment - Date - Sampling method - Materials used	The study reported only a subset of the required characteristics (e.g., date, location, materials used), however is still fairly reproducible.	No/ insufficient reporting of sampling methods.	**2**	**2**	**2**
Sample size	**Surface & ground water:** > 500 L **WWTP:** - Influent: 1L - Effluent: >500 L or until sieve clogging *Sample volume may be smaller if target microplastic sizes are smaller*	**Surface water:** < 500 L "with good cause" (high concentrations e.g.) Trawls without reporting volume is acceptable. **WWTP:** If insufficient volume, sampling till clogging	**Surface water:** < 500 L **WWTP:** Insufficient sampling volume.	**0** 7.5 L at CW influent, 100 L CW effluent in sieve, not stated until clogging	**2** From 10 L to 230 L until sieve clogging	**1** 150 L at CW influent, 300 L at other locations
Sample processing and storage	Sample storing shortly after sampling; any sample handling was avoided before arriving in the laboratory. Sample containers should be rinsed with filtered water. *Sieving in the field is acceptable if sample volume is large. Precautions*	Standards only partially met or containers are pre-rinsed with samples. Citizen science approach with validation	Samples are handled outside. Storage not mentioned. Citizen science approach without validation	**0** Sieves are rinsed outside	**0** Sieves are rinsed outside	**0** Sieves are rinsed outside

	should be taken to prevent contamination.					
Laboratory preparation	Cotton lab coat or non-synthetic clothes Equipment and lab surfaces wiped and rinsed	Solely wiping laboratory surfaces and equipment or not wearing a lab coat **IF** negative samples were run in parallel and examined for contamination.	No precautions.	**2**	**2**	**2**
Clean air conditions	Clean room or laminar flow cabinet	Mitigation of airborne contamination by carefully keeping samples closed as much as possible **IF** negative samples were run in parallel and examined for occurring contamination.	No regard of airborne contamination, or solely use of *fume hood.*	**1** No mention of laminar flow cabinet	**2**	**1** No mention of laminar flow cabinet
Negative control	Controls (in triplicate) treated and analysed in parallel to actual samples. Sample concentrations need to be reported accounting for controls.	Insufficient form of a control, e.g. the filtration of air, or the sole examination of petri dishes/ soaked papers placed next to the samples.	No negative controls.	**1** Not in triplicate	**1** Sole examination of mesh screens placed next to samples	**2**
Positive control	Controls (triplicate) with an added amount of microplastic particles treated the alongside the samples, and for which the particle recovery rates are determined.	Insufficient form of a positive control (e.g. if only a part of the protocol is tested).	No positive controls.	**0**	**0**	**1** Not in triplicate

Sample treatment (only for surface water and WWTP samples)	Digestion of complete sample using a protocol with KOH, WPO and/or enzymes. If another chemical was used, effects on different polymers should be tested before application. All sample treatments to be carried out below 50°C to prevent any damage to microplastics.	If proof is missing that polymers are not affected by protocol (e.g. heated KOH) **OR** in case studies exclusively focus on the bigger microplastics by sieving the samples (mesh size ≥ 300µm). If WPO is carried out without cooling.	No digestion of sample.	**1** Sample heated to 70 °C	**1** Sample heated to 90 °C	**2**
Polymer identification	Per study; analysis of all particles when numbers of pre-sorted particles are <100. For particle numbers >100, 50% should be identified, with a minimum of 100 particles. Per sample; analysis of all particles up to a maximum of 50 particles per sample. Per filter: ≥25% of the surface area.	Insufficient polymer identification, potentially resulting in an unrepresentative subsample. Identification with SEM/EDX to distinguish polymer vs non-polymeric materials.	No polymer identification.	**1** Five particles verified with ATR-FTIR	**0** No reporting of number of particles verified by ATR-FTIR	**1** 12 particles >1 mm identified by FTIR

1.10 Introduction to phthalates

Plasticizers are chemical substances that are added to materials, especially plastics, to increase their flexibility, durability, and workability. By reducing the intermolecular forces between polymer chains, plasticizers make these materials softer and more pliable, improving their performance in a wide range of products (Wypych, 2017). Without plasticizers, many plastic products would be brittle and inflexible. Phthalates are the most common plasticizer and are widely studied due to their ubiquity, human health effects, and environmental impacts. A google scholar search revealed 5,120 published articles with phthalates in the title (as of October 2024).

Phthalates, which are esters of phthalic acid, are a group of chemicals that are primarily employed to improve the properties of plastics, particularly polyvinyl chloride (PVC), by increasing their transparency, flexibility, durability, and longevity. Additionally, phthalates are used as lubricants and solvents in numerous applications. At room temperature, most phthalates exist as oily liquids with melting points generally below -25 °C and boiling points ranging from 230 to 486 °C, a characteristic that significantly enhances their effectiveness as plasticizers (Staples *et al*., 1997).

Phthalates can be found in a wide array of consumer and industrial products, demonstrating their versatility and extensive utility. These products include, but are not limited to, toys, vinyl flooring, detergents, wall coverings, piping, packaging materials, lubricating oils, electrical wires, food packaging, pharmaceuticals, blood bags, nail polish, personal care items, hair sprays, aftershave lotions, soaps, perfumes, and shampoos.

Phthalates are not chemically bound (covalently bonded) to the materials they are used in, meaning they can readily leach out. For example, phthalates have been shown to be released from food (Bradley *et al*., 2013), food packaging (Fasano *et al*., 2012), personal care products (Parlett *et al*., 2013), medical products (Wang and Kannan, 2023), and some dietary supplements (Romano *et al*., 2019).

Given their propensity to leach, potential exposure pathways for phthalates are diverse and include inhalation, ingestion, skin absorption, and intravenous injection (Schettler, 2006). This widespread exposure results in almost ubiquitous human contact with phthalates, leading to significant health concerns. Research has shown that phthalates can have adverse effects on human health, particularly impacting male reproductive development (Swan *et al*., 2005). In a recent study, Trasande *et al*. (2024) identified that phthalate exposure was responsible for approximately 10% of preterm births in the United States in 2018, with the associated economic costs ranging from 1.63 to 8.14 billion USD. The current state of the evidence on human health impacts caused by phthalate exposure is well reviewed in Eales *et al*. (2022).

In Europe, the most widely used phthalates, in order, are DEHP, DIDP, DINP, and DBP (Peijnenburg, 2008). No exact production numbers of phthalates were identifiable in the literature, although production was estimated at 4.3 million tonnes year^{-1}, 90 % of which were used in plasticizers (Peijnenburg, 2008).

1.11 Fate of phthalates in aquatic environments and removal by WWTPs

Phthalates have half-lives ranging from hours to months in surface waters (Staples *et al*., 1997). Abiotic degradation of phthalates is slow in the aquatic environment. The hydrolysis of phthalates first produces the monoester and an alcohol, then an acid and alcohol. At neutral pH, hydrolysis of phthalates is minute, while acidic hydrolysis is possible but approximately four orders of magnitude slower than alkaline hydrolysis rate constants (Staples *et al*., 1997). Hydrolysis of phthalates therefore plays little role in their degradation in the environment.

Limited data is available regarding photo degradation of phthalates, although it is not considered a significant process (Staples *et al*., 1997). Photolysis can be mediated either by the direct absorption of UV radiation (290-400 nm), or by the reaction of activated species (for example hydroxyl radicals) with phthalates. Gledhill *et al*. (1980) observed less than 5 % degradation of 1 mg L^{-1} BBP after exposure to sunlight for 28 days and determined an aqueous photolysis half-life of >100 days. Howard (1991) estimated aqueous photooxidation half-lives of 2.4 to 12 years for DBP, and 0.15 to 1.5 years for DEHP.

The most significant removal process of phthalates in aquatic, sediment, and soil environments is biodegradation, when phthalates are used as a source of carbon and energy for anaerobic and aerobic microbes (Lertsirisopon *et al*., 2009). Biodegradation rates of phthalates are higher in eutrophic waters (Rubin *et al*., 1982) and under aerobic conditions (Table 1.2). DBP has been shown to degrade in nutrient rich constructed wetland sediment, with a half-life of 1.4 days in surface (0–5 cm) soil and 4.0 days in subsurface (20–25 cm) soils (Zhou *et al*., 2005). One reason for this difference may be that the deeper soil is more anoxic than the surface, limiting phthalate degradation rates there (Zhou *et al*., 2005). Degradation products can also be environmentally relevant, for example mono 2-ethylhexyl phthalate (MEHP), the degradation product of DEHP, is more toxic than the parent compound (Zhu *et al*., 2019).

Table 1.2 Biodegradation half-lives (days) of selected phthalates in aquatic environments. Re-produced from Lertsirisopon *et al*., 2009

Condition	DBP	BBP	DEHP	Type
Aerobic	0.5–10.1	0.5–10.1	7.3–27.5	Unacclimated river sediment samples
	2–5	4–11	6–21	Unacclimated pond water samples at 28 degrees
Anaerobic	2– 3	2–3	>208	Natural sediment microcosms at pH 7 and 28 degrees
	11.7–18.9	99–25.5	29.9–39.1	Unacclimated river sediment microcosms at pH 7 and 30°C

Phthalates generally have high octanol-water partition coefficients (K_{OW}) (Table 1.3), meaning they are hydrophobic and will readily adsorb to organic material, suspended solids and sediment. Phthalates with higher log K_{OW} values are preferentially removed during the WWTP process, with most removed during primary treatment (screening, grit removal, and sedimentation) by adsorbing to solids (Figure 1.11). Phthalates are reduced during secondary treatment by biodegradation and adsorption to solids, while tertiary treatment is generally the least effective removal process (Bai *et al*., 2022). Other more advanced WWTP technologies are evaluated in Bai *et al*. (2022), although there is no clear best option for phthalate removal. WWTPs can therefore be a significant source of phthalates to rivers (Tran *et al*., 2015). Globally, phthalate concentrations are variable in WWTP influent (0.10 to 2488.31 µg L^{-1}) and effluent (0.12 to 30.99 µg L^{-1}), while DEHP was the most frequently detected phthalate in many countries, such as France, China, and Denmark (Bai *et al*., 2022). Average global removal in WWTPs was 69.5 % for DMP, 70.6 % for DEP, 82.2 % for DBP, 81.6 % for BBP, 67.9 % for DEHP, and 73 % for DNOP (Bai *et al*., 2022). The lowest phthalate removal rate recorded was 14.2 % for DEHP in Nigeria, and the highest was 99.82 % for DMP in China.

Table 1.3 Physical-chemical properties of selected phthalate esters. Data from PubChem (2023).

Phthalate ester	Abbreviation	LogK_{OW}	Alkyl chain length	Water solubility (mg L^{-1})	Molecular weight (g mol^{-1})
Dimethyl phthalate	DMP	1.60	1	4000	194.2
Dibutyl phthalate	DBP	4.50	4	11.2	278.3
Benzyl butyl phthalate	BBP	4.73	4, 6 (aromatic ring)	2.69	312.4
Dicyclohexyl phthalate	DCHP	6.20	6	4	330.4
Di(2-ethylhexyl) phthalate	DEHP	7.60	8	0.003	390.6
Diisononyl phthalate	DINP	9.37	9	0.2	418.6
Diisodecyl phthalate	DIDP	10.36	10	0.28	446.7

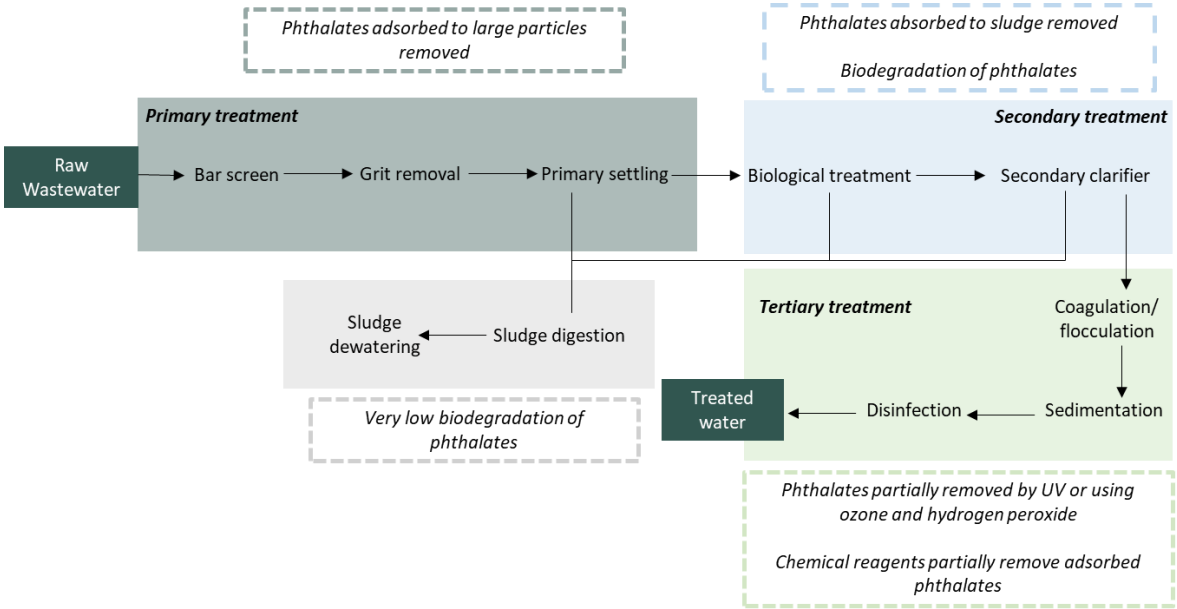


Figure 1.11 Fate of phthalates in conventional WWTP processes

1.12 Potential environmental impacts of phthalates in aquatic environments

Phthalates are known endocrine disruptors. They have been shown to alter the expression of peroxisome proliferator-activated receptors (particularly phthalate monoesters) (Bility *et al*., 2004). The phthalates DEHP, BBP, DBP, and MBP have been demonstrated to disrupt amphibian thyroid hormone genes (Mathieu-Denoncourt *et al*., 2015). Thyroid hormone disruption by phthalates (of all alkyl chain lengths) can lead to development abnormalities

(Horie *et al*., 2022). Phthalates can also have impacts on reproduction and sex ratios in aquatic species (Mathieu-Denoncourt *et al*., 2015).

The US Environment Protection Agency's (EPA) phthalate action plan address eight phthalates (Table 1.4) for "their toxicity and the evidence of pervasive human and environmental exposure to them" (U.S. Environment Protection Agency, 2012, page 1). Of these, "BBP, DEHP, and DBP elicit the most toxicity to terrestrial organisms, fish, and aquatic invertebrates" (U.S. Environment Protection Agency, 2012, page 5). The European Chemicals Agency (ECHA) lists 12 phthalates as substances of very high concern, with toxicity for reproduction and endocrine disrupting properties given as their reason for inclusion. Additionally, DEHP (and its isomers) is the only phthalate on the EU Water Framework Directive (WFD), with a maximum allowable annual average concentration of 1.3 µg L^{-1} in inland surface waters (EUR-Lex, 2013).

Table 1.4 Phthalates on the US EPA phthalate action plan and ECHA substances of very high concern.

US EPA phthalate action plan (2012)	**ECHA substances of very high concern (2023)**
Dibutyl phthalate (DBP)	Dibutyl phthalate (DBP)
Diisobutyl phthalate (DIBP)	Diisobutyl phthalate (DIBP)
Butyl benzyl phthalate (BBP)	Butyl benzyl phthalate (BBP)
Di-*n*-pentyl phthalate (DnPP)	Bis(2-methoxyethyl) phthalate
Di(2-ethylhexyl) phthalate (DEHP)	Di(2-ethylhexyl) phthalate (DEHP)
Di-n-octyl phthalate (DnOP)	Dicyclohexyl phthalate (DCHP)
Diisononyl phthalate (DINP)	Dihexyl phthalate (DHP)
Diisodecyl phthalate (DIDP)	Diisobutyl phthalate (DIBP)
	Diisohexyl phthalate (DIHP)
	Diisopentyl phthalate (DIPP)
	Dipentyl phthalate (DPP)
	n-pentyl-isopentyl phthalate

1.13 Current evidence of phthalate retention by constructed wetlands

Few studies have investigated how effectively constructed wetlands retain phthalates. Fifteen phthalates were assessed by Diepenheim *et al*. (2020) at a horizontal subsurface flow constructed wetland in Oregon, USA, receiving 11,356 m^3 of WWTP effluent per day (from 40,000 people). The wetland was fully operational in May 2018 and covers 364,000

m^2, with a hydraulic residence time of 5.5 days. *Typha latifolia* and *Typha angustifolia* were the dominant plant species. Samples were collected from five locations, including where WWTP effluent was discharged into the wetland and at various locations between ponds. Phthalates were analysed by GC-MS. In water samples the greatest concentrations of $\sum_{15}$ phthalates were found near the inlet of the CW, and the least near the exit of the CW, equating to a 68 % reduction in concentration (Figure 1.12). A negative relationship was found between $\sum_{15}$ phthalate concentration and dissolved oxygen in water that was attributed to enhanced microbial activity and biodegradation of phthalates in oxidising conditions. DEHP concentrations in water samples ranged from not detectable to 0.21 µg L^{-1} at the inlet of the CW. DEHP was found in *Typha* shoots at each sampling location (0.22–1.17 µg g^{-1} dry weight), indicating that plant uptake of DEHP in CWs is an important removal mechanism (additionally, more accumulation was expected in the plant roots and rhizome). DEHP content in sediment ranged from not detectable to 0.34 µg g^{-1} dry weight. DBP concentrations were 0.063–0.27 µg L^{-1} in water, not detectable to 1.87 µg g^{-1} dry weight in sediments, and 0.088–2.02 µg g^{-1} dry weight in *Typha* (Diepenheim *et al*., 2020). DBP content in sediment increased by 87 % from the inlet of the wetland to near the outlet. DEP and DMP were mostly found in water and *Typha* samples. This study to an extent shows concentration changes of individual phthalates over the course of the constructed wetland, although there are limited details of this in the text. Additionally, the authors remark that the sample sizes used were small, the variance was large, and that further study should investigate seasonal variation in phthalate retention.

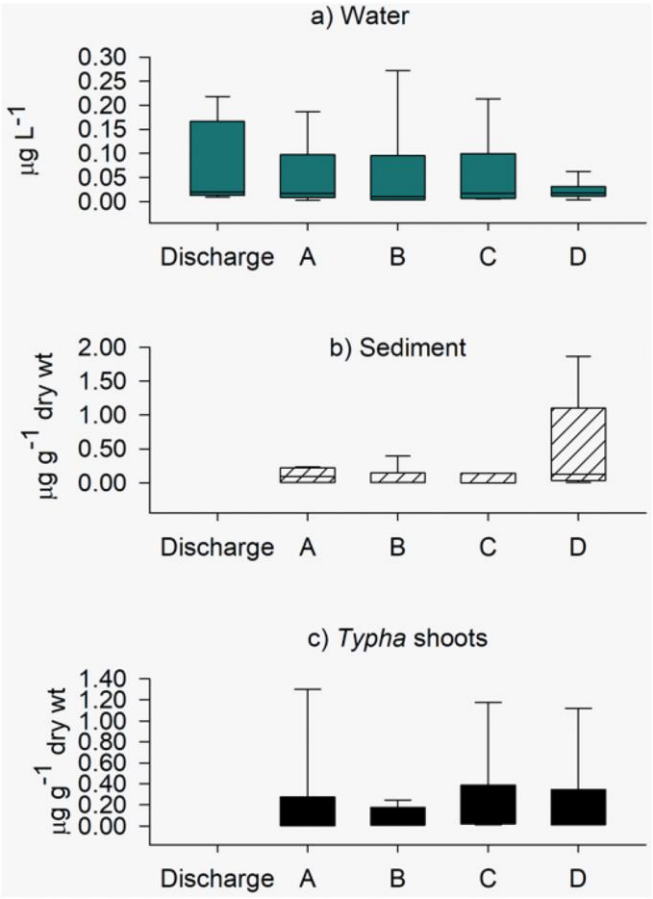


Figure 1.12 ∑Phthalates (n=15) in a constructed wetland from A) water µg L^{-1} B) sediment µg g^{-1} C) Typha shoots µg g^{-1} dry weight at different locations in the wetland (discharge indicates WWTP effluent, A-D represents distance along wetland). From Diepenheim *et al*. (2020)

Xiaoyan *et al*. (2015) investigated removal efficiency of six phthalates in lab scale surface flow constructed wetlands (SFCWs), including planted and unplanted units. In planted SFCWs, removal rates for the six phthalates were as follows: DMP: 53–71 %; DEP: 48–57 %; DBP: 42–47 %; BBP: 49–60 %; DOP: 31–37 %; and DEHP: 23–31 %. Phthalates with short ester hydrocarbon chains (e.g., DMP & DEP) are more readily biodegradable and mineralized than those with longer ester chains (e.g., DEHP & DOP). In unplanted SFCWs, average phthalate concentrations were slightly lower than planted ones, although not statistically significantly so.

In a large, constructed wetland in eastern China covering 2,260,000 m^2 and receiving approximately 300,000 m^3 of WWTP effluent daily, Xu *et al*. (2019) investigated retention of 10 phthalates in summer and winter. Unlike other studies that have reported phthalate retention by CWs, the concentration of phthalates increased from the inlet to outlet of the wetland by 60.3 % in summer and 26.4 % in winter. Removal performance was thus worse in summer and the reason for this was not clear.

Nas *et al*. (2022) investigated phthalate removal in a surface flow constructed wetland in Konya, Turkey. The CW receives 210 m^3 day^{-1} of domestic wastewater effluent, pre-treated in a septic tank serving 2000 people. No data is provided on the size of the wetland. Samples were collected monthly over a year. Influent concentrations were: BBP: 0.11 µg L^{-1}; DEHP: 0.45 µg L^{-1}; and DNOP: 0.09 µg L^{-1}. There was little change in influent phthalate concentrations throughout the year. Removal efficiencies varied significantly over the sampling period: BBP: -23 % to 5 %; DEHP: -53 % to 47 %; and DNOP: -497 % to 100 %. Removal efficiencies were worse (i.e., concentrations were higher at the outlet of the CW than the inlet) in March for BBP and DEHP, and June for DNOP. There were no clear seasonal changes in removal efficiency for any of the three phthalates (Table 1.5). Nas *et al*. (2022) concluded that the constructed wetland had no significant effect on the removal of these phthalates.

Table 1.5 Phthalate removal during each month in a surface flow constructed wetland. Removal percentage refers to wetland inlet and outlet concentrations. N/A indicates no data available. Based on supplementary materials in Nas *et al*. (2022).

	Phthalate removal (%)		
Month	**BBP**	**DEHP**	**DNOP**
January	-1.8	N/A	N/A
February	-1.1	N/A	-34.5
March	-23.9	-52.6	N/A
April	0.2	-25.1	N/A
May	0.2	-4.6	34.8
June	2.7	29.7	-497.7
July	0.6	90.1	82.8
August	-1.2	47.0	-425.7
September	N/A	27.4	N/A
October	-3.4	-37.4	N/A
November	-1.0	N/A	100
December	N/A	N/A	-148.2

Phthalates can leach out of plastics over a long duration (Henkel *et al*., 2022), thus any microplastic retained in constructed wetlands may begin to leach phthalates and cause phthalate concentrations to increase over the course of a wetland, potentially resulting in negative removal efficiencies. For example, the leaching half-life of DEHP from PVC plastic under average river conditions was estimated to be 122 years, and >900 years in slow flow conditions (Henkel *et al*., 2023). In ICWs, phthalate leaching rates may be increased by

high dissolved organic carbon (DOC) concentrations and decreased by slow flow conditions and the formation of biofilms on microplastic particles (Wang *et al*., 2019).

In summary, despite increasing recognition of ICWs as sustainable solutions for wastewater treatment, significant research gaps remain that limit their optimization and policy integration. Notably, no studies have comprehensively examined the long-term (12-month) retention of microplastics and anthropogenic fibres in ICWs or evaluated retention on an hourly scale. Understanding how these pollutants behave over extended periods and at larger temporal resolutions is crucial for assessing the full capability of ICWs in removing and mitigating the environmental impacts of emerging contaminants. Additionally, sediment samples within ICWs have largely been overlooked, preventing the identification of specific storage areas where pollutants might accumulate. This is a critical omission, as understanding the spatial distribution of pollutants could inform better management and design of ICWs. Furthermore, the retention of phthalates, a group of harmful endocrine-disrupting chemicals, has not been assessed within ICWs. Filling these research gaps is important for informing policies that promote the use of ICWs, particularly in addressing microplastic and phthalate pollution, which are of growing regulatory concern. Without this knowledge, policymakers lack the robust evidence needed to confidently promote ICWs as a comprehensive, nature-based solution for pollutant retention and environmental protection.

1.14 Research aim

To assess whether integrated constructed wetlands are an effective pollution mitigation solution for anthropogenic fibres, microplastic fragments and phthalates discharged into rivers from sewage effluent.

1.15 Research objectives

i. Develop a method to reliably quantify microplastic fragments and anthropogenic fibres in water samples and organic rich sediment samples from integrated constructed wetlands (ICWs) receiving sewage effluent.
ii. Assess the microplastic and anthropogenic fibre retention efficiency of two ICWs (Ingoldisthorpe and Northrepps) receiving WWTP effluent over a 12-month period.
iii. Assess the concentration of microplastic fragments and anthropogenic fibres in fine bed sediment of ICWs.
iv. Assess the phthalate removal performance of Northrepps ICW over a 6-month period.

1.16 Wider significance

This study is important because there is increasing pressure on water companies to maintain cost-effective wastewater treatment, partially driven by population growth and sub-optimally performing infrastructure, meaning interest has grown regarding the application of integrated constructed wetlands to provide a potential nature-based solution to the challenges of conventional wastewater treatment. Emerging contaminants of concern, including microplastics, anthropogenic fibres and phthalates, present new challenges and it is essential to determine how well integrated constructed wetlands can retain these if they are to meet the requirements of future wastewater treatment. This research therefore has real-world impact by informing Anglian Water on the potential pros and cons of wider ICW rollout at other WWTPs across the region, as well as informing Norfolk Rivers Trust on potential wetland design modifications to optimise management for microplastics.

Chapter 2

Study Location

2.1 Ingoldisthorpe Integrated Constructed Wetland (ICW)

The Ingoldisthorpe ICW lies on the River Ingol (52°51′53″N 0°31′18″E, Norfolk, UK) (Figure 2.1). Ingoldisthorpe ICW was operational in April 2018 and covers 10,788 m^2 across four shallow (20–30 cm) unlined cells with areas of 1972 m^2 (cell 1), 2450 m^2 (cell 2), 3560 m^2 (cell 3) and 2806 m^2 (cell 4) (Figures 1.9–1.13). Emergent vegetation cover in cell 1 is >90 %, and 30–50 % in cells 2–4. The wetland was planted with 25,000 native aquatic plants, including *Glyceria maxima, Iris pseudacorus, Juncaceae sp., Cyperaceae sp., Typha latifolia,* and *Caltha palustris.*

Prior to discharge into the ICW, sewage effluent is treated with primary settlement tanks, trickling filters, humus tanks, and a nitrifying sand filter. The WWTP also contains a storm overflow tank, meaning there is no combined sewer overflow. When effluent discharge rates are high from the WWTP, an overflow pipe diverts excess water directly into the river to prevent the wetland from flooding. The WWTP serves 6258 people, and total capital costs were £194,000 for Ingoldisthorpe ICW, equating to approximately £31 per person served (Cooper *et al.*, 2020).

Baffles were installed in summer 2023 at the Ingoldisthorpe ICW (in cells 2, 3 and 4) in an attempt to increase the hydraulic residence time and minimise flow short circuiting. Researchers at the University of Warwick have found that residence times in wetland cells are much lower than anticipated (sometimes as low as three hours). Vegetation clearance also occurred in cell 1 during December 2023, with the aim of increasing the effective volume for increased residence times. There has been no sediment removal at the Ingoldisthorpe ICW.

Figure 2.1 Locations of Ingoldisthorpe and Northrepps ICWs, as well as River Mun and River Ingol water courses (blue line) and their catchments.

The River Ingol is a 10.3 km long, predominately groundwater fed lowland calcareous river in Norfolk, England, with a 35.3 km^2 catchment. It provides a rare habitat for a diverse group of fauna and flora and is one of only 200 chalk rivers in the world, characterised by clear alkaline water that is stable in temperature, flowing over exposed chalk or gravel beds. Parts of the river have Special Area of Conservation (SAC), Site of Special Scientific Interest (SSSI), and Ramsar status. The river runs near to the Norfolk coast, an area of international natural importance providing habitat for rare or endangered migrating birds and breeding waders. The river exits into the sea near to an RSPB nature reserve (Snettisham). Prior to installation of the ICW, the river water quality was being degraded by WWTP effluent (Cooper *et al.*, 2020).

The area experiences a temperate maritime climate, with a mean annual temperature of 10.5 °C and a mean annual precipitation total of 684 mm (1991–2020) (Meteorological Office, 2023).

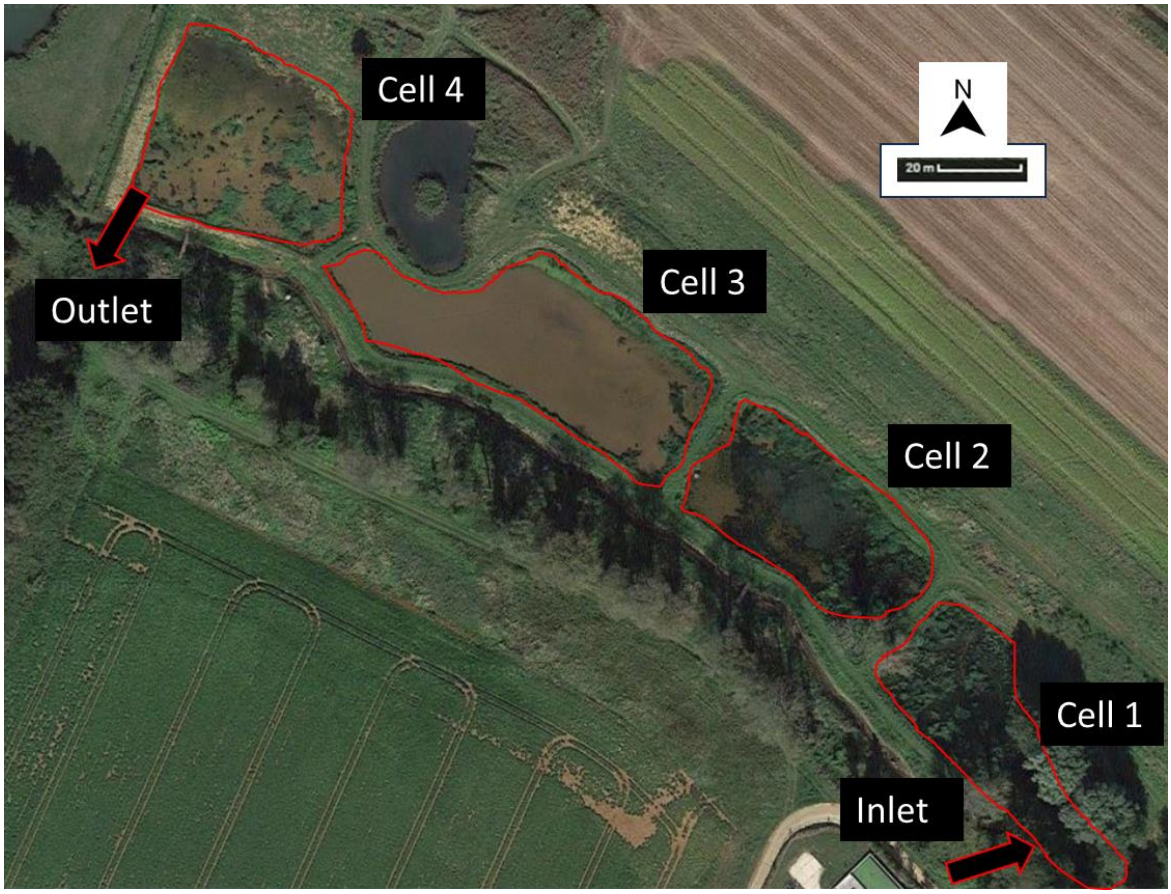


Figure 2.2 Google Maps satellite imagery of the Ingoldisthorpe ICW, with cells approximately outlined in red.

Figure 2.3 Aerial view of the Ingoldisthorpe wetland, with the WWTP visible in the bottom left corner. Taken in June 2022 by Derek Lawrence using a DJI drone.

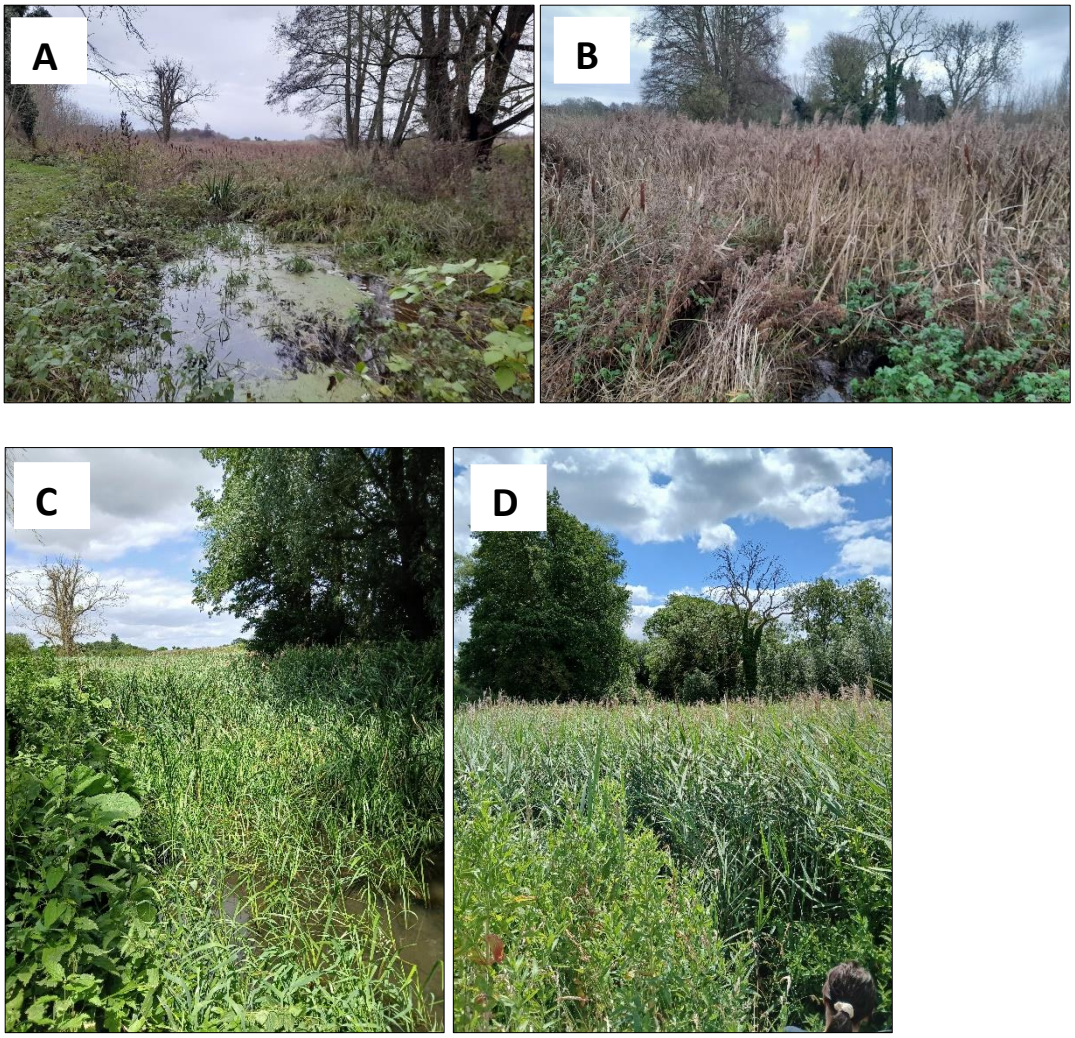


Figure 2.4 Photos from the beginning (A&C) and end (B&D) of the first cell of Ingoldisthorpe ICW. Photos A&B taken December 2023, photos C&D taken July 2024.

Figure 2.5 Photos from the beginning (A&C) and end (B&D) of the second cell of Ingoldisthorpe ICW. Photos A&B taken December 2023, photos C&D taken July 2024.

Figure 2.6 Photos from the beginning (A&C) and end (B&D) of the third cell of Ingoldisthorpe ICW. Photos A&B taken December 2023, photos C&D taken July 2024.

Figure 2.7 Photos from the beginning (A&C) and end (B&D) of the fourth cell of Ingoldisthorpe ICW. Photos A&B taken December 2023, photos C&D taken July 2024.

2.2 Northrepps integrated constructed wetland

The Northrepps ICW lies on the River Mun, (52°53′46″N, 1°20′41″E, Norfolk, UK) a 7.9 km long groundwater dominated lowland river with a 22 km^2 catchment, located 55 km east of the River Ingol. Parts of the river are designated as County Wildlife Sites, but it is rated as being in 'poor' condition for fish and overall ecological health under the EU Water Framework Directive. The Anglian Water WWTP at Northrepps contributes approximately 70% of the discharge in the headwaters of the River Mun. The ICW was operational in October 2014 and covers 2900 m^2 across three shallow (~30 cm) unlined cells with areas of 1600 m^2 (cell 1), 700 m^2 (cell 2), and 600 m^2 (cell 3) (Figures 1.13–1.16). Emergent vegetation cover is >95 % in each cell. The wetland was planted with 15,000 native aquatic plants including *Carex riparia, Iris pseudacorus, Alisma plantago-aquatica, Sparganium erectum, Veronica beccabunga, and Mentha aquatica.*

Prior to discharge into the ICW, sewage effluent undergoes secondary treatment with an aeration tank and a final settlement tank (Figures 2.12–2.14). The WWTP serves a population of 770 people, and total capital costs were £30,021 for the Northrepps ICW, equating to £39 per person served (Cooper *et al.*, 2020).

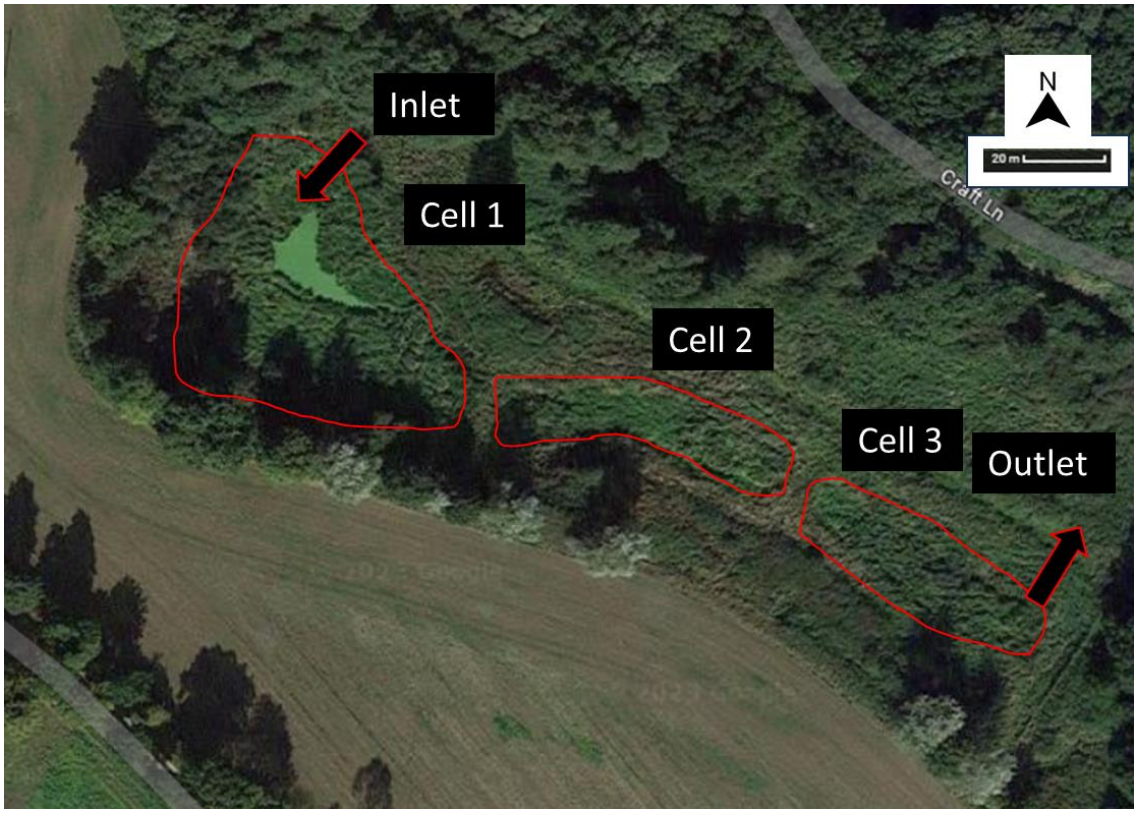


Figure 2.8 Google Maps satellite imagery of the Northrepps ICW, with cells approximately outline in red.

Figure 2.9 First cell at Northrepps ICW, outlined in red. Taken August 2023.

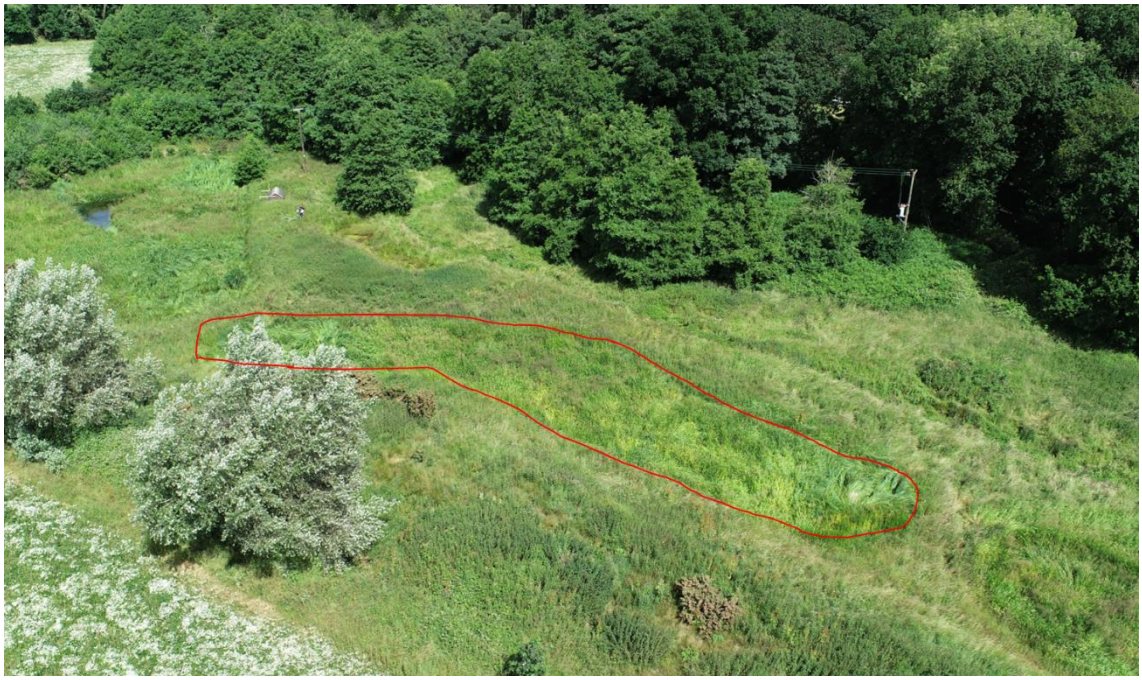


Figure 2.10 Second cell at Northrepps ICW, outlined in red. Taken August 2023.

Figure 2.11 Third cell at Northrepps ICW, outlined in red. Taken August 2023.

Figure 2.12 Photograph of aeration tank at Northrepps WWTP. Taken June 2024.

Figure 2.13 Photograph of final settlement tank at Northrepps WWTP. Taken June 2024.

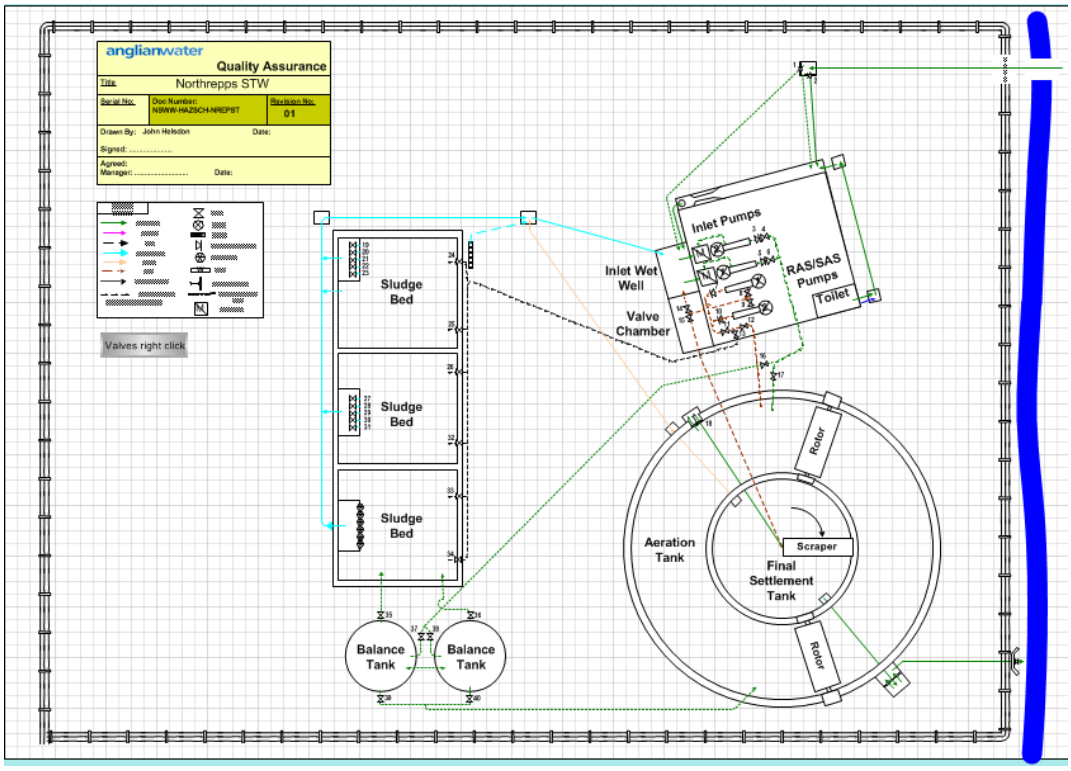


Figure 2.14 Schematic diagram of the Northrepps WWTP. Supplied by Anglian Water.

2.3 Nutrient removal performance in Northrepps and Ingoldisthorpe ICWs

The data used in this section to demonstrate the nutrient removal performance at Northrepps and Ingoldisthorpe ICWs were gathered from April–September 2019, June–August 2022, and March–October 2023. The 2019 data was used in Cooper *et al*. (2020).

Across the entire sampling period, the Ingoldisthorpe ICW was effective at reducing nutrient concentrations, with a 30.3 % average decrease in nitrate concentrations (Figure 2.15) between the wetland inlet and outlet and a 19.3 % average decrease in phosphate concentration (Figure 2.16). The Northrepps ICW was more effective still, with a 64.1 % average decrease in nitrate concentrations between the wetland inlet and outlet and a 57.8 % average decrease in phosphate concentration. The Northrepps ICW received higher average concentrations from WWTP effluent of both nitrate (53 mg N L^{-1} compared to 35 mg N L^{-1}) and phosphate (6.9 mg P L^{-1} compared to 2 mg P L^{-1}) relative to the Ingoldisthorpe ICW.

The EU WFD physicochemical status (UKTAG, 2013) of the River Ingol can be classified as 'moderate' upstream and 'bad' downstream of the ICW, whilst the River Mun can be classified as 'poor' upstream and 'bad' downstream of the ICW with respect to phosphate concentrations. For nitrate concentrations, both the River Ingol and River Mun can be classified as 'bad' upstream and downstream of the ICW. This suggests that, despite the high treatment efficiency of the ICWs, sewage effluent remains a significant contributor to eutrophication in these river systems.

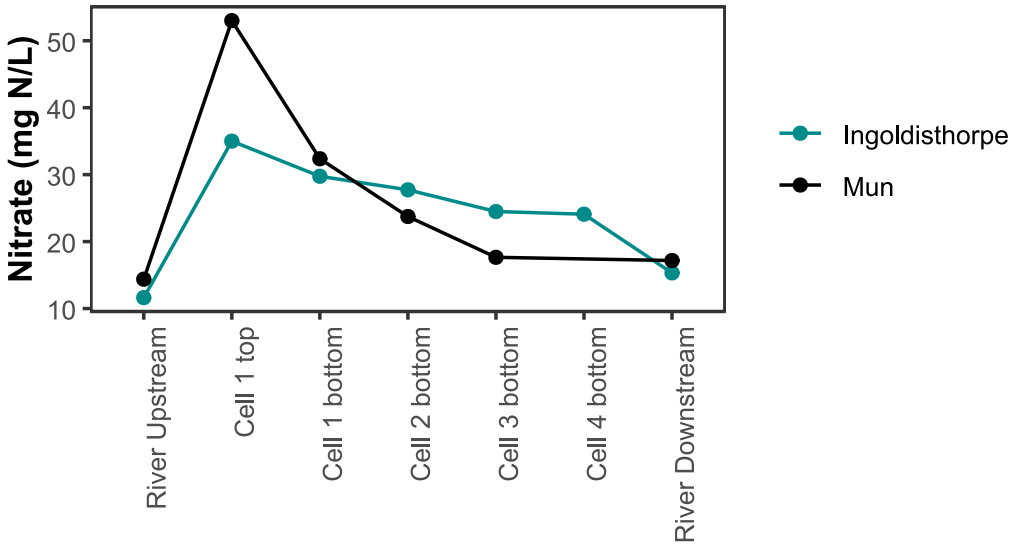


Figure 2.15 Average nitrate concentrations across Ingoldisthorpe and Northrepps ICWs recorded between April 2019 and October 2023.

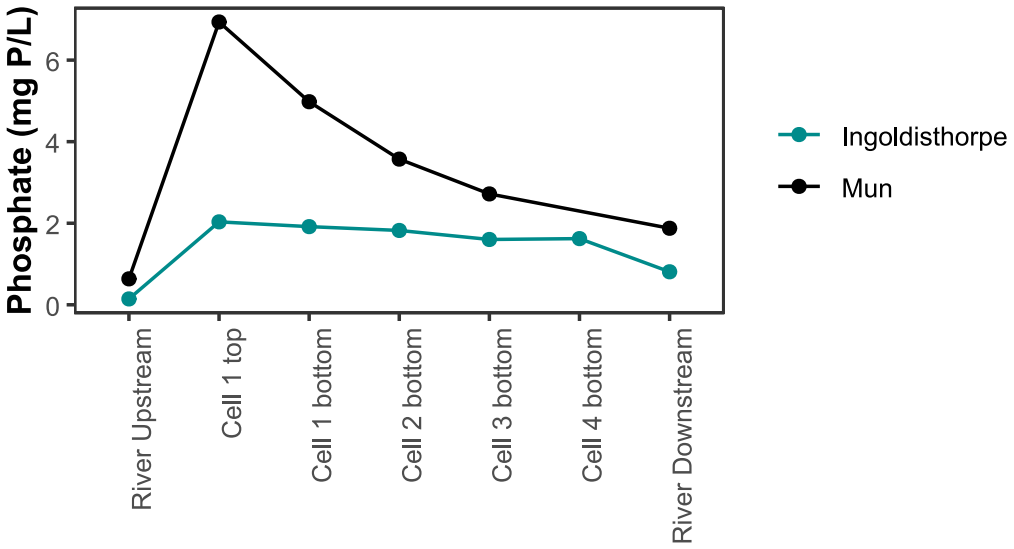


Figure 2.16 Average nitrate concentrations across Ingoldisthorpe and Northrepps ICWs recorded between April 2019 and October 2023.

Chapter 3

Developing an improved methodology for identifying microplastic fragments and anthropogenic fibres in water and organic-rich sediment samples

3.1 Introduction

3.1.1 Context

In microplastic research, two primary methods are generally used for identification: dye staining and visual identification. Several dyes can be used for microplastic identification, although Nile Red is the most common (Primpke *et al*., 2020) and was first applied to microplastic identification by Andrady (2011). Nile Red is a solvatochromic dye that can fluoresce when sorbed to plastics (Maes *et al*., 2017). The principle behind its use lies in its lipophilic properties, which make it highly selective for hydrophobic materials such as plastics. When Nile Red binds to microplastics, its fluorescence is activated. The dye fluoresces under specific wavelengths of light (typically under UV or blue light), allowing the microplastics to be easily identified and imaged under a fluorescence microscope or detected using other fluorescence-based techniques. The exact fluorescence emitted by Nile Red can vary depending on the type of plastic and the solvent used in the staining process.

Compared to visual inspection, Nile Red dye staining methods are significantly faster, relatively cheap, and the criteria for selecting particles can be consistently replicated with each sample regardless of operator proficiency because the particles counted as suspected microplastic are those that fluoresce. Additionally, staining may identify microplastic particles <100 µm that are difficult to both see under light microscopes and isolate for further analysis with spectroscopy. Nile Red has been used to identify microplastics from a range of environments, including estuarine sediment (Vermeiren *et al*., 2020), marine sediment (Bakir *et al*., 2023), drinking water (De Frond *et al*., 2022), and biological samples (Prata *et al*., 2021b). Prata *et al*. (2021a) were able to report microplastic fragments down to 2 µm in river water samples and found average concentrations at different sites ranging from 265 fragments L^{-1} downstream of a WWTP to <1 fragments L^{-1} in a rural stretch. The dominant size fraction (85 % of total) in these river water samples was <40 µm (Figure 3.1).

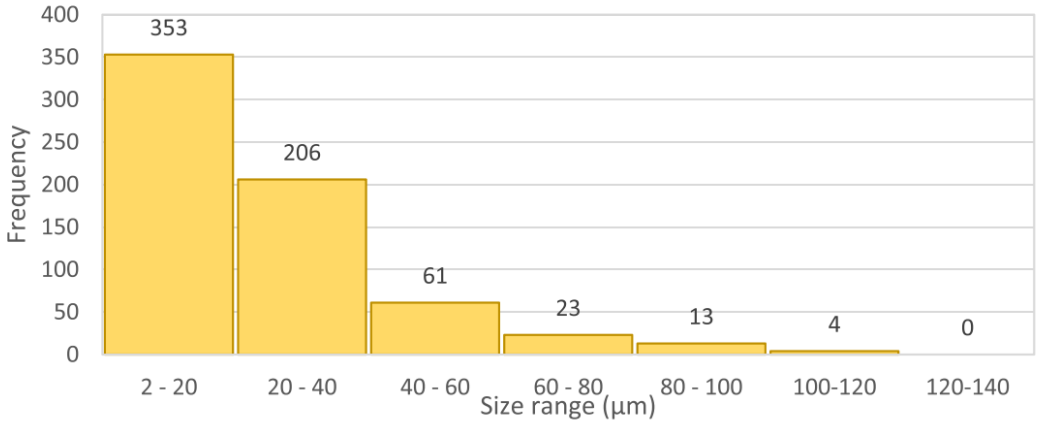


Figure 3.2 Size ranges of microplastic fragments found in water samples from Douro River, Portugal, using a Nile Red staining-based methodology (Prata *et al*., 2021a).

Despite these advantages, the Nile Red approach to microplastics identification has significant problems. In particular, the dye used to colour plastics appears to impact the fluorescence response of Nile Red, and it is questionable which textile fibre colours are identifiable using a Nile Red based identification method. Stanton *et al*. (2019) found that Nile red did not stain black polyester, blue acrylic, or red polyamide fibres. White and translucent microplastic particles and textile fibres may fluoresce under UV light when stained with Nile Red (Maes *et al*., 2017). However, Prata *et al*. (2021a) suggested that individual fibres do not fluoresce because of the refraction of fluorescent emissions at their surface, so fibres were identified visually under white light in their samples. If only white and translucent fibres are identifiable, the Nile Red protocol may not stain a significant proportion of textile fibres in environmental samples, and importantly those that are likely to be found in the present studies' WWTP effluent derived samples. For example, Erdle *et al*. (2021) found that the most common colours of microfibres in WWTP effluent were blue (32 %), translucent (29 %), and black (22 %). Additionally, plastic fragment colour can significantly impact fluorescence intensity when stained with Nile Red (Prata *et al*., 2023; Shruti *et al*., 2022). Of 60 plastic fragments tested, 81.6 % of produced medium to high fluorescence when stained with Nile Red (10 µg mL^{-1} in acetone) (Prata 2023). Plastics were characterized as 66.7% PE, 30.0% PP, and 3.3% PS, while particle colours were blue (33.3%), red (16.7%), green (15.0%), white (10.0%), grey (10.0%), yellow (6.7%), orange (5.0%), and brown (3.3%) (Prata 2023). Blue and green plastics produced the least fluorescence, while orange, yellow and white plastics were the most fluorescent. Pigments were determined to be a more significant factor influencing plastic particle fluorescence than polymer type (Prata 2023). The results of Prata (2023) give some indication as to how well Nile Red stains a variety of plastics, however the size of particles tested was 1.6–16.7 mm and the imaging conditions were different to those that would be used in the present study to identify microplastics in real environmental samples with Nile Red.

The laboratory at UEA had previously developed expertise in microplastic identification using a Nile Red dye staining protocol, which was inherited as the foundation for the analysis methods aspect of this PhD research. However, due to critiques in the literature regarding the method's accuracy and specificity, it was necessary to evaluate its effectiveness and explore potential improvements, particularly regarding the extent to which Nile Red can stain a broad range of polyester fibre colours and microplastic fragments, before using this method in the present study.

3.1.2 Aim

Develop a method to reliably quantify microplastic fragments and anthropogenic fibres in organic rich water and sediment samples from integrated constructed wetlands (ICWs) receiving sewage effluent.

3.1.3 Objectives

1. Assess the standard Nile Red methodology's ability to quantify anthropogenic fibres.
2. Assess the standard Nile Red methodology's ability to quantify microplastic fragments in real-world water samples.
3. Describe and justify a method to identify anthropogenic fibres in organic-rich freshwater samples.
4. Describe and justify a method to identify microplastic fragments in organic-rich submerged sediment samples.

3.2 Methods

To generate fibres for testing with Nile Red staining, polyester sewing thread was cut into 25 pieces, each approximately 3–5 mm in length, and placed into a petri dish. To generate microplastic fragments for testing with Nile Red staining, a file was used to produce fragments (from macroplastic) that were sieved to 250–750 µm and placed into a petri dish, except for plastic films that were manually cut. Fibres and fragments were then vacuum filtered onto polycarbonate filters (0.45 µm pore size). Each filter was incubated at room temperature for 15 minutes in 5 mL of Nile Red solution (10 µg mL^{-1} in ethanol). This Nile Red concentration was chosen based on previous work by Maes *et al*. (2017) and is also used by Centre for Environment, Fisheries and Aquaculture Science (CEFAS) for analysis of microplastics in biota. Filters were left to dry for at least 1 hour before imaging. Stained particles/fibres were photographed under 420–470 nm (CRIME-LITE 2) using a digital camera (Canon EOS 600D, shutter speed 1/25, ISO 1600, F7.1) coupled with an orange camera lens filter (HOYA YA3 Pro Orange) and under visible light without the orange filter.

Images of stained polyester fibres were manually sorted into three groups based on the visibility of the fluorescent thread by eye: visible, barely visible, and not visible. The visibility could be slightly enhanced by increasing the brightness of the image, but this was avoided because in doing so the background noise signal goes up, meaning the number of false positives may increase. To determine whether the standard Nile Red staining approach was able to quantify anthropogenic fibres, the images of the stained polyester threads were analysed in ImageJ, an image analysis/editing software, based on the microplastic visual analysis tool (MP-VAT) method (Prata *et al*., 2019). This approach uses an automatic threshold using the maximum entropy method, and an updated version of the MP-VAT uses a Renyi entropy automatic threshold (Prata *et al*., 2020).

Test samples were taken from the Northrepps integrated constructed wetland (ICW) to determine whether microplastics could be detected in real-world water samples. To do so, a 100 L water sample from the outlet of the Northrepps ICW (collected May 2022) was poured through a 38 µm sieve that was then sealed and transported back to the laboratory. The contents were then rinsed into a beaker to the 100 mL mark, and 100 mL of NaClO was added (creating a 1:1 dilution) and incubated at 40 ˚C for 16 hours. The contents were then vacuum filtered onto polycarbonate filters (0.45 µm pore size), stained with Nile Red solution (10 µg mL^{-1} in ethanol) for 15 minutes, left to dry for at least 1 hour, and then imaged.

To assess the recovery rate of the water sampling method, a spiked field control sample was collected. 30 pink polyester fibres (thread code 23, see Figure 3.2) were peeled from the end of the thread and cut to approximately 2-5 mm in length (below this length was unpractical because fibres were too difficult to handle and therefore to spike a known number into the sample). These fibres were stored in a glass beaker in water and then poured through a 38 µm sieve, following which the standard water sampling method was followed (explained in section 3.3.3). This was performed three times to achieve a more reliable average recovery rate.

To assess recovery for sediment samples, a spiked field control sample was collected. Pink polyester fibres were prepared (as for the water samples) and microplastic fragments were generated by using a file on macroplastic items to generate small fragments that were then sieved to 250–750 µm for use in recovery experiments (it was prohibitively difficult to extract particles below this size). 30 pink polyester fibres, 30 dark blue PVC fragments and 30 yellow PP fragments were mixed in with 10 g of organic rich sediment from the Northrepps ICW. This sediment was collected from the third cell where there was unlikely to be any microplastics that visually resembled those in spiked samples. By using real wetland sediment, the recovery rates are more specific to the actual samples than for example using beach sand or pond sediment that has different characteristics that may influence recovery

rate (such as organic matter content). These two plastic materials were used to assess the method's recovery rate for microplastic fragments because they have different densities (PP is ~0.9 g cm^{-3} and PVC is ~1.48 g cm^{-3}) that may impact recovery rates, particularly during density separation. After spiking the sediment, the standard sediment sampling method was then followed (explained in section 3.3.4). This was performed three times to achieve a more reliable average recovery rate. The size of the microplastic fragments found after recovery was recorded by measuring the longest dimension of each particle using ToupView software.

3.3 Results and Discussion

3.3.1 Nile Red's ability to detect and quantify anthropogenic fibres

Of the 30 different polyester thread colours, 13 were not visible, six were barely visible, and 11 were visible with the Nile Red method used (Figure 3.2). The threads that were most visible were all brightly coloured, while none of the darkest shades produced any fluorescence. The fluorescent images captured of each polyester thread are shown in Figure 3.3 and demonstrate the poor visibility of most threads. Additionally, polyester threads will be easier to see in fluorescent images than individual fibres because they are larger (Prata *et al*., 2021), as shown in Figure 3.4, meaning the results presented here are potentially a best-case scenario.

Figure 3.2 Polyester sewing threads that were used to assess Nile Red's ability to stain fibres. Number indicates thread code for correspondence with Figure 3.3

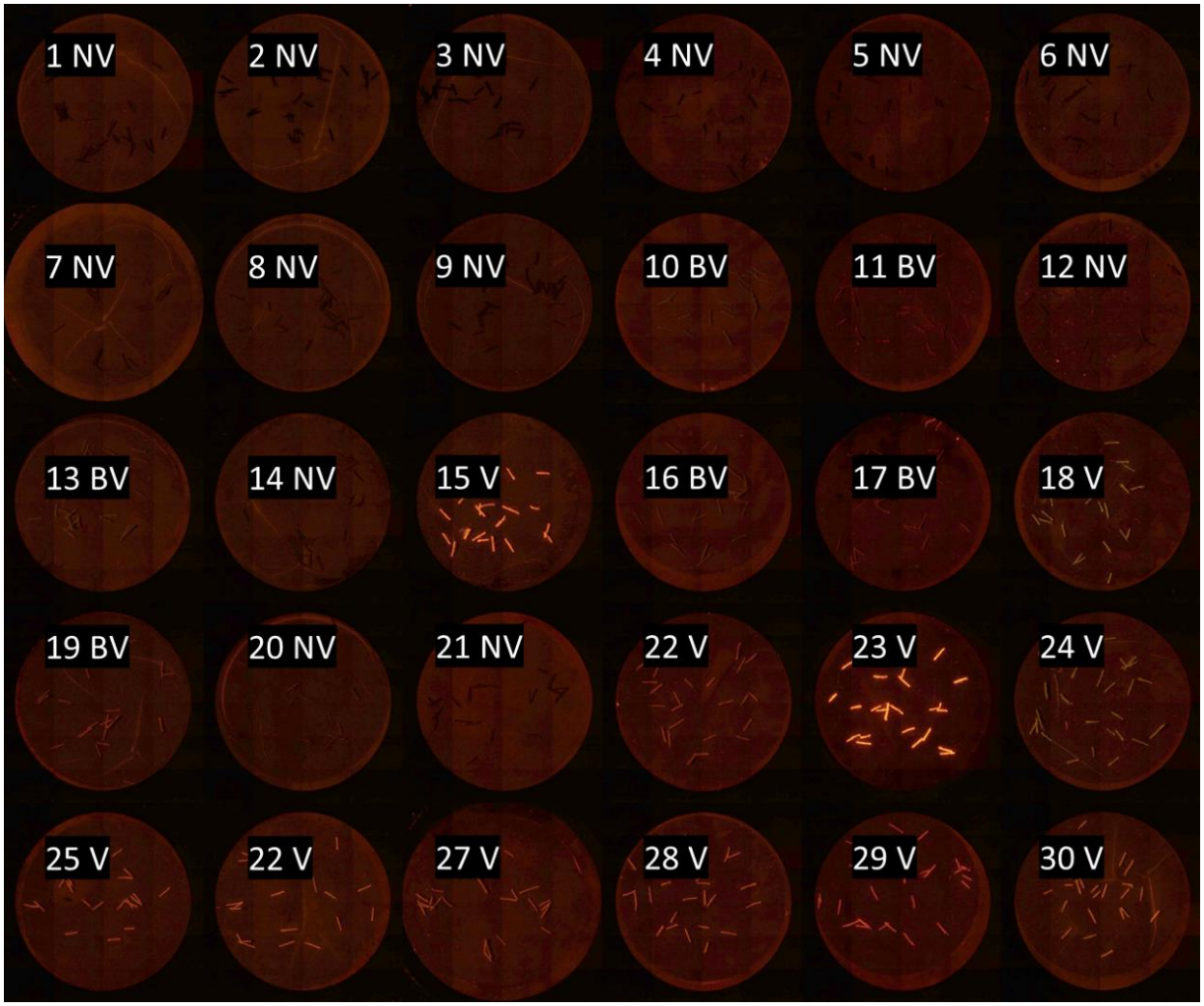


Figure 3.3 Fluorescent images of the polyester threads shown in Figure 3.2 when stained with Nile Red dye. Images 40 % brightened for presentation purposes. NV = not visible, BV = barely visible, V = visible.

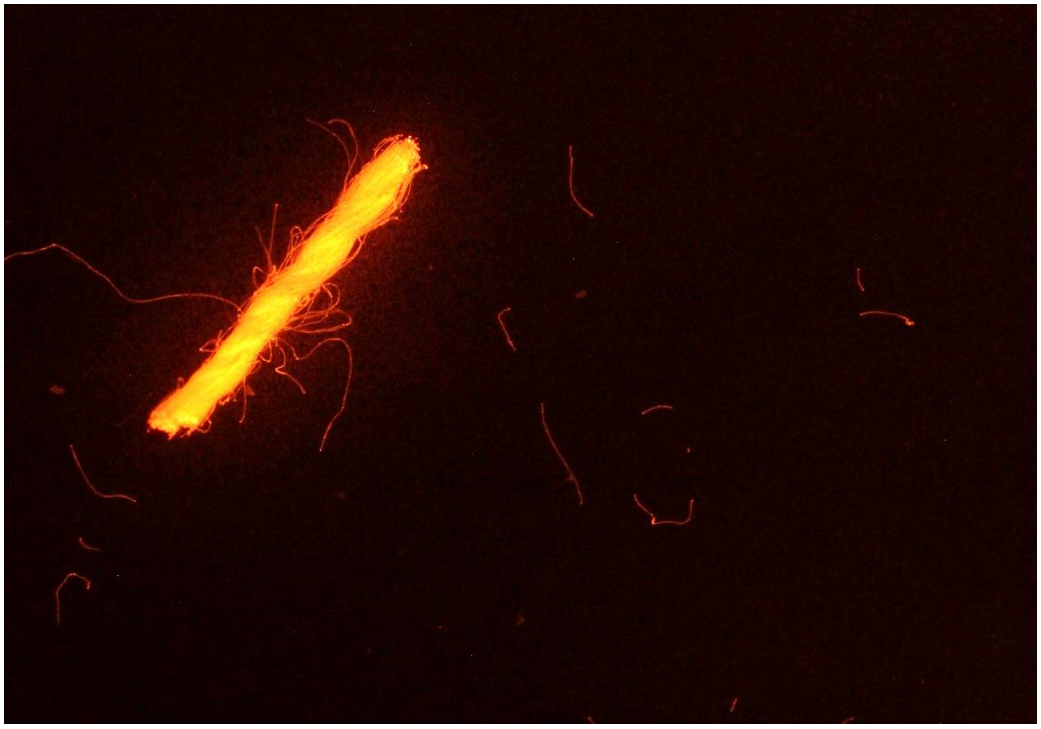


Figure 3.4 Close up image of polyester thread code 23 (as shown in Figure 3.2 and 3.3). The thread can clearly be seen to be fluorescing brighter than individual fibres beside it. Waveguiding of internally reflected photons in individual fibres can also be seen where parts show greater fluorescence intensity. Image brightened by 40 % for display purposes.

If fibres do fluoresce when stained with Nile Red, they may be quantified manually. However, this reduces some of the intended benefits of a Nile Red dye staining approach to microplastic/fibre analysis; being that it is a rapid method. It is common for an automatic quantification tool to be used, such as that developed by Prata *et al*. (2020): the MP-VAT v1.0 and v2.0. However, only two of the 30 thread colours are fully detectable using a maximum entropy and Renyi entropy threshold on ImageJ (thread number 15 and 23), so the MP-VAT v1.0 and v2.0 would not work to detect most polyester fibres. Even then, Figure 3.5 shows that polyester fibres exhibit variable fluorescence intensities along their length, so when these thresholds are applied to individual fibres they register and are counted as multiple fibres (overestimating the number present), meaning this method is unlikely to be capable of consistently producing accurate fibre counts.

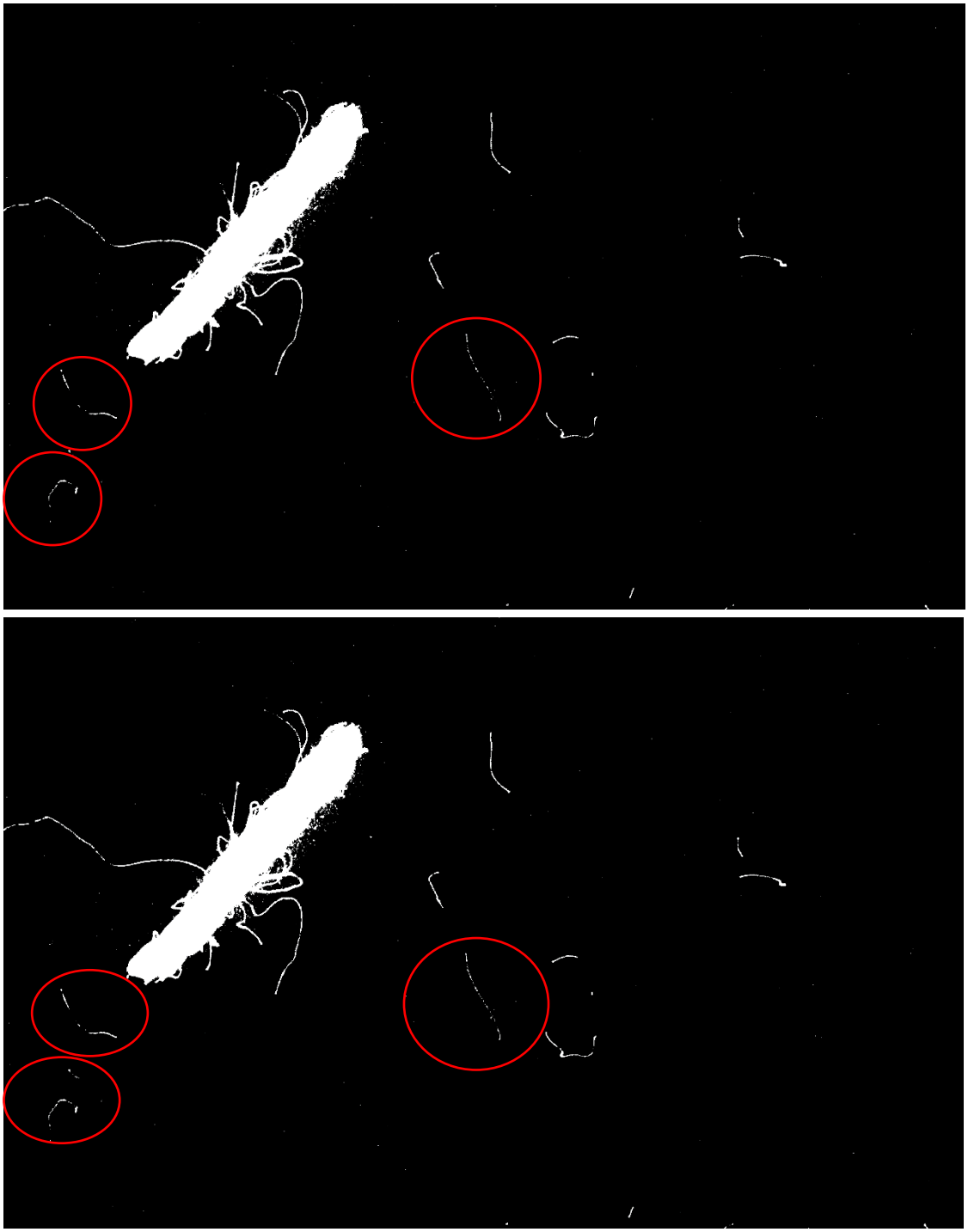


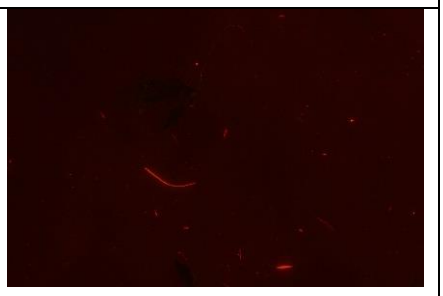
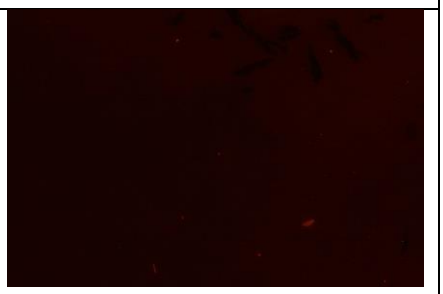
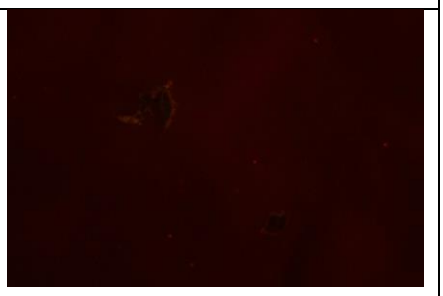
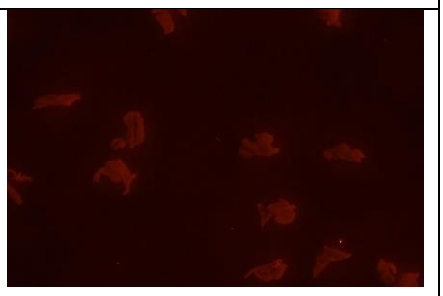
Figure 3.5 Close up image of polyester thread code 23 (as shown in Figure 3.1 and 3.2) after a MaximumEntropy (top) and RenyiEntropy have been applied on ImageJ. Some individual fibres (circled) can be seen to have been separated with these thresholds applied, leading to inaccurate reporting of fibre counts.

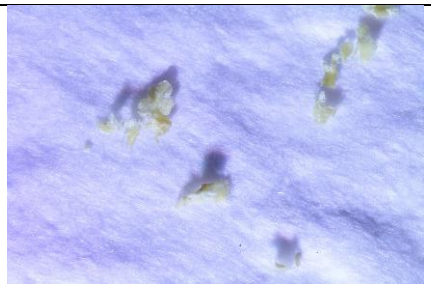
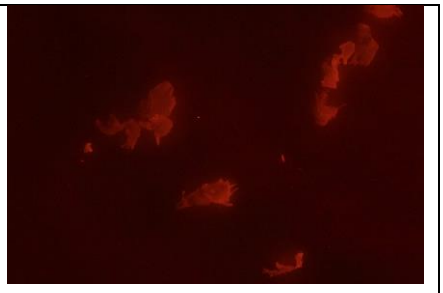
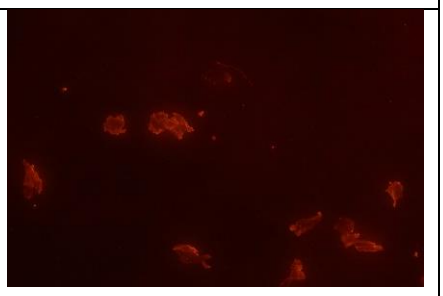
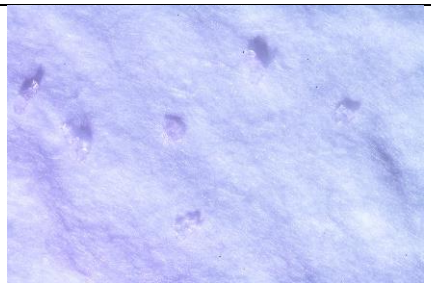
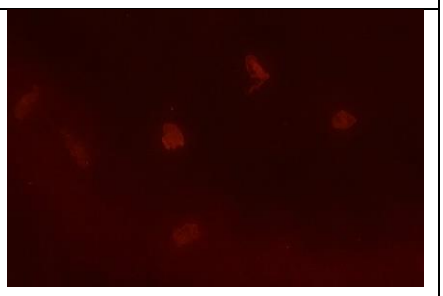
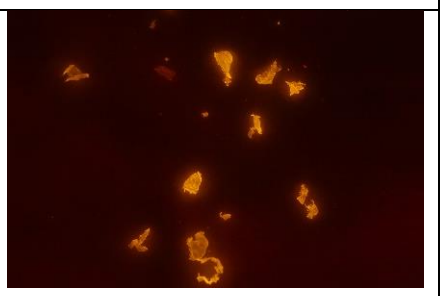
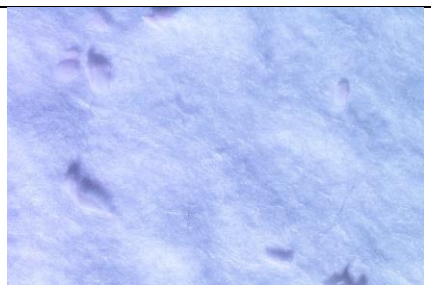
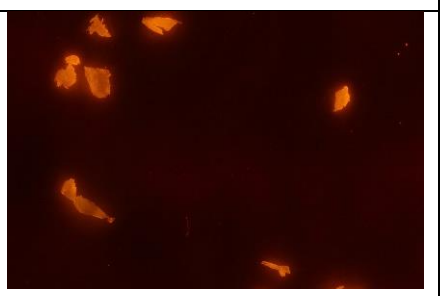
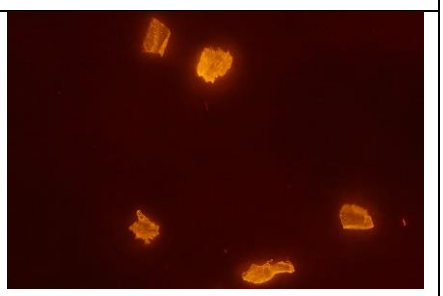
3.3.2 Nile Red's ability to detect microplastic fragments

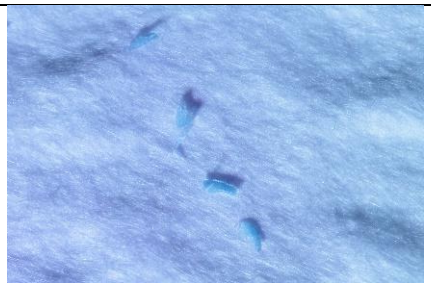
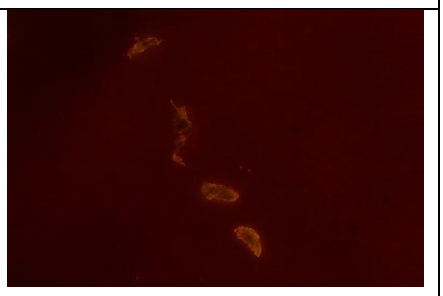
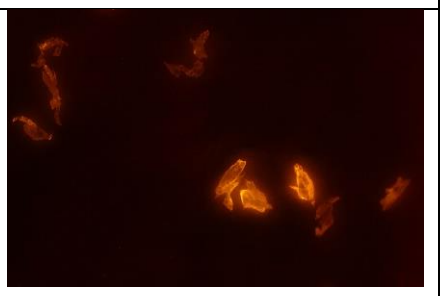
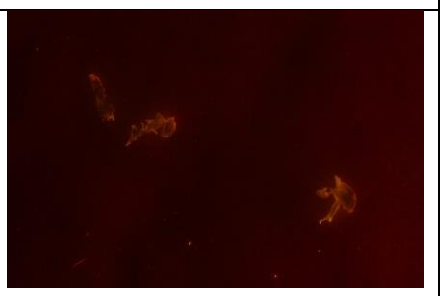
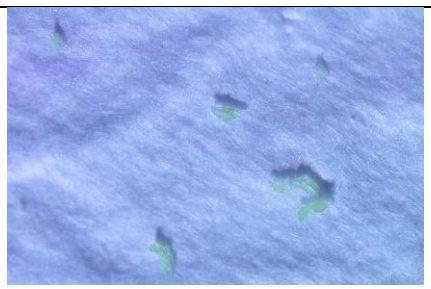
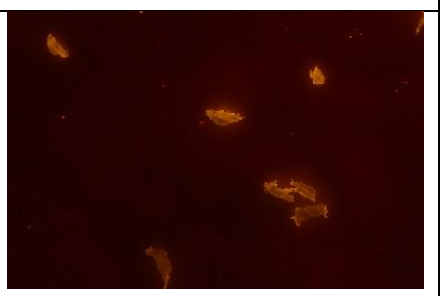
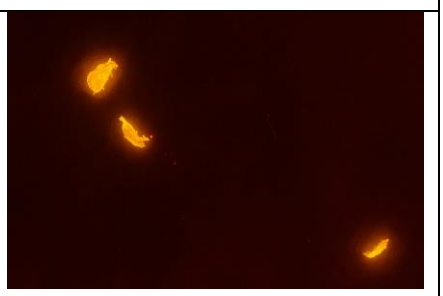
Of the 20 plastic particles stained with Nile Red, 15 % were not visible, 40 % were barely visible, and 45 % were visible. All those that were not visible were black particles, confirming that Nile Red cannot detect black plastic particles due to lack of fluorescence. Generally,

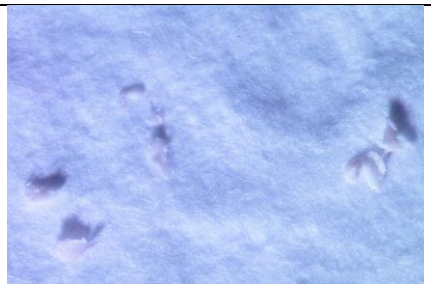
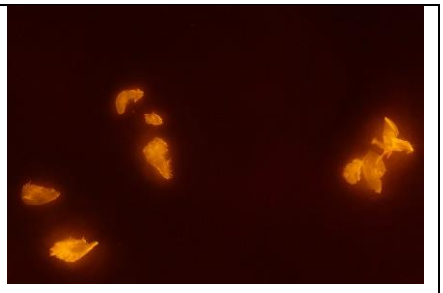
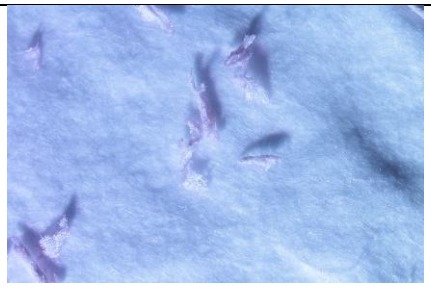
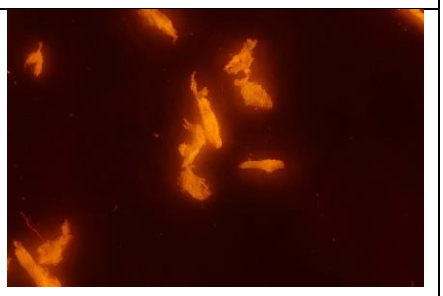
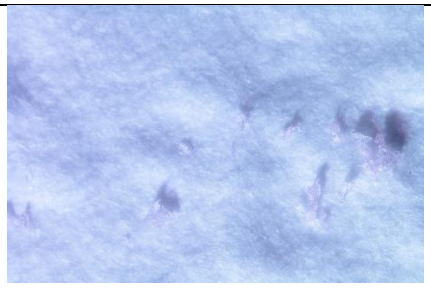
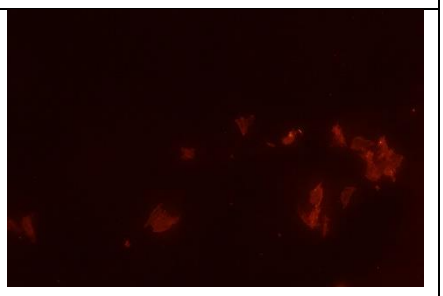
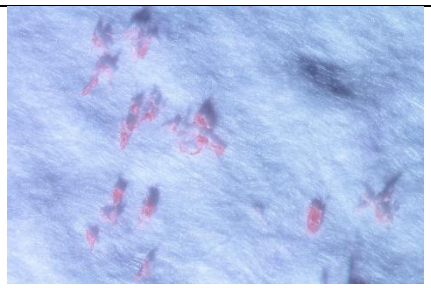
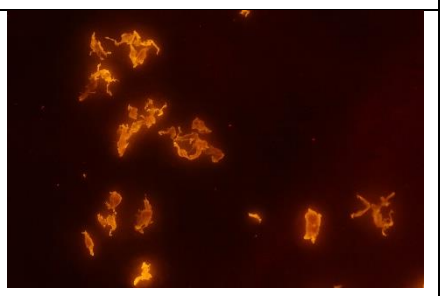
clear and white plastics produced the highest fluorescence, except for clear PVC and PET that were barely visible. Overall, too few plastics were tested to reveal statistically significant relationships, although some insights can be made. Most importantly, variations in fluorescence intensity are shown for the same polymer type, and the same polymer colour. For example, some fragments of clear PS were obviously more fluorescent than others. The use of different pigments in plastics may have shifted the fluorescent emission based on polarity or changed the affinity of Nile Red. Different additives can be used in dyes of the same colour, for example blue can be produced using copper phthalocyanine, iron blue, cobalt aluminate blue, and ultramarine blue (Prata, 2023).

Table 3.1 White light and fluorescent images of various microplastics after staining with Nile Red. NV = not visible, BV = barely visible, V = visible.

Material and colour	Fluorescence visibility	White light image	Fluorescent image
LDPE Black	NV		
PP Black	NV		
PP Black	NV		
PVC Gray	BV		

PP Yellow	BV		
PS Red	BV		
PVC Clear	BV		
LDPE Clear	V		
PP Cream	V		
HDPE Dark blue/purple	V		

HDPE Clear	V		
PP Blue	BV		
PS Clear	V		
PP Brown	BV		
PP Lime green	BV		
PP Clear	V		

LDPE White	V		
PS White	V		
PET Clear	BV		
PP Red	V		

Nile Red also stains other organic material, potentially leading to significant overestimates of microplastic numbers in real world samples (Stanton *et al*., 2019). For example, in two out of three river water samples (River Soar, UK), Nile red significantly ($p<0.05$) overestimated microplastic counts by an average of 48.4 % at 40x magnification and 54.5 % at 100x magnification (Stanton *et al*., 2019). Some organic material fluoresces with similar intensity to that of plastics when stained with Nile Red. For example, Figure 3.6 shows a fragment of *Lemna Minor* after being digested in 50 % NaClO for 16 hours at 40°C, stained with Nile Red, and imaged under fluorescent light. This level of fluorescence was particularly problematic in the present study because *Lemna minor* are abundant in constructed wetland samples and can be resistant to chemical degradation, thus the potential for false positives was high.

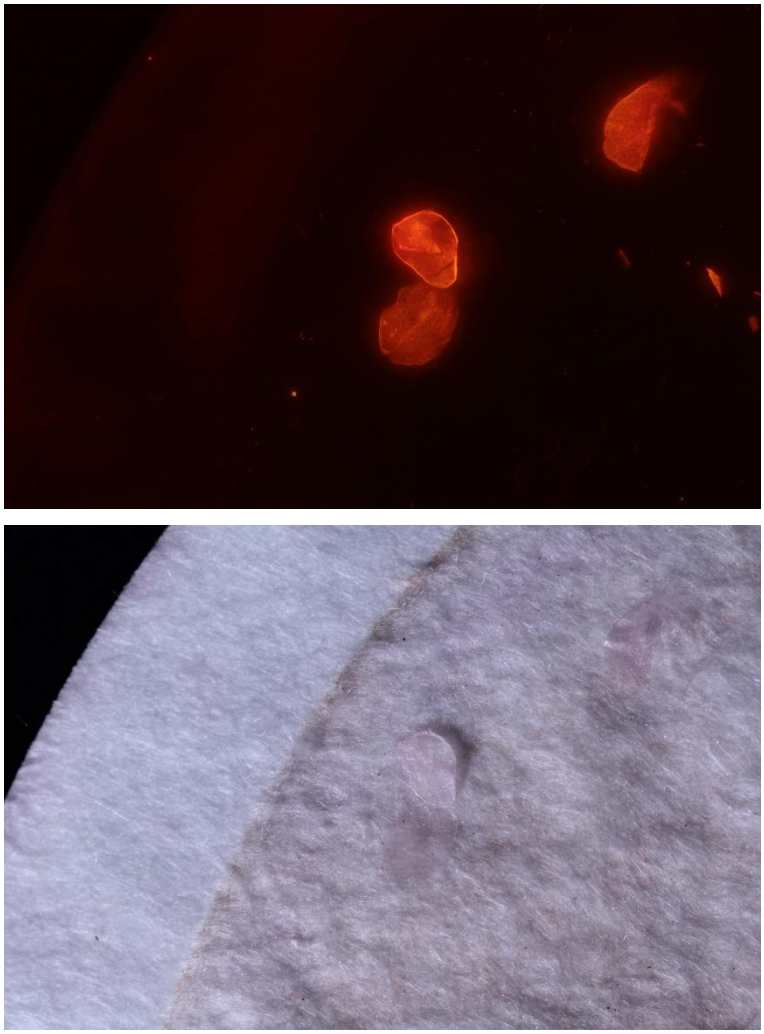


Figure 3.6 Fluorescent (top) and white light (bottom) image of *Lemna Minor* after digestion with 50 % (v/v) NaClO at 40°C for 16 hours.

Although an organic matter reduction step is normally used, typically chemically with an acid (Tuttle and Stubbins, 2023), base (Thiele, Hudson and Russell, 2019), or oxidiser (Hurley *et al*., 2018), it is unlikely that all organic material can be removed (so that no false positives are identified) while maintaining the integrity of polymers in the sample, particularly if the sample is relatively large (which it may need to be to ensure that plastic counts are above the limit of detection).

Figure 3.7 shows a large number of fluorescent particles in a 100 L water sample from the outlet of the Northrepps ICW in May 2022. Using the MaxEntropy threshold method on ImageJ 179 particles were counted (5 or more pixels), and with RenyiEntropy 200 particles counted. The concentration reported here was therefore approximately 1.8–2.0 microplastic fragments L^{-1}. This is a higher concentration than expected, for example, Bydalek *et al*. (2023) found 0.04 microplastic fragments L^{-1} in water samples at the outlet of the Cromhall ICW. Additionally, when visually inspecting the white light image of the filter (Figure 3.7), no particles were identified that also fluoresced which obviously resembled microplastic fragments (appearing artificially coloured) (Figure 3.8). Validating the fluorescent particles

by ATR-FTIR was somewhat biased because only the brightest fluorescent particles can be reliably located and identified by the ATR-FTIR. Also, ensuring that only the particles counted as microplastics were selected for verification was not easily done, especially for small particles that were weakly fluorescing.

The Nile Red protocol therefore appears less reliable than a visual inspection method to identify microplastic fragments in organic rich samples because it can underestimate polyester fibres and various microplastic fragments (that do not produce fluorescence), and it can also overestimate counts by including false positive fluorescent non-plastic particles. The use of Nile Red to detect microplastics in organic rich samples is therefore not suitable unless a high spectroscopic material verification rate can be achieved. In doing so, the false positives can be reliably accounted for, and the final results can be considered an underestimate because of unstained microplastics. To achieve such a high spectroscopic verification rate would be challenging and may only be possible if the entire filter (containing the sample) can be scanned with either Raman or FTIR, and the resultant particle material results aligned with the fluorescent image. This would also require few particles to be present on a filter, presenting additional sample processing complications (such as increased steps and therefore potential for increased contamination and losses). A method based on visual inspection was therefore developed.

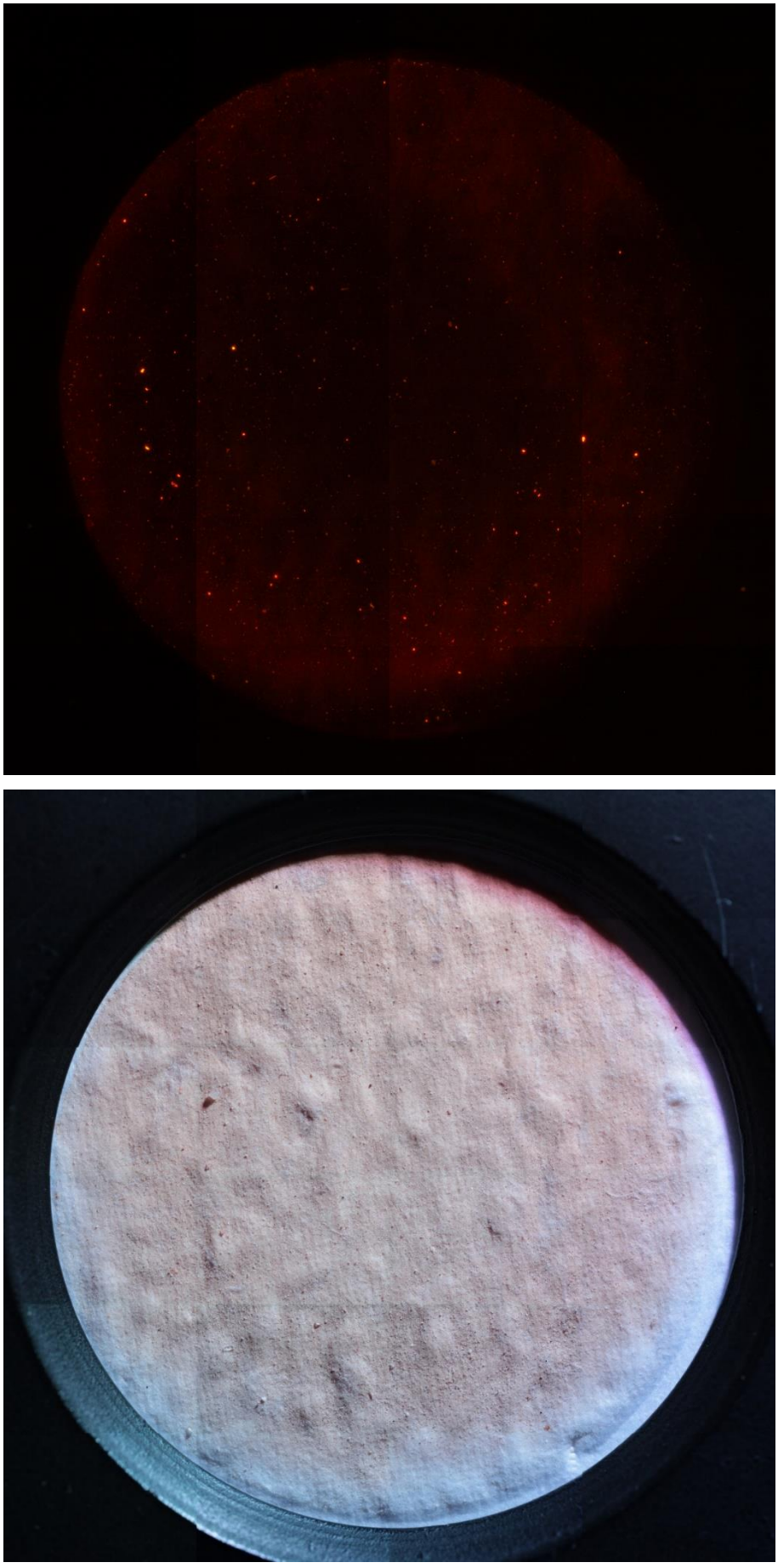


Figure 3.7 Fluorescent image (top) and white light image (bottom) of a 47 mm filter *c*ontaining residual particulates from a 100L water sample (after digestion with NaClO)

from the outlet of the Northrepps ICW. Orange, fluorescent particles indicate potential microplastics.

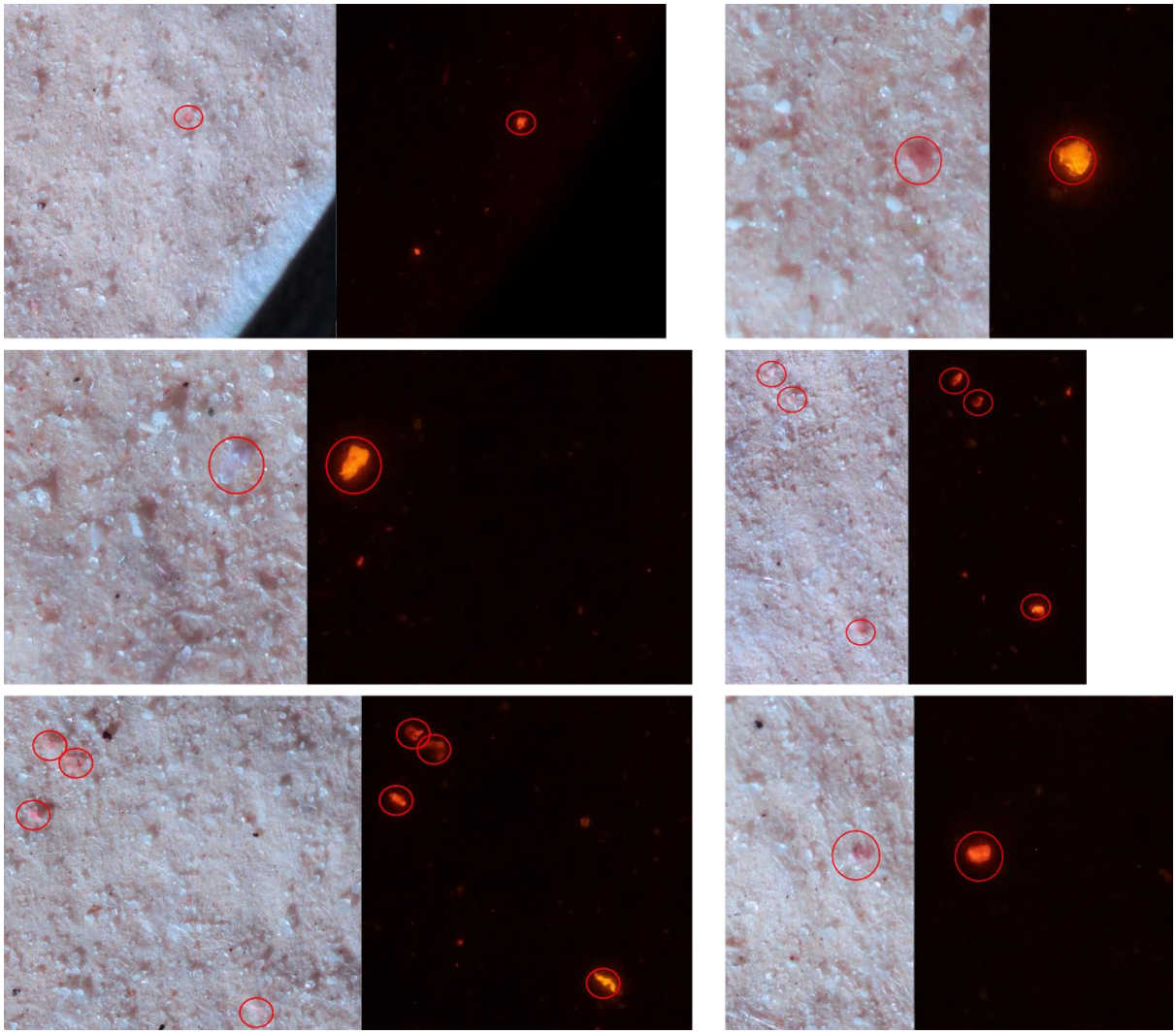


Figure 3.8 Side by side view of fluorescent particles and the white light image of these (circled)

3.3.3 Developing a new method to identify anthropogenic fibres in freshwater samples

3.3.3.1 *Sample collection*

Almost all microplastic studies report contamination in their procedural blank samples, and anthropogenic fibres constitute most of these (Prata *et al*., 2021). It was therefore soon determined that contamination would be inevitable, despite best efforts to reduce it, and that a large sample volume was desirable so that anthropogenic fibres were quantifiable at concentrations above the limit of detection. Therefore, simply taking a water sample in a glass jar was deemed inadequate because the sample volume would be too low (logistically limited to 10 L for each sample). For example, in a similar ICW, Bydalek *et al*. (2023)

reported concentrations at the outlet of 0.26 fibres L^{-1}. The advantage of taking a smaller sample in a glass container is that the sampling time is low, so contamination risk is lower. Additionally, the water sample can be directly vacuum filtered using small pore size filter paper (for example 0.45 µm), meaning small microplastics could be identified in samples. However, visual identification and material composition verification (using micro-ATR-FTIR) of particles <25 µm would be challenging, thus the benefit of this is insignificant.

In the present study, to sample as larger a water volume as possible, a sieving method was established, whereby a known volume of water was passed through a 38 µm stainless steel sieve (200 x 50 mm) until it began to lightly clog (this point was determined when water would take ~30 seconds to completely filter through). This approach enabled large sample sizes to be achieved, up to 800 L depending on the turbidity of the water. By stopping sampling before the sieve completely clogged, the amount of material collected was optimal for a method that avoids a density separation step. Too much material collected on the sieve may complicate sample processing by requiring either increased steps to remove the inorganic portion (density separation), or by increasing the final number of filters per sample (increasing contamination risk). There are two main downsides to this sampling technique: firstly, sampling takes longer so contamination risk increases (compared to using bottles), and secondly that anthropogenic fibres with diameter below 38 µm may pass through the sieve and therefore be removed from the sample. However, given that few anthropogenic fibres were expected at the outlet of the constructed wetlands, it was deemed more important to obtain a large sample volume so that there was a higher probability that fibre concentrations would be quantifiable above the limit of detection in samples.

After sampling, the sieve was sealed with a stainless-steel lid and base and transported back to the laboratory. A new sieve was therefore used for each sampling location. This meant sieves did not have to be processed in the field which is preferred from a contamination perspective (Koelmans *et al*., 2019).

3.3.3.2 Sample processing

Reducing anthropogenic fibre contamination was considered of high importance when processing water samples. As a result, a minimalist philosophy was adopted whereby the essential requirements of the method were established, and then the simplest way of achieving them was determined. The suspended particulate matter within constructed wetlands consists mostly of low-density organic material, meaning a density separation step (that removes high density inorganic material) was not required. On the other hand, this meant that an organic matter digestion step was necessary to enable visual identification and FTIR verification of anthropogenic fibres (that would otherwise be coated in organic material).

Upon return to the laboratory the same day as sample collection (to prevent material inside from drying and being un-extractable), the contents of the sieves (containing suspended particulate matter to be analysed) were rinsed into glass beakers (400 mL). The exact volume of water required to thoroughly rinse the sieve contents was variable for each sample, but it was always less than 100 mL. To simplify the method, MilliQ water was added to the 100 mL mark.

NaClO was chosen to remove organic material from the sample because it has been shown to be effective at doing so without destroying microplastics (Bottone *et al*., 2022) and can perform better than alternative digestion solutions, such as Fenton reagent and potassium hydroxide (Monteiro *et al*., 2022). As such, 100 mL NaClO (minimum 14 % free chlorine) was applied to the sample to create a 1:1 dilution. A weaker 10 % (v/v) NaClO solution was tested, as used in Monteiro *et al*. (2022), but this concentration did not remove enough organic matter from the sample. To enhance the organic matter digestion, the beaker was placed in a shaker incubator (Orbital Shaker Incubator ES-80) at 40 °C and 90 rpm for 16–20 hours. By incubating at 40 °C, microplastic degradation is unlikely to occur (Munno *et al*., 2018), yet the organic matter removal rate is enhanced compared to incubating at room temperature. The motion of the shaker incubator was also found to prevent the particulate material from settling quickly and therefore improved digestion efficiency. After digestion, this solution was vacuum filtered onto one or two separate 47 mm diameter cellulose nitrate filters (pore size 3 µm), depending on the amount of sediment present (only a thin layer on each filter was required to reduce the need to search for anthropogenic fibres). By not collecting samples until the sieves clogged heavily and filtering onto two separate filters, a density separation step was not required. This is important because the risk of contamination and microplastic losses is likely reduced by having a simpler method involving fewer steps. Given that low microplastic numbers were expected in some samples, reducing any potential losses was considered of high importance.

3.3.3.3 Sample analysis

Each 47 mm cellulose nitrate filter was transferred to a microscope (Leica CMA) and fibres were identified by visual inspection (with 4x objective). A fine tip sewing needle was used to gently poke fibres that may not be anthropogenic (without a smooth texture) and if the fibre broke then it was deemed too brittle to be anthropogenic (Rochman, 2021). Fibres were categorised based on their colour and estimated length grouped into three categories: small (38–250 µm), medium (250–800 µm), and large (>800 µm). These measurements represent an estimation because often a portion of a fibre was hidden beneath another layer on the filters and attempting to excavate each one would risk dislodging other material (including other fibres) from the filter. The precise length of each fibre was not measured

because this would be extremely time consuming given the large number of fibres (>5,000) identified in this research.

Microplastic fragments were not searched for on water sample filters, mainly because the method was optimised for the detection of anthropogenic fibres, since the majority of plastic material found in sewage effluent is often fibrous (Viitala *et al*., 2022).

3.3.4 Developing a method to identify microplastic fragments in organic-rich sediment samples

3.3.4.1 Sample collection

The collection of sediment samples from densely vegetated integrated constructed wetlands was challenging. The main problem was that the plant stems and their litter formed a dense heterogeneous subsurface 'filter'. The sediment was therefore not accessible by traditional means such as submerged coring devices because it was too wet. This subsurface 'net' of plant stems and litter was also of interest to be sampled because microplastics may have settled on top of it given the very low water velocities in places. An isolation agitation sampling method based on Woodward *et al*. (2021) was therefore adopted. A stainless-steel cylinder (300 x 900 mm) was pushed down as firmly as possible approximately 5 cm into the sediment, sometimes requiring vegetation to be carefully pulled apart. The exact depth of penetration could not be measured because the depth of fine bed sediment varied between sampling locations. The fine bed sediment (FBS) samples (the loose surface flocculent layer) were agitated into suspension using a stainless-steel saucepan for 30–60 seconds. Turbid water samples were poured (with the stainless-steel saucepan) through a sieve stack of 2 mm and 38 µm until enough sediment could be extracted from the 38 µm sieve to approximately fill a glass jar (480 mL). The contents of the 2 mm sieve were extracted into separate jars to be analysed for macroplastics. All glass jars were sealed with aluminium foil to prevent contamination. Any mud stuck to the metal sampling cylinder was washed off with the water in the wetland then lightly rinsed down between sampling locations with MilliQ water, meaning any plastics stuck to the metal should not have been transferred to the next sampling location.

3.3.4.2 Sample processing

To determine the content of microplastics and anthropogenic fibres in samples, the content per unit dry mass was used (as per Woodward *et al*., 2021). A test sample was first dried in a vacuum oven at 40 °C, although this produced a hard conglomerate resembling a hockey puck that was unsuitable for further processing. Freeze drying was therefore tested, and this produced a much finer dry sediment that could be processed.

A similar minimalist philosophy was applied to the processing of sediment samples as for water samples, although a density separation step was this time essential given the

presence of dense inorganic material. FBS samples were freeze dried (Scanvac Coolsafe) until a constant dry weight was achieved. Due to equipment availability, for some samples drying was completed in the vacuum oven at 40 °C (after freeze drying). This did not impact the texture of the final dry sediment. Before a density separation step was performed, the organic material was first removed. This was important because a large proportion (estimated between 50–95 %) of the fine sediment collected was low density organic material that would float in a high-density salt solution, meaning the density separation would have been largely ineffective at reducing the sample volume. To remove much of the organic material, 400 mL of NaClO (50 % dilution in water) was added and left for 16–20 hours at 40 °C in a shaker incubator (Orbital Shaker Incubator ES-80) at 90 rpm. The solution was then filtered through a 38 µm stainless steel sieve and rinsed thoroughly. However, a large amount of partially degraded organic material remained after this. A second digestion was therefore performed for 16–20 hours at 40 °C in a shaker incubator at 90 rpm, after sieving the contents of the first. This was chosen instead of a higher concentration digestion solution to prevent degradation of microplastics in the sample.

Following organic matter removal, a density separation step was required. To do so as simply as possible, the sample containing digestion solution was filtered through a 38 µm stainless steel sieve and rinsed into the same glass beaker to reduce potential losses. However, to prevent dilution of the high-density salt solution, the sample (containing water from rinsing the sieve) was placed into a vacuum at 40 °C oven to near dryness (until the water level was less than ~25 mL). Completely drying the sample was avoided to prevent crisps of sediment forming that would disrupt the density separation process. Zinc chloride (1.5 g mL^{-3}) was added to the same glass jar containing the 'dried' sample and left on a shaker at 90 rpm for at least 30 minutes to better separate any sediment agglomerates. The homogenised sample was then added to the density separator and topped with $ZnCl_2$ (1.5 g mL^{-3}).

The density separator used was custom made and based on the design of Vermeiren *et al*. (2020), although the diameter tubing was slightly different, 63 mm in the present study compared to 67 mm. The unit had a volume of 935 ml until overflow (Figure 3.9). The units were filled close to the top and left for a minimum of three hours before $ZnCl_2$ (1.5 g mL^{-3}) was added to overflow the units. After three hours, there was visibly no suspended sediment in the column, indicating that further time was unnecessary. The overflow was stopped after approximately 100 mL of $ZnCl_2$ had overflowed into the glass beaker. The density separator was then left for a minimum of three hours again after agitation with a magnetic stirrer for 60 seconds before another 100 mL was overflowed. Agitation was required between overflows to minimise the number of microplastics/anthropogenic fibres 'trapped' in inorganic sediment. After density separation, the sample (combined overflow) was vacuum filtered onto a 47 mm cellulose nitrate filter (0.45 µm pore size) before analysis of the filter.

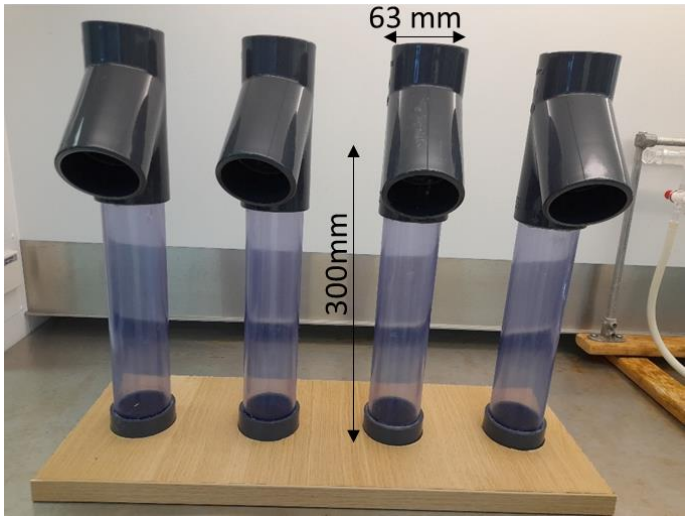


Figure 3.9 Custom made density separators.

A more common density separator design is the sediment microplastic isolator (SMI) unit (Coppock *et al*., 2017). Density separation was attempted with one of these, but it was found cumbersome to use and if the components were not perfectly fitted together then significant leakage could occur. Additionally, there were visible scratch marks on the central plastic ball valve indicating that significant contamination occurred from these units as sediment particles were trapped in the valve and ground against the plastic. Cleaning these SMI units is also more difficult because often sediment can get stuck in the ball valve. Instead of the SMI, the overflow unit was used. For the overflow density separator, Vermeiren *et al*. (2020) reported mean recovery rates over 90 % for four polymers tested (polypropylene, polyethylene, polyethylene terephthalate, polyvinyl chloride) and found the recovery rates similar to those with the SMI. The overflow density separator is easier to use than the SMI because it is already fixed in place. However, a potential downside is that when applying $ZnCl_2$ to the density separator, some disturbance can occur, and fine sediment can re-suspend. To minimise this the density separator was filled as close to the top as possible (by viewing from above) so that the unit started overflowing very soon after applying $ZnCl_2$. Additionally, $ZnCl_2$ was squirted around the back edges of the unit so that the flow did not directly hit the surface liquid and cause unnecessary disturbance.

The same density of $ZnCl_2$ was also used as in Vermeiren *et al*. (2020), that being 1.5 g mL^{-3}, although some plastics (PVC) have a higher density (Table 3.2) than that used (meaning they may not be captured in the sample). At higher density $ZnCl_2$ solution becomes harder to work with because it becomes increasingly viscous so takes longer to filter when recycling the solution. Given its relatively high cost, recycling was important in the present study. Additionally, the density of plastics is also impacted by agglomeration with other particles and biofilm growth, which may reduce the average density (depending upon the density of the agglomerating material) (Guo and Wang, 2019).

Table 3.2 Densities of common plastics. Data from Hidalgo-Ruz *et al*. (2012)

Polymer	Density (g cm^{-3})
Polyethylene	0.917–0.965
Polystyrene	1.04–1.1
Polypropylene	0.9–0.91
Polyamide	1.02–1.05
Polyethylene terephthalate	1.37–1.45
Acrylic	1.09–1.20
Polyvinylchloride	1.16–1.58
Polyurethane	1.2

3.3.4.3 Sample analysis

Each 47 mm cellulose nitrate filter was transferred to a microscope (Leica CMA) and fibres were identified (with 4x objective) in the same way as for water samples. Tweezers and a 33-gauge syringe needle were used to extract suspected microplastic fragments into a beaker for spectroscopic validation. An attempt was made to extract approximately every tenth suspected microplastic fragment for chemical extraction. Suspected microplastic fragments were identified by the following criteria:

- Fragment appearing artificially coloured or shiny (resembling glitter)
- Fragment dark in colour with sharp edges and smooth surface

A significant omission then was the counting of white and clear microplastic fragments. The problem was that there were too many false positives on each filter, particles that were clear or white that could conceivably be microplastic. While some clear microplastic films or larger white plastics may have been identifiable, it is crucial to recognize that the method used may only be capable of identifying certain types of clear and white microplastics. This limitation must be considered to avoid misrepresenting the microplastic composition in the samples. Example images of filters are shown in Figure 3.10. The reasons why a large amount of these false positive particles were present is because of the large amount of residual organic material and the large sample size. To have identified white and clear microplastic fragments would require a method with increased steps to reduce the amount of material on the final filter (such as a third digestion), or a smaller initial sample size of < ~10 g. However, some ATR-FTIR spectra already showed signs of degradation (peaks at 660 cm^{-1} indicative of chlorination in polyethylene) by the digestion step, so adding another would have degraded plastics further and required extra sample processing time in an already long procedure. A smaller sample size was also inappropriate because enough microplastics needed to be counted on each filter to be above the limit of detection (that

would be unchanged). The use of Nile Red may aid detection of translucent and white microplastic fragments because these would fluoresce and thus be easier to identify. However, the relatively large amount of organic material on the filters would mean that many particles would fluoresce anyway, so the use of the Nile Red would not be worthwhile. There was also limited laboratory equipment available to achieve this.

Figure 3.10 Images of a typical sediment sample filter (taken with 4x objective) with potential false positive clear, white and black particles circled.

3.3.5 Attempting to identify microplastics and anthropogenic fibres adhered to submerged plant surfaces

An attempt was made to assess the number of microplastics and anthropogenic fibres that were attached to submerged living plant stems within 5 m of the inlet pipe in the first cell of Northrepps ICW. This information was of interest because if a large number of microplastics/fibres were found strongly attached to plant stems via biofilms, the retention of these items may be improved compared to if they had just settled and could perhaps be more easily resuspended (Goss *et al*., 2018). Additionally, wetland management in the form of vegetation removal may be influenced by the extent of microplastic/fibre contamination on removed plants.

To sample submerged plants, plant stem samples were cut at the water surface and near to the bottom of the fine bed sediment using sheers. Plants were collected within 3 m of the inlet pipe (Figure 3.11). This was complicated by the low water levels at the time of sampling, July 2023 (the first attempt), because most of the plant was in a layer of loose mud, not the water column, with a floc layer taking up most of the depth. Floc is comprised of decaying plant material, microbes, algae, and soil/sediment particles. This layer has low solids content and is therefore soupy in texture and pourable (Kadlec, 2020). The formation of the floc layer is particularly prevalent near the inlet due to the elevated nutrient concentrations from WWTP effluent (Kadlec, 2020). For example, although the water depth looks shallow in Figure 3.11, when wading in the depth is approximately 30 cm to firm ground.

When sampling, the plant stems were therefore covered in mud instead of being relatively clean as they would be if the water was deeper. Five stems were placed in a black bin liner (previously unused to prevent contamination) and transported back to the laboratory. Under the laminar flow hood, the mud on the stems was gently rinsed off and disposed of. A stem after initial cleaning is shown in Figure 3.12. The stem was then rinsed strongly using a squeezy wash bottle (with MilliQ water) and the surface layer carefully scraped with a knife while rinsing to extract any microplastic/fibres that were attached in biofilms to the plant surface. Five stems were combined into one sample. This sample was then digested in 50 % (V/V) NaClO at 40 °C for 3 hours and then vacuum filtered onto cellulose nitrate filters (0.45 µm pore size) and analysed under the microscope. A shorter digestion time was used, compared to water and sediment samples, because the organic material scraped/rinsed off plants was visibly removed by digestion within this time.

After processing five stems into one sample: 78 anthropogenic fibres and 19 suspected microplastic fragments were visually identified. A procedural blank sample was also run in parallel: 26 anthropogenic fibres and four suspected microplastic fragments were identified. Significant contamination occurred, probably because the time the sample was exposed

during processing was high (approximately 10 minutes was spent rinsing/scraping each stem). It was therefore decided not to pursue sampling plant stems further because the contamination rate was too high and the stems too muddy. To date, it appears that no other studies have attempted to quantify microplastics/fibres on submerged plant stems in such wetlands, possibly due to the methodological difficulties of doing so.

A second attempt was made to sample submerged plants for attached microplastics/fibres in January 2024. Plants were collected in the same way as before. The sampling was no easier, although the water level was likely marginally higher (due to winter rainfall), the plants at the inlet were still submerged within a muddy floc layer, not a ‘clear’ water column. The plants removed were thus muddy and required rinsing upon return to the laboratory. Instead of scraping the plant stems to remove biofilms, which required a high sample exposure time increasing contamination, the stems would be first rinsed to remove mud and then placed whole in digestion solution. They were to be left for a short period that would allow for the chemical digestion of the attached biofilms (not the entire stem), meaning the proceeding rinsing step would be simpler and scraping would be avoided. In this way, contamination would be reduced. However, this did not work because the digestion process caused unintended degradation of the plant material, making it difficult to separate biofilm residues from the sample.

Figure 3.11 Photograph showing vegetation and floc within 5 m of the inlet in cell one at Northrepps ICW. Taken March 2023.

Figure 3.12 A submerged plant stem sample in a glass beaker after being initially rinsed.

3.4 Call for standardised methods

Like many others, the importance of standardizing microplastic processing and analysis methods is emphasized to enable more accurate comparisons between studies (Pérez-Guevara et al., 2022). However, it is acknowledged that the equipment available to laboratories will significantly influence their chosen methods. Therefore, an exact standardization of methodology is not being called for, but instead a recommendation for all future microplastic studies to define (with evidence) precisely what it is their method is capable and not capable of identifying in their samples. The ability to consistently be able to identify a target particle type (e.g. size, shape, colour, material) in each sample is essential to allow accurate comparisons between samples, and would allow comparisons between studies, even if the studies used different methods. Some aspects of analytical methods could benefit from standardization, particularly the use of consistent chemical digestion conditions, although adjustments may still be necessary based on the organic matter content in the samples. The advantage of employing a standardized chemical digestion solution is that it allows for a comprehensive assessment of its effects on polymers, such as potential degradation, particle generation, or changes in the interpretability of spectra.

It is also recommended to consider the use of multiple sample analysis methods for different microplastic types and sizes. For example, most studies are not able to detect small microplastics (<20 µm) due to methodological limitations (such as sieve mesh size). Generally, studies that do identify small microplastics can only sample a small volume,

meaning quantifying microplastics at concentrations above the limit of detection may be less likely due to inevitable contamination. This could drastically reduce the accuracy of anthropogenic fibre results because it is easy to contaminate a sample due to the ubiquitous presence of fibres. For example, Tagg *et al*. (2015) were able to extract microplastics down to 0.2 µm, however their maximum sample volume their method could process was merely 0.25 L. It is emphasised that there is not a 'one size fits all' microplastic analysis method.

3.5 Conclusions

In summary, the standard Nile Red protocol was not selected to identify microplastics and anthropogenic fibres in freshwater and organic rich sediment samples because:

- The method did not identify a range of coloured polyester fibres.
- The method is likely to overestimate counts of microplastic fragments due to the organic rich nature of the samples.
- The method is likely to underestimate an unknown proportion of microplastic fragments, owing to low fluorescence intensity.

As a result, a new method was developed that was based on the principles of visual inspection rather than fluorescence staining. Compared to the standard Nile Red method, the benefits of the new developed method are:

- Ability to detect all coloured anthropogenic fibres in samples.
- Ability to consistently detect coloured microplastic fragments in samples without overestimating.
- Ability to achieve a higher ATR-FTIR validation rate because fragments/fibres are easier to identify and extract from samples for spectroscopic validation.

Some limitations remain with the developed method. The most significant of which is that the method was ineffective at identifying non-coloured microplastic fragments due to the prevalence of false positives on filters. Future research could address this by testing the impacts of adding more steps to the method (such as centrifugation and different chemical digestion conditions) to determine whether cleaner filter papers can be achieved and if false positives of clear, white, and dark fragments can therefore be reduced. A superior method would also achieve a high spectroscopic validation rate, although this was not possible given the laboratory equipment at the time. For example, the spectral acquisition time using a Raman microscope is significantly less than micro-ATR-FTIR, and the resolution of the microscope is higher, meaning the entire sample could be analysed using the Raman microscope and suspected fragments/fibres could be selected for spectral verification with zero disturbance to the sample.

Chapter 4

Sewage Derived Microplastic and Anthropogenic Fibre Retention by Integrated Constructed Wetlands

4.1 Introduction

High microplastic and anthropogenic fibre loads can be discharged into rivers from wastewater treatment plants (WWTPs) with primary, secondary, and tertiary treatment (Ziajahromi *et al.*, 2017, Blair *et al.*, 2019, Napper *et al.*, 2023). Generally, microplastics in WWTP influent are derived from a variety of sources (Prata, 2018), while washing machine emissions are a dominant source of anthropogenic fibres (Browne *et al.*, 2011). Assuming working operation, an average of 72 % of microplastics are removed after primary treatment, 88 % after secondary treatment, and 94 % after tertiary treatment (Iyare *et al.*, 2020), although removal rates can be as high as 99.9 %, as reported by Carr *et al*. (2016). Tertiary treatment is expensive and is typically used in WWTPs discharging into sensitive waterbodies and serving population equivalents >10,000 (Bunce *et al.*, 2018), thus limited cost-effective measures are available to resolve the problem of microplastic and anthropogenic fibre release from WWTPs. Additionally, WWTPs do not always perform as they are intended to (Hammond *et al.*, 2021) and releases of untreated wastewater contribute a significant source of microplastics and anthropogenic fibres to waterbodies, particularly from combined sewer overflows during rainfall events (Woodward *et al.*, 2021). Hence, WWTP discharge exports significant microplastic loads to the sea (Siegfried *et al.*, 2017). Risk assessment for microplastic particles is complicated (Koelmans *et al.*, 2023), especially given the diversity of microplastics (Rochman *et al.*, 2019). However, microplastics act as vectors of other pollutants enhancing their transport (Wagstaff *et al.*, 2022) and leach chemical additives from within the plastic itself, such as endocrine disrupting plasticizers (Meeker *et al.*, 2009).

In addition to microplastics, WWTP discharges can elevate nutrient concentrations in rivers (Cooper *et al.*, 2022) and increase eutrophication risk (Jarvie *et al.*, 2006). As a result, integrated constructed wetlands (ICWs) have been applied to enhance the water quality of WWTP effluent prior to release into surface waterbodies (Scholz *et al.*, 2007). ICWs generally consist of a series of connected surface flowing ponds containing shallow water year-round supplied entirely from WWTP effluent. Their implementation balances ecological aims of wetland restoration and engineering targets for economically and consistently enhancing water quality (Babatunde *et al.*, 2008). The two ICWs investigated in the present study, Northrepps and Ingoldisthorpe, Norfolk (UK), have been shown to effectively retain nutrients and reduce eutrophication risk from WWTP discharges: mean nitrate and

phosphate concentrations were reduced by ~63 % and ~30 % across the Northrepps and Ingoldisthorpe ICWs respectively (Cooper *et al.*, 2020). The dense stands of emergent vegetation in ICWs decrease water velocities and promote sedimentation of suspended material, meaning ICWs may also be well placed to cost-effectively reduce microplastic loads in receiving waters.

Few studies have assessed microplastic retention by constructed wetlands, and those that do are mostly subsurface flow constructed wetlands (Xu *et al.*, 2022). In a surface flow constructed wetland (SFCW) in Northern China, the Lingang Ecological Wetland Park, average microplastic removal rates were 29.4 % from September to October 2020 (Zhou *et al.*, 2022). In a nearby SFCW, Konggang, microplastic removal rates were 43.7 % (Zhou *et al.*, 2022). These removal rates are based on the numbers of microplastics (including fibres) down to a reported size of 20 µm. In the Lingang SFCW, larger particles (>100 µm) were better retained than smaller (20–100 µm) particles. Fibres were most well retained, and fragments least well retained in both the Lingang and Konggang SFCW. However, the surface flow wetlands studied by Zhou *et al.* (2022) are not comparable in design to those in the present study: they are part of a much larger wetland system combined with subsurface flow constructed wetlands. The most similar work to the present study identified in the literature is that of Bydalek *et al.* (2023). Their study aimed to assess microplastic fate in a surface flow constructed wetland at the Cromhall ICW, Gloucestershire, UK. Here the loading rate was 790 m^3 day^{-1} from a secondary treatment WWTP serving 2000 people (Bydalek *et al.*, 2023). Although the ICW is relatively similar to those in the present study, the sampling campaign by Bydalek *et al.* (2023) occurred only during summer months in July and August 2021, so seasonal variation in ICW performance was not addressed. Constructed wetland plant biomass (including underground biomass) is lowest during winter months (Zhang *et al.*, 2022), meaning the microplastic filtering capacity of these wetlands may be reduced in winter when loading rates from WWTP effluent are higher due to increased rainfall. Additionally, the material composition of suspected microplastics and fibres found was not investigated in detail by Bydalek *et al.* (2023), with only 12 particles >1 mm verified by FTIR. Furthermore, microplastic fragments and anthropogenic fibres have not to date been quantified in fine bed sediment of integrated constructed wetlands treating WWTP effluent.

The present study addresses these research gaps and aims to assess the microplastic and anthropogenic fibre retention efficiency of two ICWs receiving treated WWTP effluent (Ingoldisthorpe and Northrepps) over a long duration by:

1. Quantifying anthropogenic fibre retention in both ICWs over a 12-month period (May 2022 to June 2023) by analysing approximately monthly water samples from the inlet and outlet;

2. Assessing how the concentration and size of microplastic fragments and anthropogenic fibres in fine bed sediment samples changes with distance from the inlet in the Northrepps ICW; and
3. Using ATR-FTIR to ascertain the material composition of microplastics and anthropogenic fibres entering and within ICWs.

4.2 Methods

4.2.1 Field campaigns

Water samples were collected over a 12-month period at Ingoldisthorpe and Northrepps ICWs at each wetland's inlet and outlet (Figure 4.1), sampling at approximately monthly intervals between July 2022 and June 2023 at Ingoldisthorpe, and between June 2022 and May 2023 at Northrepps (Table 4.4). The exact timing of sampling was not considered to be of high importance because insufficient replicates could be obtained to enable monthly comparisons between wetlands in anthropogenic fibre concentrations.

Figure 4.1 Sampling locations A) Northrepps inlet B) Northrepps outlet C) Ingoldisthorpe inlet D) Ingoldisthorpe outlet.

At the Northrepps inlet, water samples (*n* = 14) were taken from an inspection point approximately halfway along the 150 m pipe supplying the wetland from the WWTP (Figure 3.1). At the Northrepps outlet (*n* = 14), Ingoldisthorpe inlet (*n* = 13), and Ingoldisthorpe outlet (*n* = 13), samples were collected by holding a plastic bucket (8 L capacity with 500 mL graduations) beneath the pipe, meaning the entire flow of the pipe was sampled (note: only fibres were analysed in these water samples, thus use of a non-fibrous plastic bucket was deemed low-risk). Known volumes of water samples were then poured from the bucket through a 38 µm stainless steel sieve (200 x 50 mm) until the sieve began to lightly clog (this point was determined when water would take ~30 seconds to completely filter through). Sampling volume therefore varied considerably at each location, depending on the turbidity of the water sample (Table 3.4). The average sample volume at the Ingoldisthorpe ICW inlet was 436 (standard deviation (SD) = 176) L and 300 (SD = 176) L at the outlet. The average sample volume at the inlet of Northrepps ICW was 44 (SD = 15) L and 314 (SD = 57) L at the outlet. After sampling, the sieves were sealed with a stainless-steel lid and base and transported back to the laboratory. A new sieve was used for each sampling location. Sampling was performed during dry weather only, although loading rates (from WWTP effluent) were variable (Table 4.1).

Table 4.1 Discharge rates of WWTP effluent entering Ingoldisthorpe and Northrepps ICWs. The data from Northrepps on 13/03/2023 are incorrect: on this date there was a blockage at the WWTP and the flow was the highest ever observed when sampling. This blockage at the WWTP must have impacted the discharge reading device at this time.

Wetland	*Date*	*Discharge (L/s)*
Northrepps	06/06/2022	4.70
Northrepps	24/06/2022	1.24
Northrepps	12/07/2022	1.05
Northrepps	31/08/2022	1.53
Northrepps	03/10/2022	1.17
Northrepps	04/11/2022	1.27
Northrepps	04/01/2023	1.17
Northrepps	13/02/2023	1.20
Northrepps	13/03/2023	0.91
Northrepps	26/04/2023	1.24
Northrepps	09/05/2023	1.68
Ingoldisthorpe	11/07/2022	14.12
Ingoldisthorpe	25/07/2022	14.5
Ingoldisthorpe	01/08/2022	14.68
Ingoldisthorpe	20/09/2022	13.71
Ingoldisthorpe	17/10/2022	12.88
Ingoldisthorpe	22/11/2022	26.21
Ingoldisthorpe	29/11/2022	13.65
Ingoldisthorpe	09/01/2023	16.41
Ingoldisthorpe	20/02/2023	16.94
Ingoldisthorpe	27/03/2023	17.52
Ingoldisthorpe	23/04/2023	18.02
Ingoldisthorpe	21/05/2023	16.63

Fine bed sediment (FBS) samples were collected on 11 and 20 December 2022 from Northrepps ICW and on 22 November 2022 from Ingoldisthorpe ICW. Three FBS samples were collected from Ingoldisthorpe ICW: one in cell 1 (10 m from the inlet) and two in cell 4 (at the beginning and end of the cell). In the first cell at Northrepps ICW, 51 FBS samples were collected at approximately 2 m intervals (by stride) along three transects (Figure 4.2). Eight samples were collected from cells two and three at Northrepps (Figure 4.2). FBS

samples were collected using an isolation agitation method based on Woodward *et al.* (2021). See section 3.3.4 for full details on collection methods.

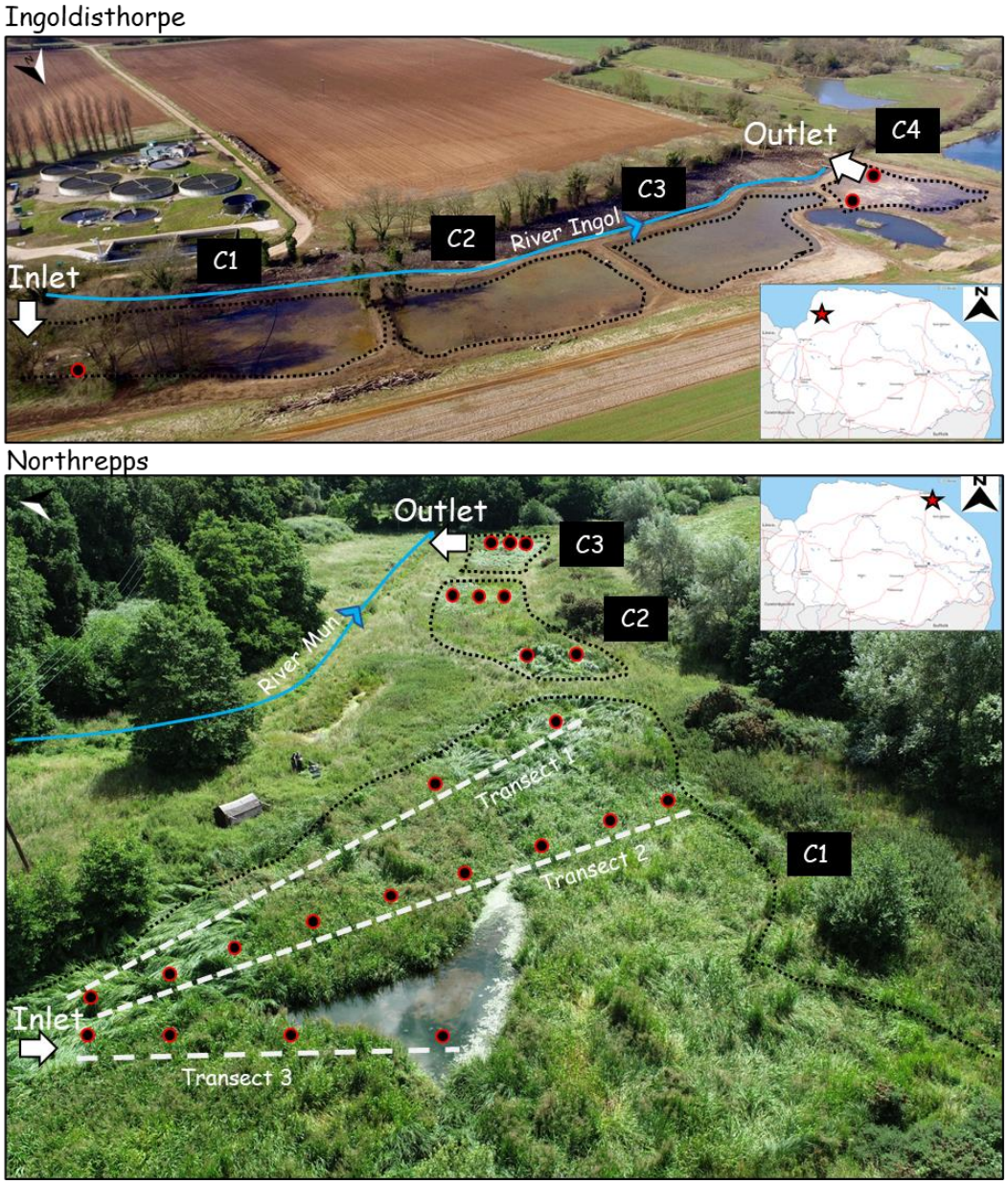


Figure 4.2 Aerial view of Ingoldisthorpe and Northrepps ICWs. Black dots with red outlines indicate approximate sediment sampling locations. Location of approximate transects highlighted in the first cell. Ingoldisthorpe image (top) taken before vegetation was planted (Credit: Norfolk Rivers Trust). Northrepps image (bottom) taken 6 July 2023. Cells labelled as 'C1' etc. Cells are outlined with dashed black line. 'Inlet' shows location where the wetland is supplied by WWTP effluent. 'Outlet' shows location of wetland outflow.

4.2.2 Laboratory analysis

4.2.2.1 Water samples

See section 3.3.3 for full details on the water sampling. Briefly, a chemical digestion was applied to particulate matter collected on 38 µm sieves before vacuum filtration onto 47 mm cellulose nitrate filters.

Each filter was transferred to a microscope (Leica CMA) and fibres were identified by visual inspection (with 4x objective). Fibres were categorised based on their colour and estimated length grouped into three categories: small (38–250 µm), medium (250–800 µm), and large (>800 µm). These measurements represent an estimation because often a portion of a fibre was hidden beneath another layer on the filters and attempting to excavate each one would risk dislodging other material (including other fibres) from the filter. The precise length of each fibre was not measured because this would be extremely time consuming given the large number of fibres (>5,000) identified in this research.

4.2.2.2 Sediment samples

See section 3.3.4 for full method details. Briefly, sediment samples were freeze dried until a constant dry weight was achieved, two chemical organic matter digestion were performed using NaClO, before a density separation step with $ZnCl_2$ and final filtration onto 47 mm cellulose nitrate filters. Anthropogenic fibres were identified as for the water samples, but in addition microplastic fragments were identified by the following criteria:

- Fragment appearing artificially coloured or shiny (resembling glitter)
- Fragment dark in colour with sharp edges and smooth surface

4.2.2.3 ATR-FTIR

Selected fibres and suspected microplastic fragments were extracted with either tweezers or a 33-gauge syringe needle into a glass beaker containing water. An attempt was made to extract approximately every tenth fibre and fragment found (on each water and sediment sample filter, respectively) for chemical identification. In total, 369 fibres and 140 suspected microplastic fragments were validated by ATR-FTIR (5.3 % and 11.6 % of total identified in all samples, respectively). The samples were then vacuum filtered onto 25 mm silver coated filters (0.45 µm pore size). Filters with only fibres were lightly coated with a spray-on glue ('Crafter's Companion Stick and Spray') before filtration to prevent fibres blowing away when handling the filter and performing FTIR. No glue was used with the fragments because they generally remained in place. All particles and fibres found on each of these filters were analysed by a micro-ATR-FTIR microscope (Bruker Hyperion 2000, 20 X ATR objective, resolution = 4.0 cm^{-1}, 64 scans $sample^{-1}$). The spectra acquired were analysed using Open Specy (Cowger *et al.*, 2021) to determine the best library match. Default pre-processing

settings were used for threshold signal-noise, smoothing, intensity adjustment, baseline correction and flatten region (removing CO_2 peaks) options. Wavenumber range selection of 0–3500 cm^{-1} was applied. Identification was performed using the 'Cor: FTIR Deriv' option. A spectral hit quality score (Pearson correlation coefficient) of 0.7 was set as the threshold, below which all samples were considered unknown to avoid bias in spectral interpretation.

4.2.3 Quality control

During sampling a 100 % woollen jumper was worn (woollen fibres were removed in sample processing by chemical digestion with NaClO) or a closed weave shirt in warm weather to prevent contamination from clothing fibres. This is important because a significant amount of contamination can come from clothing worn during sample processing and collection (Scopetani *et al.*, 2020). All sieves and glass jars used to collect samples were pre-cleaned in the laboratory with MilliQ water and sealed (either with a sieve lid or aluminium foil) prior to sampling. Sediment sampling equipment was rinsed with MilliQ water between sampling locations.

Unless otherwise stated, all solutions used in sample processing were pre-filtered through 0.45 µm polycarbonate filters. Laboratory work was undertaken within a laminar flow cabinet that was vacuumed and wiped down with paper towel before use. Microscope analysis was done in a room with managed airflow to minimise airborne contamination and was regularly cleaned.

4.2.4 Positive controls

To assess the recovery rate of the water sampling method, 30 individual pink polyester (PET) fibres were peeled from a sewing thread and cut to approximately 2–5 mm in length. These fibres were stored in a glass beaker in water and then poured through a 38 µm sieve, following which the standard water sampling method was followed. This was performed three times to achieve a more reliable average recovery rate.

To assess the recovery rate of the sediment sampling method, a spiked field control sample was collected. Pink polyester fibres were prepared in the same way as for the water samples, while microplastic fragments were generated by filing macroplastic items to generate small fragments that were then sieved to 250–750 µm for use in recovery experiments. 30 pink polyester fibres, 30 blue polyvinyl chloride (PVC) fragments and 30 yellow polypropylene (PP) fragments were mixed in with 10 g of fine bed sediment from the third cell of the Northrepps ICW. PP (~0.9 g cm^{-3}) was used to represent low density plastics and PVC (~1.48 g cm^{-3}) was used to represent high density plastics. After spiking the sediment, the standard sediment sampling method was then followed. This was performed three times. The size of the microplastic fragments found after recovery was recorded by measuring the longest dimension of each particle using ToupView software.

4.2.5 Negative controls

A total of 19 procedural blank samples were taken over the course of the 12-month water sampling campaign (Table 4.2). To do so an empty sieve was placed beside the sampling location to assess airborne fibre contamination while sampling. This sieve was then sealed, transported back to the laboratory, processed, and analysed following the standard water sampling method. Anthropogenic fibres were found in every procedural blank: averaging 4.5 (SD = 2.6) fibres sample^{-1}. A total of 85 fibres were found in the procedural blanks: most were clear (68 %) or dark (25 %). Additionally, 32 % of fibres found were approximately >800 µm, 58 % were 800–250 µm, and 10 % were 38–250 µm. The limit of detection (LOD) was calculated as 12 fibres sample^{-1}, and this value was therefore subtracted from each sample fibre count (as done by Dawson *et al.* (2023)).

Table 4.2 Anthropogenic fibres found in procedural blanks for water samples across the sampling campaign.

Sampling Date	Anthropogenic fibre count
06/06/2022	7
25/07/2022	12
01/08/2022	5
31/08/2022	9
20/09/2022	4
03/10/2022	4
17/10/2022	5
04/11/2022	5
22/11/2022	4
29/11/2022	5
04/01/2022	2
09/01/2023	1
13/02/2023	1
20/02/2023	2
13/03/2023	4
27/03/2023	4
23/04/2023	3
26/04/2023	4
09/05/2023	4

A total of five procedural blank samples were taken for the sediment samples. Fibres were found in every blank sample (Table 4.3), with an average of 11.2 (SD = 8.6) fibres sample^{-}

[1]. A total of 56 fibres were found in these blank samples: most were clear (57 %) or dark (29 %). Additionally, 30 % of fibres found were approximately >800 µm, 52 % were 800–250 µm, and 18% were 38–250 µm. The LOD was calculated as 37 fibres sample^{-1}, and this value was therefore subtracted from each sample fibre count. Suspected microplastic fragments were found in three out of five blank samples, with an average of 2 (SD = 2.5) fragments sample^{-1}. A total of 10 suspected microplastic fragments were found in these blank samples (Figure 4.3). The LOD was calculated as 10 fragments sample^{-1}, and this value was subtracted from the sample counts.

Table 4.3 Anthropogenic fibres and suspected microplastic fragments found in procedural blank samples for sediment samples.

Blank	Anthropogenic fibre count	Suspected MP fragment count
1	25	3
2	8	1
3	13	6
4	8	0
5	2	0

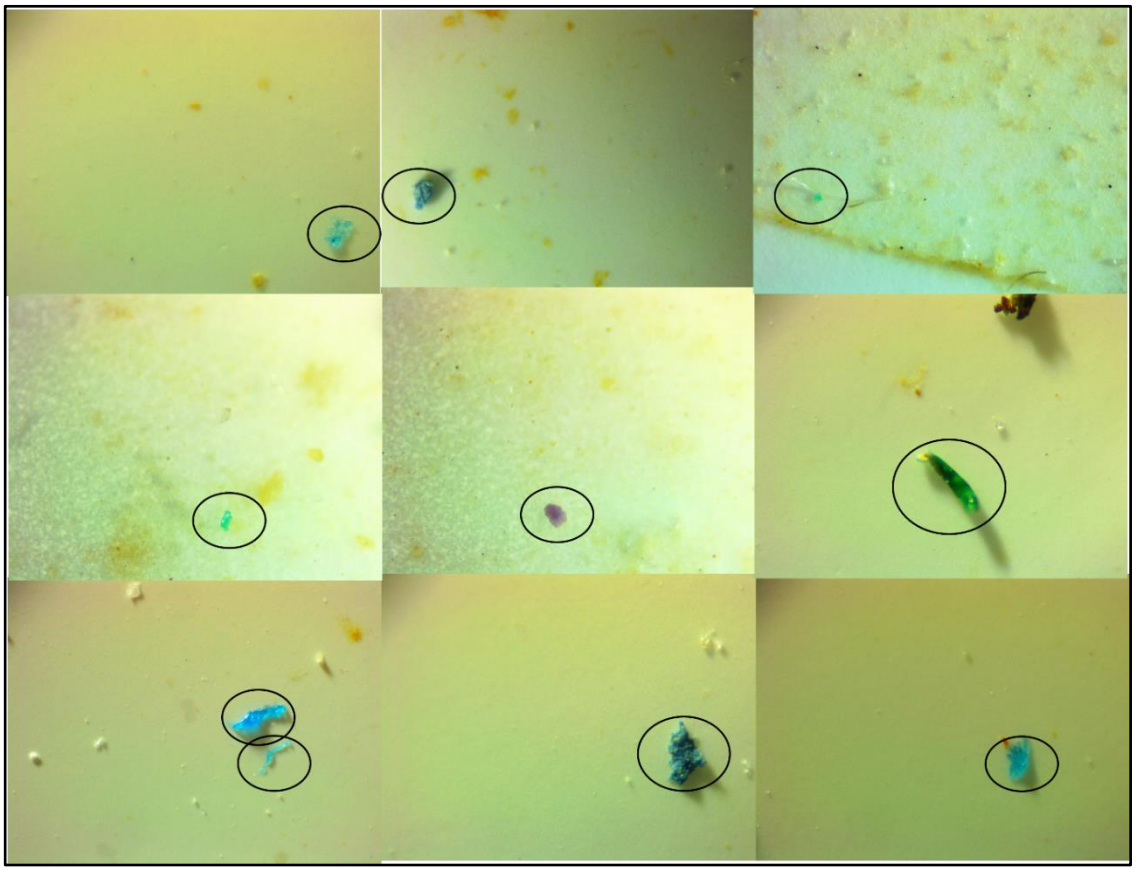


Figure 4.3. Suspected microplastic fragments found in procedural blank samples (circled). Images from Leica CMA microscope with 4x objective.

4.2.6 Data analysis

The limit of detection (LOD) was calculated for both fibres and fragments separately (for sediment samples) and subtracted from the total value of each sample.

$$\text{LOD} = \text{Mean blank} + (3 \times \text{standard deviation blank})$$

This correction method was chosen based on the findings by Dawson *et al.* (2023), where LOD methods were recommended for microplastic studies.

Fibre retention rates were calculated as:

$$\text{Retention (\%)} = \left(1 - \frac{Conc.outlet}{Conc.inlet}\right) \times 100$$

Areal removal (AR) rates were calculated as:

$$\text{AR (items m}^{-2}\text{ day}^{-1}\text{)} = \frac{(conc.inlet - conc.outlet) \times Q}{S}$$

where Q is the discharge at the inlet (m^3 day^{-1}) and S is the surface area (m^2). Discharge data provided by Anglian Water.

Data analysis was performed in Microsoft Excel. A Shapiro-Wilk test was used and indicated that the data did not meet the normality assumption for parametric tests. Consequently, a Mann-Whitney U Test was used to compare mean fibre concentrations at the inlet and outlet of Northrepps ICW. Standard deviation is reported in parenthesis after average values.

Error propagation was applied to fibre loading rate (Z) calculations using the equation below (Fantner, 2013):

$$\frac{\sigma_Z}{Z} = \sqrt{\left(\frac{\sigma_x}{x}\right)^2 + \left(\frac{\sigma_y}{y}\right)^2}$$

where x is fibre concentration (fibres L^{-1}) and y is discharge (L s^{-1}). The propagated error is denoted with '±' in parenthesis.

4.3 Results

4.3.1 Ingoldisthorpe anthropogenic fibre retention

Mean fibre concentrations at the inlet of Ingoldisthorpe ICW across the entire sampling period were 0.01 (SD = 0.02) fibres L^{-1}, thus fibres appeared to be passing through the treatment plant and entering the wetland in low concentrations (Table 4.4). Average discharge from WWTP effluent entering the wetland for the period May 2022 to June 2023 was 11.01 (SD = 7.25) L s^{-1}, equating to an average loading rate of 0.11 (± 0.23) fibres s^{-1}, or 9504 (± 19,872) fibres day $^{-1}$. However, fibres were not continuously released in significant numbers from the WWTP into the wetland because in seven out of 13 of these samples, fibres were not detected above the LOD. In no outlet samples were microplastic concentrations detected above the LOD. Fibre retention at the Ingoldisthorpe wetland therefore appears consistently 100 %. However, the low fibre concentrations at the inlet show that it was the WWTP that was highly effective at retaining fibres, meaning the wetland was not overloaded with high fibre numbers.

Table 4.4 Microplastic concentrations and removal performance of the Northrepps and Ingoldisthorpe ICW, derived from the water sampling campaign. NA indicates no available data.

Wetland	Sampling date	Inlet		Outlet		Fibre retention (%)
		Volume (L)	Fibres L^{-1} (LOD subtracted)	Volume (L)	Fibres L^{-1} (LOD subtracted)	
Northrepps	06/06/2022	40.5	3.46	228	<LOD	100
Northrepps	24/06/2022	49.5	1.07	375	0.06	94.8
Northrepps	12/07/2022	39	2.90	225	<LOD	100
Northrepps	12/07/2022	39	3.92	NA		NA
Northrepps	12/07/2022	39	4.28			
Northrepps	02/08/2022	54	3.74	300	<LOD	100
Northrepps	31/08/2022	45	4.36	200	0.08	98.3
Northrepps	03/10/2022	40	2.23	300	<LOD	100
Northrepps	04/11/2022	60	2.37	315	0.03	98.9
Northrepps	04/01/2023	60	2.90	350	<LOD	100
Northrepps	04/01/2023	NA		350	<LOD	NA
Northrepps	13/02/2023	69	3.28	350	<LOD	100
Northrepps	13/03/2023	6	39.00	350	0.04	99.9
Northrepps	26/04/2023	30	1.10	350	0	100
Northrepps	26/04/2023	NA		350	0	NA
Northrepps	09/05/2023	45	2.16	350	0	100
Ingoldisthorpe	11/07/2022	600	0.01	252	<LOD	100
Ingoldisthorpe	25/07/2022	630	0.06	NA		NA
Ingoldisthorpe	01/08/2022	750	0.01	300	<LOD	100
Ingoldisthorpe	20/09/2022	350	<LOD	350	<LOD	NA
Ingoldisthorpe	17/10/2022	350	0.06	175	<LOD	100
Ingoldisthorpe	22/11/2022	140	<LOD	140	<LOD	NA
Ingoldisthorpe	29/11/2022	700	<LOD	350	<LOD	NA
Ingoldisthorpe	09/01/2023	400	<LOD	350	<LOD	NA
Ingoldisthorpe	09/01/2023	NA		350	<LOD	NA
Ingoldisthorpe	20/02/2023	350	<LOD	350	<LOD	NA
Ingoldisthorpe	27/03/2023	350	<LOD	350	<LOD	NA
Ingoldisthorpe	23/04/2023	350	0.01	350	<LOD	NA
Ingoldisthorpe	21/05/2023	350	<LOD	350	<LOD	NA
Ingoldisthorpe	12/06/2023	350	<LOD	350	<LOD	NA

4.3.2 Northrepps anthropogenic fibre retention

Fibres were found in concentrations above the LOD in all samples from the Northrepps inlet (Table 4.4), with mean fibre concentrations across the entire sampling period of 5.48 (SD = 9.70) fibres L^{-1}. Fibres were therefore consistently passing through the treatment plant and entering the wetland. Average discharge from WWTP effluent entering the wetland over the period May 2022 to June 2023 was 0.74 (SD = 0.94) L s^{-1}, equating to an average loading rate of 4.05 (±8.84) fibres s^{-1}, or 349,920 (±763,776) fibres day^{-1}. Fibres were clear (62 %), dark (29.9 %), red (4.2 %), blue (2.4 %), and light (1.5%) in colour (light includes white and cream), while 27.2 % were approximately >800 µm, 56 % were 800–250 µm, and 16.8 % were 38–250 µm at the Northrepps inlet.

The highest fibre concentration was observed on 13 March 2023 at 39 fibres L^{-1}: a clear outlier in the dataset (Table 4.4). On this date there was a blockage at the WWTP that was cleared approximately 30 minutes before sampling; a deliberate attempt was made not to sample the initial pulse after the blockage was cleared. It was assumed after 30 minutes the flow in the pipe would become normal, and indeed it was when sampling commenced. However, after 2 L were sampled, the flow increased to a level significantly higher than normal levels observed in the pipe (Figure 4.4) and the water was also more turbid than usual, reflected in the low sample volume (Table 4.4). Excluding this sampling date, mean concentrations at the inlet were 2.90 (SD = 1.08) fibres L^{-1}. It is highly likely that the fibre concentrations reported for the Northrepps inlet are an underestimate because (not including losses during sample processing), in two inlet samples bundles of fibres were found (Figure 4.5) and the number of fibres making up each bundle was not counted.

Figure 4.4 Approximately normal flow level in inlet sampling pipe at Northrepps and high flow (right). The main pipe has a diameter of approximately 20 cm.

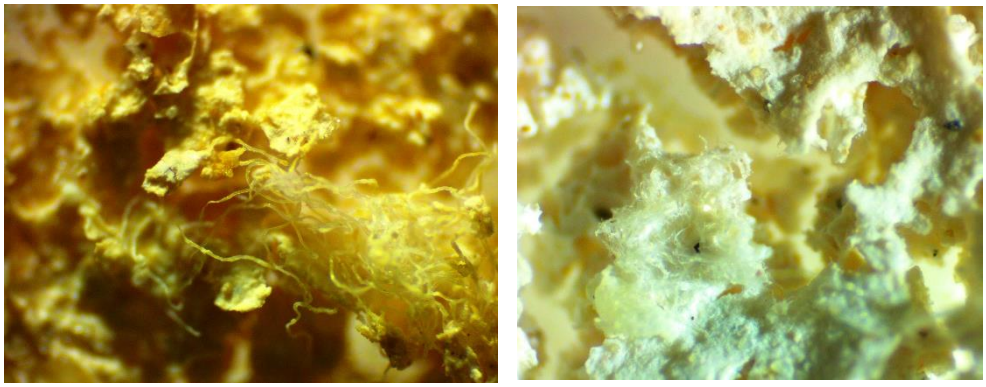


Figure 4.5 Bundle of fibres found in Northrepps ICW inlet water samples circled (magnification = 40X)

The mean fibre concentration at the outlet of the Northrepps ICW across the entire sampling period was 0.01 (SD = 0.02) fibres L^{-1}, lower (Mann Whitney U Test, U = 4, $p<0.01$), than the mean inlet fibre concentration. The average retention efficiency was 99.3 % (SD = 1.5 %) across the entire sampling period. Only in four of the 14 outlet samples were fibres detected above the LOD (Table 4.4), thus fibres appear to be released intermittently from the wetland at low concentrations. Owing to the high workload of sample processing, sample replicates were not attempted in each month, thus statistical comparisons cannot be made. However, Table 4.4 indicates no clear change in fibre retention by month or season.

4.3.3 Suspected microplastics and anthropogenic fibres in Northrepps ICW sediment samples

A total of 1203 fragments and 4540 anthropogenic fibres were found in the 23 sediment samples at the Northrepps wetland. In the first cell, average contents were 8152 (SD = 7022) anthropogenic fibres kg^{-1} and 1938 (SD = 991) suspected microplastic fragments kg^{-1} dry weight sediment. Anthropogenic fibre content declined with increasing distance from the inlet pipe (Figure 4.6), although the highest content of 22,602 fibres kg^{-1} was 22 m from the inlet. The proportion of large, medium, and small size fibres did not change significantly with distance from the inlet (Figure 4.7). As stated earlier, the size categories may not be completely accurate given the limitations of measuring fibre length when part of the fibre is buried under other material on filters. Most of the fibres found were clear (76 %) and dark (17 %), while 51 % were approximately >800 µm, 40 % were 800–250 µm, and 9 % were 38–250 µm. These values are proportionally similar to those for the inlet water samples at Northrepps which is expected because the WWTP effluent is the dominant source of anthropogenic fibres to this wetland. However, proportionally more large (>800 µm) fibres

were identified in sediment samples than inlet water samples, possibly because of the breakdown of fibrous macroplastics in the wetland into longer length fibres.

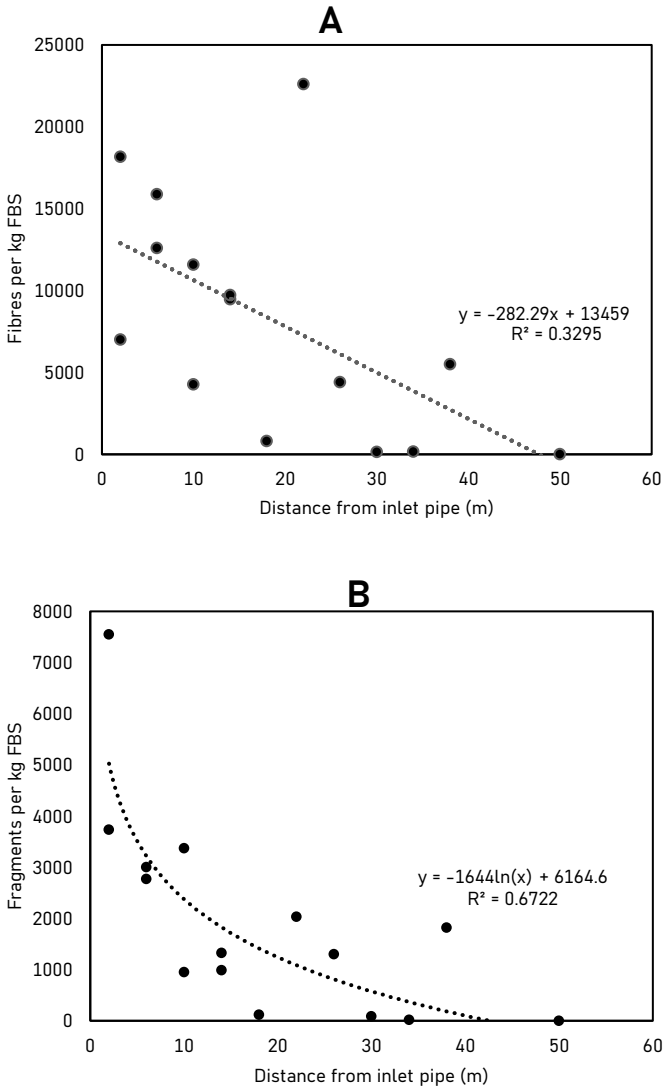


Figure 4.6 Anthropogenic fibre concentration (A) and suspected microplastic fragments (B) in fine bed sediment (FBS) samples and distance from the inlet in the first cell of the Northrepps ICW.

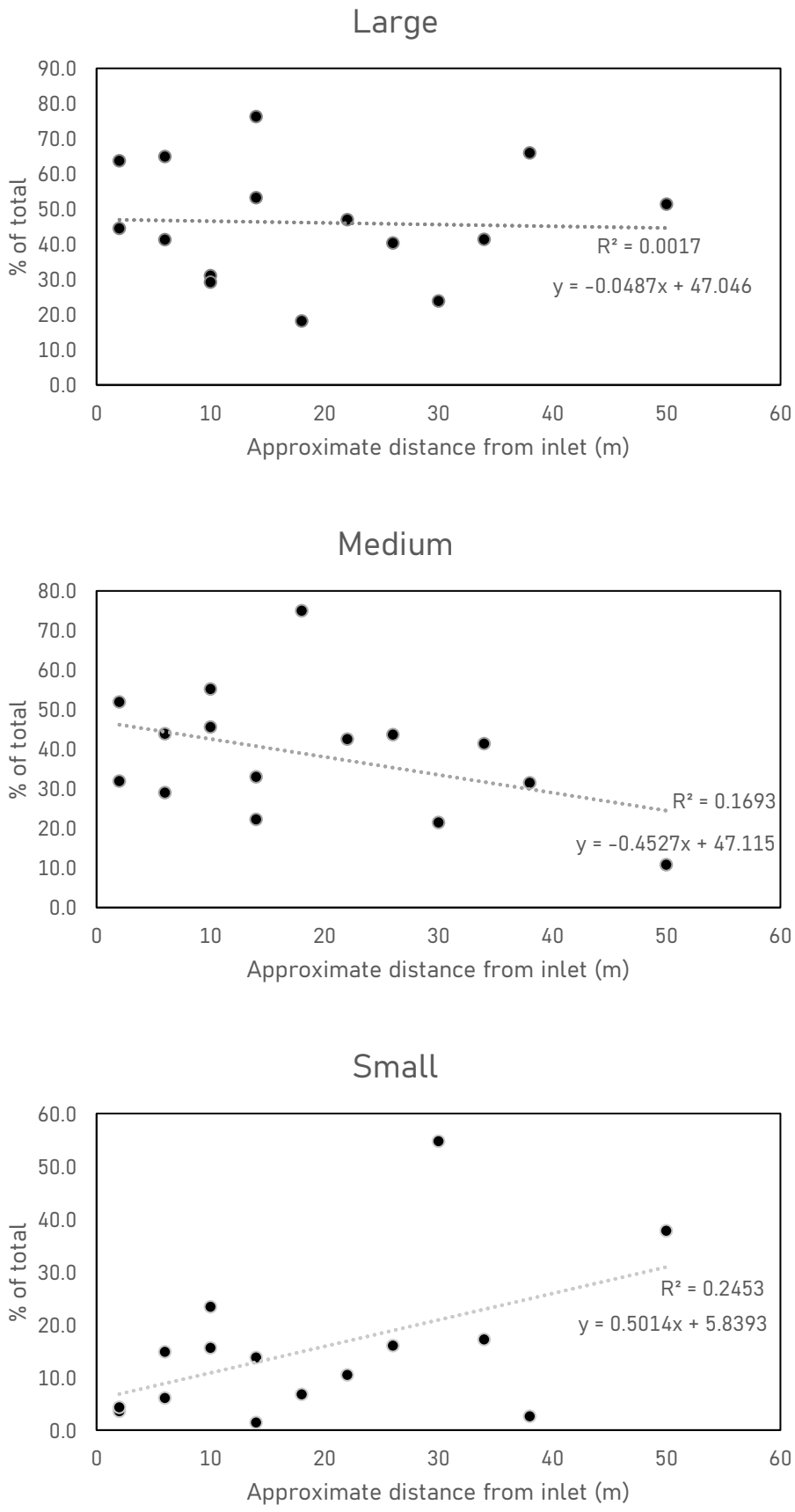


Figure 4.7 Proportion of small (38–250 µm), medium (250–800 µm) and large (>800 µm) anthropogenic fibres and distance from the inlet of the Northrepps ICW.

Microplastic fragment number decreased with increasing distance from the outlet in cell 1 (Figure 4.6). The size (longest dimension) of suspected microplastic fragments varied little with increasing distance from the inlet (Figure 4.8). A fibre bundle with a longest dimension of approximately 8 mm was found 2 m from the inlet in cell 1. This fragment was omitted from Figure 4.8. Most suspected microplastic fragments found were <100 µm or 100–200 µm (35.7 % and 37.4 %, respectively) (Figure 4.9). Most of the suspected microplastic fragments were blue or green (Figure 4.9). However, this does not reflect the actual colours of microplastics within the wetland because clear and white microplastics were not identifiable with the method applied here. Additionally, only dark fragments that were obviously suspected microplastic were counted: those with sharp edges and a smooth texture. Therefore, tyre wear particles dark in colour could have been missed, although the catchment for both WWTPs was rural with generally low speed traffic so these were not likely to occur in high concentration in the Northrepps ICW. 26 microplastic fragments were spherically shaped resembling microplastic beads (all pink or blue colour), probably deposited in the wetland before the 2018 ban of microbeads in cosmetics in the UK (Department for Environment, Food & Rural Affairs, 2018), or from leftover products containing them. Seven of these spherical beads were analysed by ATR-FTIR: all were polyethylene (PE).

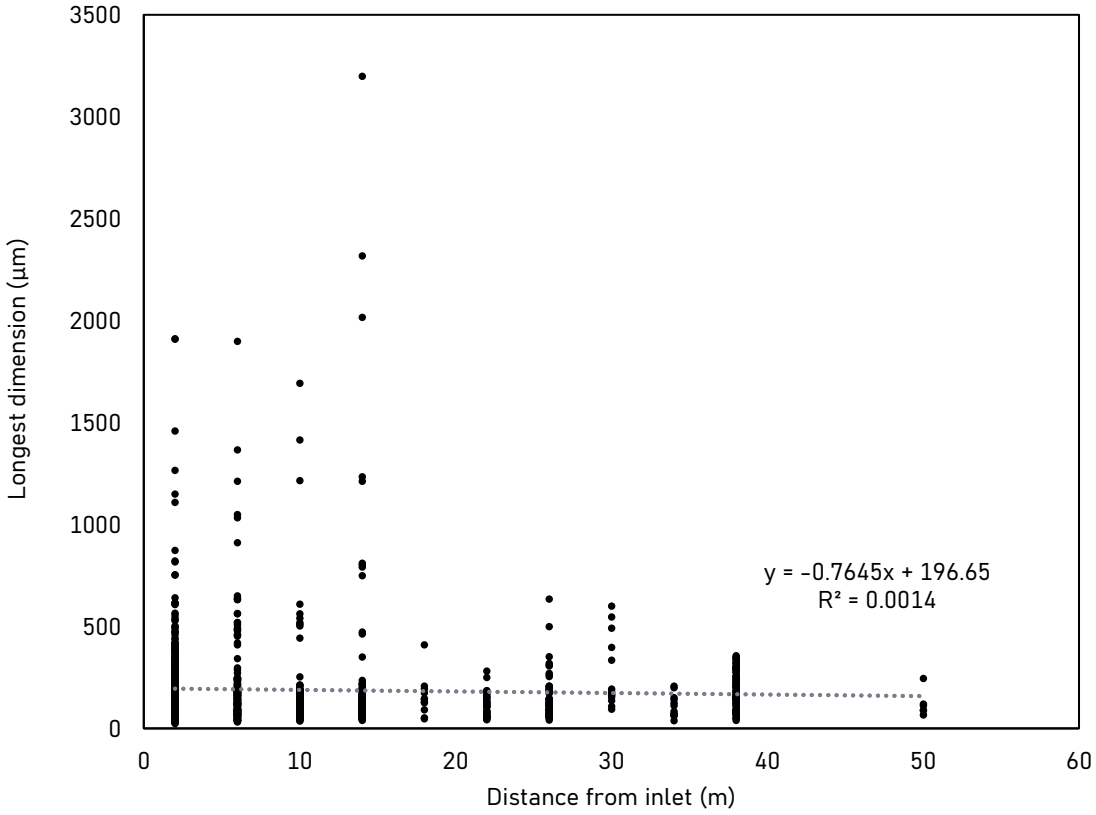


Figure 4.8 Longest dimension of suspected microplastic fragments in wetland sediment against distance from the inlet in the first cell of the Northrepps ICW.

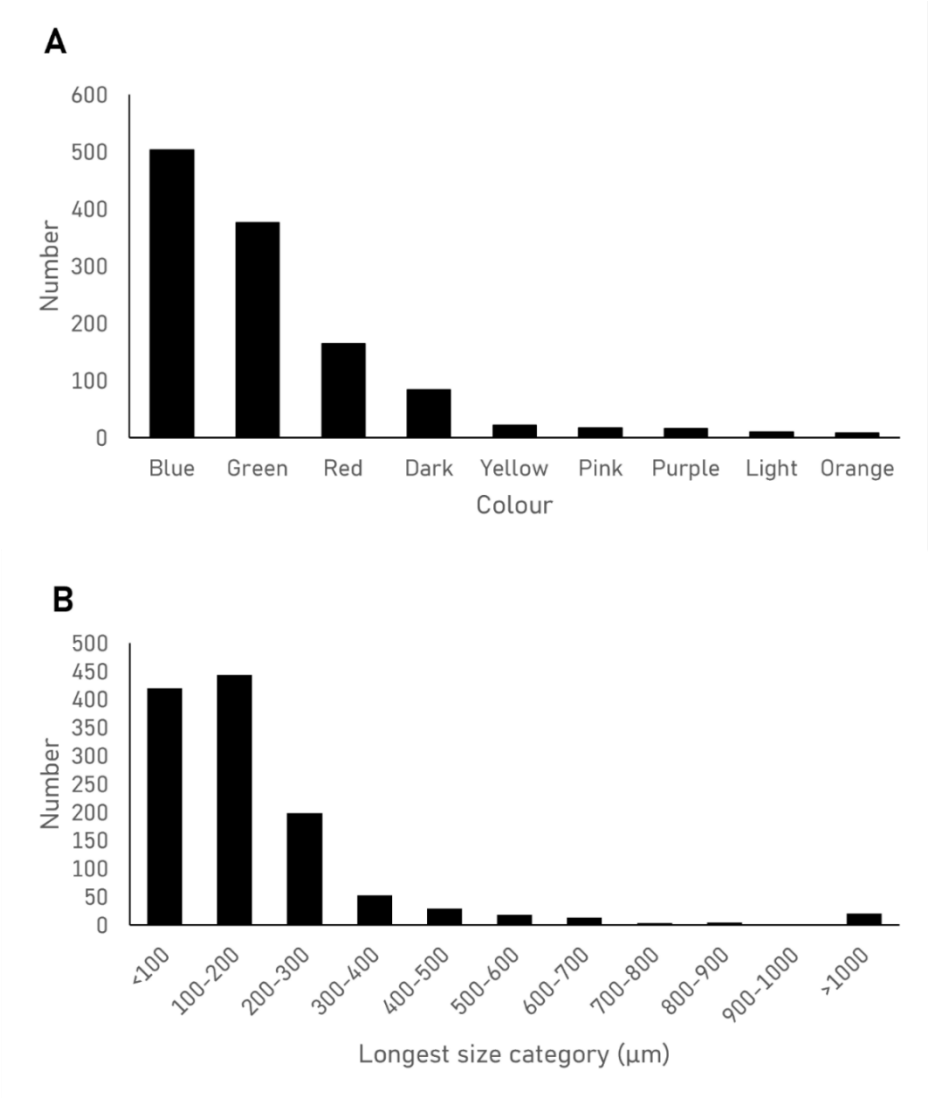


Figure 4.9 Colour and size category (longest dimension) of suspected microplastic fragments identified in sediment samples from the Northrepps ICW.

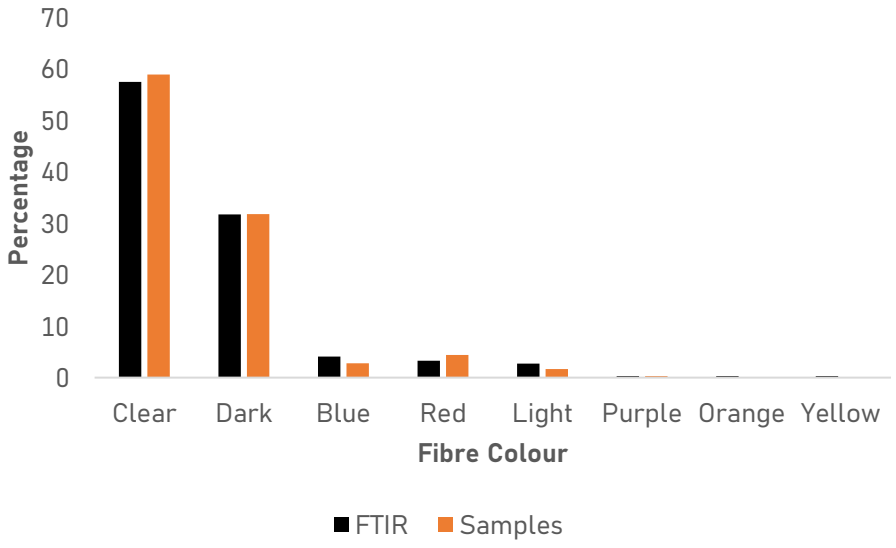
In cell 2 at Northrepps, fibres and suspected microplastic fragments were not detected at numbers above the LOD in any of the five sediment samples. Similarly, in cell 3 at Northrepps, no fibres or fragments were detected above the LOD in any of the three samples.

Microplastic and anthropogenic fibre accumulation at the Ingoldisthorpe ICW is unlikely to be significant because the treatment plant has been shown to be highly effective at removing fibres (and so it is reasonable to assume it is equally effective at retaining microplastic fragments). Three sediment samples were collected from the Ingoldisthorpe ICW: one in cell 1 (10 m from the inlet) and two in cell 4 (at the beginning and end of the cell). In none of these samples were anthropogenic fibres and microplastic fragments detected above the LOD. The microplastics and anthropogenic fibres that enter the wetland

from WWTP effluent probably are all retained in the first cell given that the area and vegetation cover are similar to the first cell at Northrepps ICW.

4.3.4 Material composition of anthropogenic fibres and suspected microplastics

To confirm the chemical composition of fibres identified, spectra were acquired for 369 fibres by ATR-FTIR. These fibres were sampled randomly for chemical identification, although FTIR validation was not performed on a filter-by-filter basis because the sample was pooled. However, the proportion of each fibre colour and size category in the FTIR validated samples and the entire sample pool is similar (Figure 4.10): in both almost 60 % of fibres were clear, 32 % were dark, and over 50 % were in the medium size category of approximately between 250 and 800 µm. These proportions suggest that the samples validated by FTIR are sufficiently representative of the entire sample pool. Additionally, the proportion of FTIR validated fibres that are cellulosic, plastic, and unknown generally plateau after ~200 samples (Figure 4.11). Therefore, the 369 fibres validated appears sufficiently high and equates to 14 % of the total fibres found in all water samples.



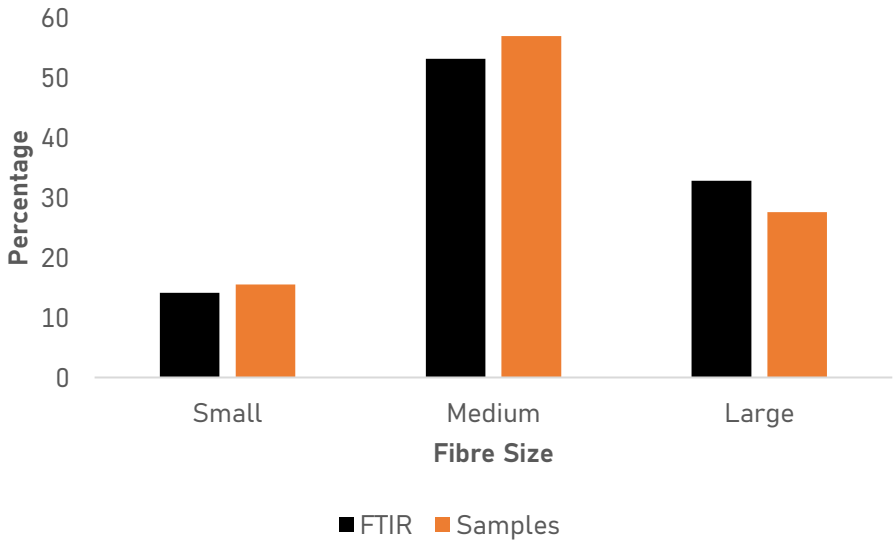


Figure 4.10 Comparing colours and sizes of fibres in the FTIR validated samples and in all samples from the 12-month water sampling campaign.

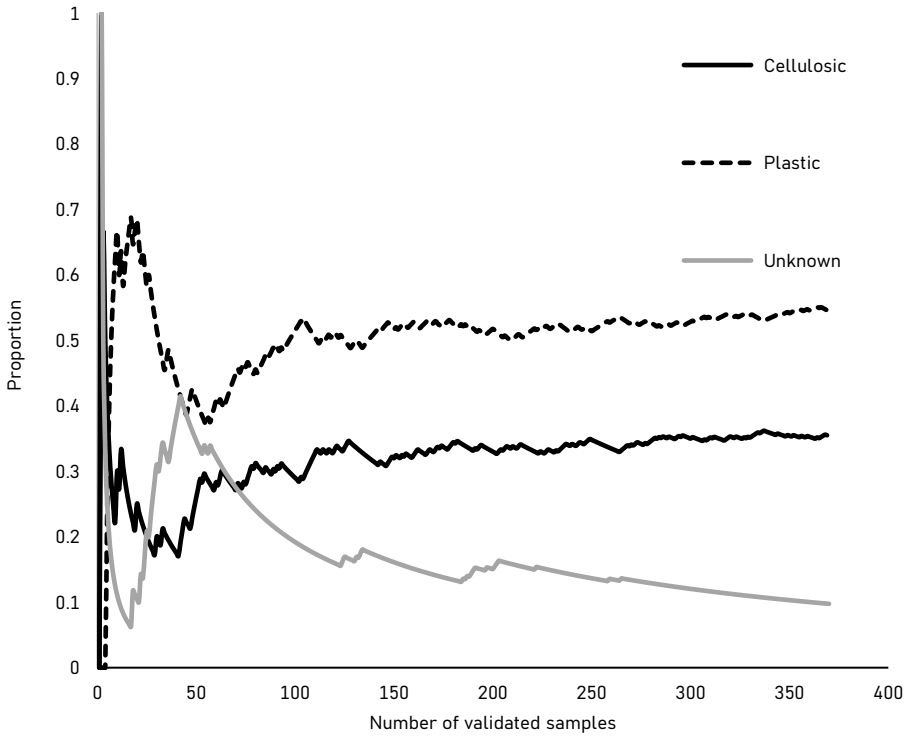


Figure 4.11 Proportion of FTIR validated fibres (from water samples) that were confirmed plastic, cellulosic or unknown.

Of the 369 fibres validated by FTIR:

• 54.7 % are plastic, of which 90.1 % are PET, 4 % acrylic, 3 % PP, 2 % PE, 1 % polyamide

• 35.5 % are cellulosic fibres, of which 89.3 % are clear or dark and 10.7 % artificially dyed.

• 9.8% of fibres are unknown, of which 3 % are unidentifiable and anthropogenic (fibre artificially dyed), 30 % are unidentifiable and not artificially dyed, and 67 % are the glue that was sprayed onto the filter (to ensure that fibres did not blow away during operation of the ATR-FTIR).

As a result of this FTIR validation, approximately 55 % of the fibres reported in water samples are plastic (dominated by PET), approximately 36 % are cellulosic and the remainder are ambiguous. No fibres were sampled from the sediment samples, although it is reasonable to assume that the fibres found are proportionally similar in their material composition because there are no other sources than the WWTP.

To confirm the chemical composition of suspected microplastic fragments identified, spectra were acquired for 140 fragments (38 µm to 2 mm) by ATR-FTIR. The FTIR validated

samples appear sufficiently representative because a variety of fragment colours, sizes and materials were identified (Figure 4.12).

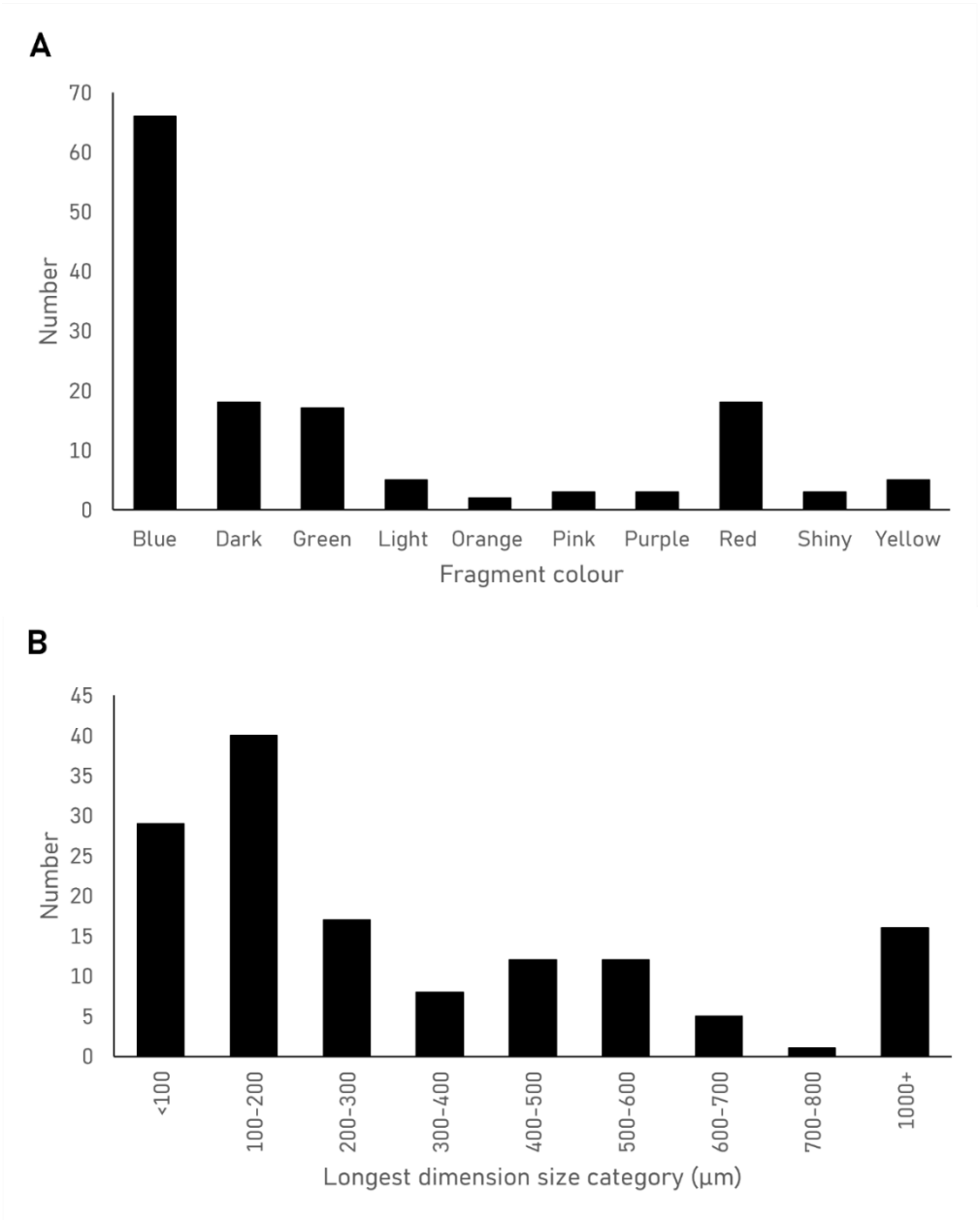


Figure 4.12 Colour (A) and size category (longest dimension) (B) of suspected microplastic fragments found in wetland sediment and analysed by ATR-FTIR.

Of the 140 suspected microplastic fragments analysed by ATR-FTIR: 73 % were plastic, 6 % non-plastic, and 21 % unconfirmed (hit quality score <0.7). Most of the fragments that were confirmed plastic were either polystyrene, polyethylene, or polypropylene (34 %, 20 %, and 23 %, respectively) (Figure 4.13). Common anthropogenic fibres and microplastic fragments are shown in Figure 4.14.

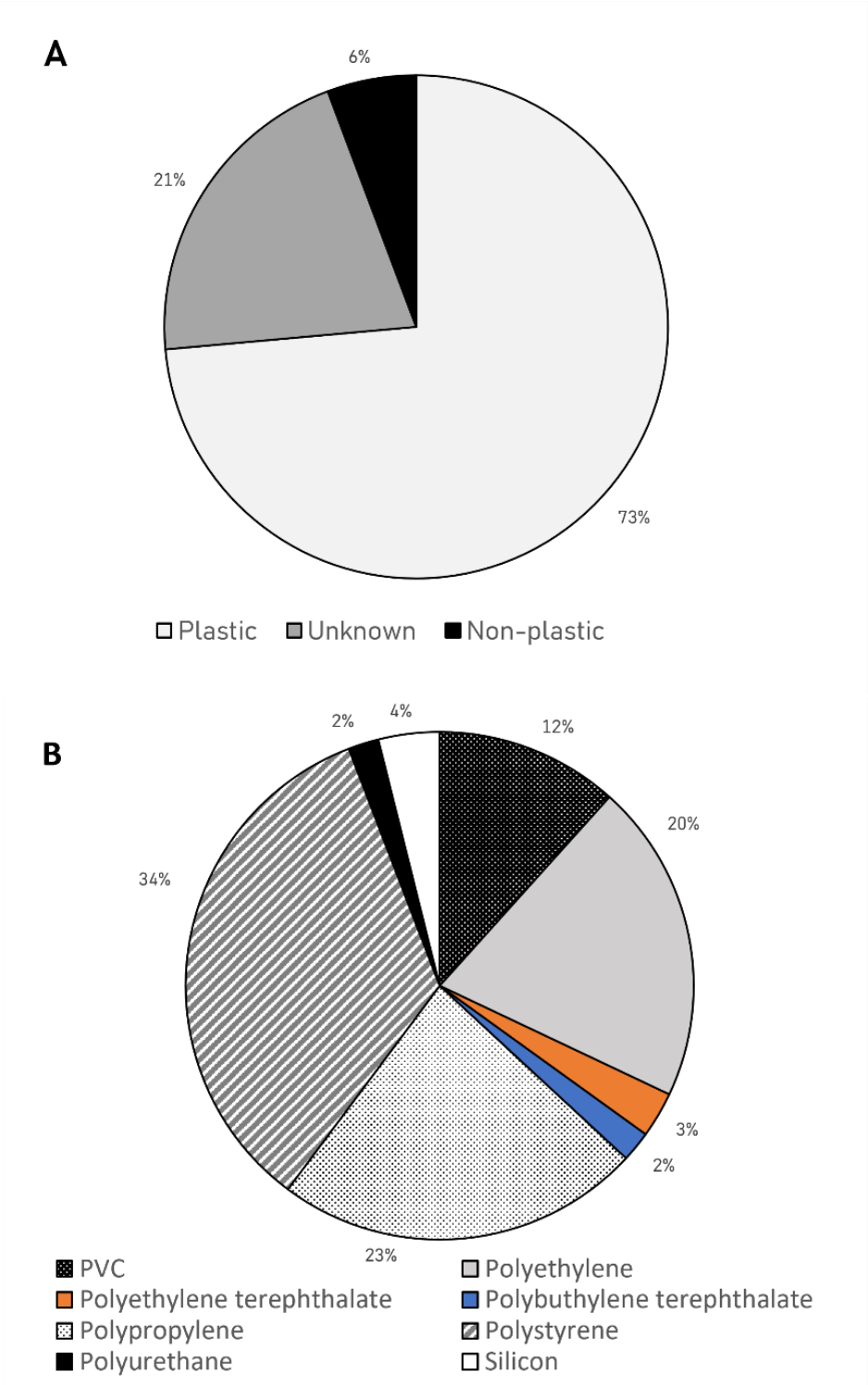


Figure 4.13 A) Material composition of the 140 suspected microplastic fragments found in wetland sediment and analysed by ATR-FTIR. B) Types of confirmed plastics in wetland sediment.

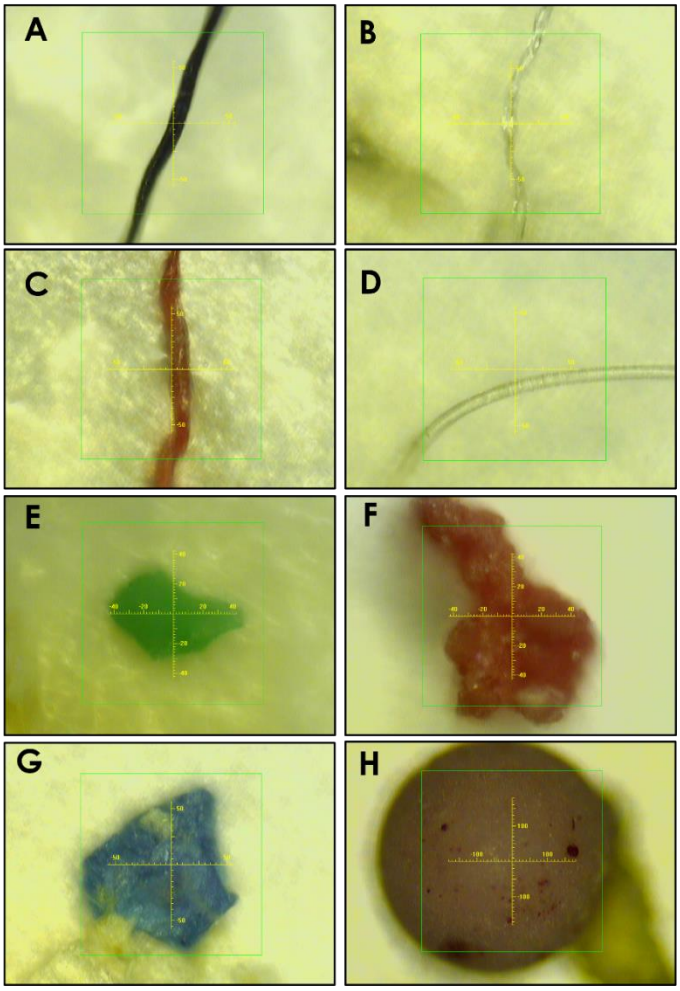


Figure 4.14 Selected anthropogenic fibres and microplastic fragments validated by ATR-FTIR. A) Polyester fibre, B) Cellulosic fibre, C) Polyester fibre, D) Polyester fibre, E) Polystyrene fragment, F) Polypropylene fragment, G) Polystyrene fragment, H) Polyethylene fragment. Scale indicated with crosshairs (µm).

4.3.5 Macroplastic in Northrepps ICW

Macroplastics were searched for in the 2 mm aperture sieve (in the laboratory after drying) for each sediment sampling location in Northrepps ICW. In total, 132 suspected macroplastic pieces were found. Of these, 97 were a white material, all visually appearing as though they were from the same source. Ten of these white plastic pieces were tested using a benchtop ATR-FTIR and revealed to be PE, likely from sanitary products: indeed, whole sanitary towels were found in the Northrepps wetland within 4 m of the inlet (Figure 4.15), thus providing evidence that untreated sewage entered this wetland. The presence of these sanitary items in the Northrepps ICW, that should not be flushed down the toilet, demonstrate the importance of individual responsibility in preventing environmental pollution. Other plastic material found included clear PP films, orange PP fragments, blue and dark fragments, and white PET fibrous material probably also from sanitary products (such as wet wipes). The approximate size of macroplastic found varied from 3 mm to fully intact sanitary towels. Most macroplastic found (103 of 132 pieces) was within 10 m of the

inlet pipe along transect 1 (Figure 4.2), indicating that there may have been a preferential flow pathway here. The furthest distance from the inlet in cell 1 where macroplastic was found was 40 m on transect 1: a 14 mm (longest dimension) piece of white plastic (resembling the same PE as found elsewhere). The prevalence of white macro plastic (73.5 % of total) highlights the extent to which microplastic fragment numbers are underestimated in the present study because white fragments were excluded due to method limitations.

Figure 4.15 Sanitary products and selected macroplastic fragments found in the first cell of the Northrepps ICW

4.4 Discussion

4.4.1 Retention of microplastics and anthropogenic fibres by vegetation

This study provides evidence in support of the hypothesis that dense vegetation in constructed wetlands acts as an efficient barrier to anthropogenic fibre and microplastic fragment transport (Helcoski *et al*., 2020). Microplastic fragment and anthropogenic fibre content declined rapidly with increasing distance from the inlet in Northrepps ICW, and entrapment occurred as soon as 2 m from the inlet pipe (no samples were taken closer than this). Anthropogenic fibres and microplastic fragments were detected up to a maximum of

38 m from the inlet pipe (along transect 1) in the first cell of Northrepps ICW, and none were detected at concentrations above the LOD in cells 2 and 3. Emergent linear-leaved vegetation planted in constructed wetlands slows water velocities encouraging sedimentation of microplastics and anthropogenic fibres. For ICWs designed with shallow water depths of 20–30 cm, emergent plants and their litter form a non-homogeneous 'filter' that takes up most of, or all, the depth of the water column (also based on observations at Northrepps ICW), encouraging interception of suspended particles. Microplastic fibres and fragments have been shown to stick to biofilms on submerged vegetation (Goss *et al*., 2018), as well as other sediment particles as small as 0.5–2.5 µm (Kadlec, 2019). Microplastics and fibres may also aggregate with flocculent suspended particulate matter in constructed wetlands, thus increasing the relative size of the particle and the likelihood of entrapment (Leiser *et al*., 2021).

It is not possible to accurately determine the vegetated area that the main flow pathway contacts in the first cell of Northrepps ICW. However, given that most macroplastics were identified along transect 1, there may be a preferential flow pathway there. Additionally, during sampling at the wetland, it was observed that flow dead zones existed where there was only wet mud. It is unlikely then that the flow pathway contacts the full 1600 m^2 of the first cell at Northrepps, potentially reducing microplastic and fibre retention efficiency.

Zhou *et al*. (2022) found that fibres were better retained by surface flow constructed wetlands than microplastic fragments, and that larger microplastics and anthropogenic fibres were better retained than smaller ones. In the first cell of Northrepps ICW, although there was little change in microplastic fragment size (longest dimension) with increasing distance from the inlet pipe, no fragments >1000 µm were detected beyond 20 m of the inlet, suggesting that these size fragments are better retained in dense vegetation. Bydalek *et al*. (2023) found a higher proportion of large fibres >1000 µm at the Cromhall wetland outlet (21 %) than at the inlet (8.3 %) and suggested that this difference could be explained by the higher buoyancy of larger fibres causing slower sedimentation. In the present study at the Northrepps wetland the proportion of fibres >800 µm were also higher at the outlet (35 %) than at the inlet (27 %). However, when subtracting the LOD from the fibre counts at the outlet, merely 32 % are then counted, thus 68 % of the datapoints making up the proportional size figures at the outlet are likely from contamination. The data are therefore not robust enough to conclude that larger fibres are more prevalent in the outlet and that vegetated wetlands preferentially retain shorter length fibres.

4.4.2 Factors influencing anthropogenic fibre retention

This study shows strong evidence that the Northrepps and Ingoldisthorpe ICW consistently retains anthropogenic fibres throughout a 12-month period. However, at Ingoldisthorpe the loading rate of anthropogenic fibres is low (9504 ± 19,872 fibres day^{-1}), meaning the areal

removal rate is also low at 0.88 ± 1.84 fibres/m^2/day. At Northrepps ICW both the loading rate (349,920 ± 763,776 fibres day^{-1}) and areal removal rate (119 ± 261 fibres m^{-2} day^{-1}) are higher than at Ingoldisthorpe. The total area covered by plants is approximately 2755 m^2 at Northrepps ICW and 4420 m^2 at Ingoldisthorpe ICW (based on minimum estimated % plant cover values). Ingoldisthorpe ICW may therefore be able to effectively retain anthropogenic fibres when loading rates are similar to those at Northrepps, if it is assumed that the total vegetation cover is the dominant factor influencing anthropogenic fibre retention. Additionally, at the Cromhall ICW, Bydalek *et al.* (2023) reported calculated anthropogenic fibre loading rates of 2,616,754 fibres day^{-1} (assuming a reported wetland inflow flow rate of 9.15 L s^{-1} and concentrations of 3.31 fibres L^{-1}), with 92.2 % of these retained, equating to areal removal rates of 310 fibres m^{-2} day^{-1} (Bydalek *et al.*, 2023). The total area of plant cover at Cromhall ICW is approximately 3957 m^2. Therefore, Ingoldisthorpe ICW may potentially retain anthropogenic fibres at loading rates similar to those reported at Cromhall ICW, assuming the total area of plant coverage is the dominant factor controlling retention efficiency.

Other wetland features also inevitably play a role in microplastic and anthropogenic fibre retention. The residence time is probably of high importance: the greater the residence time, the greater the time available for microplastic and anthropogenic fibre sedimentation. The residence time for Northrepps and Ingoldisthorpe ICWs were reported as 3.1 and 16.8 days, respectively, not accounting for preferential flow pathways (Cooper *et al.*, 2020).

Zhou *et al.* (2022) showed that the removal efficiency of a surface flow constructed wetland was 32.7 % lower on a rainy sampling day compared to a dry one. In the present study, no sampling was conducted while it rained, although on several occasions it had rained in the morning or day(s) prior to sampling. On average, WWTP effluent discharges during the period 08:00 to 12:45 (coinciding with the time when sampling was undertaken) for both wetlands were calculated on each sampling day (Table 4.1). During this sampling period, the highest discharge from the Ingoldisthorpe WWTP was on the 22 November 2022, with an average loading rate of 26.2 L s^{-1} and the lowest on 29 November 2022, with an average loading rate of 13.7 L s^{-1}. On both these days anthropogenic fibres were not detected above the LOD in the inlet and outlet samples. At Northrepps ICW, the highest WWTP discharge into the wetland during the sampling period was recorded on the 6 June 2022 (4.7 L s^{-1}), and the lowest on 12 July 2022 (1.1 L s^{-1}). The loading rates were therefore variable over the course of the sampling period at both wetlands, meaning the only aspect that may have been missed in the 12-month sampling campaign is the effect of rain droplet impact on re-suspension. However, rainfall droplets will not directly hit the water surface causing sediment (and potentially microplastic) re-suspension at the Northrepps wetland because the emergent vegetation percentage cover is so high (>95 %) in each cell. At Ingoldisthorpe, larger areas of the wetland are unvegetated (30–50 % cover in cells 2–4), meaning rainfall

may disturb sediment more, although given the low concentrations of anthropogenic fibres entering the wetland, few would be expected to be released by this mechanism.

Anthropogenic fibres were detected in water samples from the outlet of the Northrepps ICW, despite no fibres being detected above the LOD in sediment samples in cell 3 (from which the outlet flows). However, fibres may be present in fine bed sediment in concentrations below the LOD. Bioturbation may result in the movement of microplastics and anthropogenic fibres into the water column (Xue *et al.*, 2020). At the Northrepps ICW, on several occasions large mammals (deer) were observed resting in or running through the second and third cell of the wetland. Their activity may be the most likely cause of re-suspension of entrained microplastics and anthropogenic fibres at the Northrepps ICW. Additionally, the high vegetation percentage cover at the Northrepps ICW means waterfowl are unlikely to cause bioturbation.

4.4.3 Implications for wetland design

In the present study, anthropogenic fibre concentrations were not recorded at the end of each cell. However, given that anthropogenic fibre and microplastic fragment concentrations were not detected above the LOD in cells 2 or 3 at Northrepps, most is, therefore, likely retained in cell 1. Hence, a single cell with an area of 1600 m^2 and >95 % emergent plant cover appears sufficient to retain microplastics and anthropogenic fibres from WWTP effluent. Bydalek *et al.* (2023) showed that the highest areal removal rate at Cromhall ICW was in the first cell at 10,066 fibres m^{-2} day^{-1} (calculated based on supplementary material in Bydalek *et al.*, 2023). This cell had a surface area of 150 m^2, a depth of 1.5 m, was unvegetated and had a hydraulic retention time of 150 minutes and a loading rate of 9.5 L s^{-1}. If this is the maximum achievable areal removal rate (which may not be the case given this cell was unvegetated), then the first cell at Northrepps ICW could be much smaller and still retain most microplastics and anthropogenic fibres from the WWTP. Having a smaller first cell may be beneficial for wetland management, particularly regarding disposal of accumulated micro and macro plastic waste, if such a process were required in future site decontamination actions.

4.4.4 Implications for wetland management

To date, no studies appear to have assessed microplastic content in free surface flow constructed wetland (FSCW) sediment. The combined concentration of anthropogenic fibres and suspected microplastic fragments in cell 1 of Northrepps ICW was 10,090 (SD = 8519) items kg^{-1} dry sediment. This figure is comparable to the global average microplastic concentration in sewage sludge samples of 12,800 (± 5200) items kg^{-1} (Rolsky *et al.*, 2020). Given that the present study did not include white and clear microplastic fragments, and especially since a large amount of white polyethylene macroplastic was found in cell 1, the actual concentrations in sediment are probably much higher than reported here.

Additionally, Ren *et al.* (2020) found that 58 % of microplastics in sewage sludge were white. The high microplastic and anthropogenic fibre content found in the Northrepps wetland may have significant impacts on longer term management. Above-ground plant material can be harvested in constructed wetlands for the purpose of enhancing nutrient removal, although it is questionable how effective this practice is (Vymazal *et al.*, 2010). Above ground plant harvesting would presumably have minimal impact on microplastic retention in constructed wetlands because microplastics will be retained by submerged vegetation and debris only. However, Zheng *et al.* (2015) reported that the density of plants in a FSCW increased by 7.4 % a year after above surface harvesting to 175 shoots m^{-2}, compared to a 16.1 % decline in plant density without harvesting over the same time period. The slightly higher plant density may aid in microplastic retention by having more area for plastics to attach to and increasing residence times for enhanced sedimentation (Helcoski *et al.*, 2020).

Dredging is a long-term practice in constructed wetland management (Hernandez-Del Amo *et al.*, 2020). Dredging in FSCWs is recommended to a depth of 25 cm by mechanical excavation (Zhu *et al.*, 2022), thus significant microplastic and anthropogenic fibre loads will be present within this, presenting similar problems as wastewater sludge in terms of land application (Liu *et al.*, 2021). The evaluation of the EU Sewage Sludge Directive (EUR-Lex, 2023) recognises that microplastics are an increasing source of concern in sewage sludge and may pose a challenge to utilising the valuable nutrient and organic matter content of sewage sludge. Yu *et al*. (2023) found that significant changes on soil physical properties occurred at concentrations above 0.5 % w/w for polyester fibres and 2 % for polypropylene granules. Specifically, microplastics decreased bulk density and water holding capacity, and increased contact angle (i.e., wettability) and saturated hydraulic conductivity. Root penetration is enhanced by lowered bulk density, while the lower water holding capacity reduces the water available for plant growth (Yu *et al*., 2023). Microplastics presence can also alter the soil microbial community and soil fauna (de Souza Machado *et al*., 2019). Plants (including agricultural crops) can also take up nano-plastics, causing negative effects such as cytotoxicity, genotoxicity, and oxidative stress (Li *et al*., 2020). There are still relatively few studies that have assessed the potential impacts of microplastics on soil health, meaning there is currently little knowledge on the magnitude of adverse effects.

Although it was not fully quantified in the present study, the Northrepps ICW contains a large amount of macroplastic that will also be removed during dredging. The presence of this macroplastic, including many (visual observations when visiting the wetland) whole sanitary products, would likely prevent the application of dredged material to agricultural land, given the visibility of plastic contamination. The presence of sanitary products, including panty liners and nappies, also clearly indicates that this material is derived from untreated sewage.

ICWs are highly effective at retaining microplastics and anthropogenic fibres from sewage effluent, although they may be ineffective as a long-term retention mechanism: the problem of how to manage the plastic that has built up in the wetland sediment is significant. Incineration of the dredged material destroys microplastics (Vuori and Ollikainen, 2022), although this technique is prohibitively expensive (Milojevic and Cydzik-Kwiatkowska, 2021). ICWs have been reported to be cost effective measures to reduce nutrient pollution from WWTP discharges (Cooper *et al.*, 2020), but this may be reduced when costs to legally dispose deposited plastic waste are accounted for. To avoid spreading a significant amount of plastic waste to land when wetland sediment is dredged, it may be beneficial for future ICW designs to include a small first cell that is densely vegetated to retain most of the microplastic and anthropogenic fibre loads from WWTP effluent. This way, a smaller amount of material that is highly concentrated in microplastics could be disposed of at controlled landfill, and the dredged material from the remaining cells of the ICW that are less contaminated with microplastics could be safely applied to land as regulated practice dictates. Further research should be carried out to determine the optimum depth, vegetation type and hydraulic conditions of this recommended first cell to retain microplastic fragments and anthropogenic fibres most effectively in the smallest possible area.

4.5 Conclusions

The key findings of this research are summarised as follows:

1. Northrepps ICW consistently retained anthropogenic fibres over a 12-month period, with average removal efficiencies of 99.3 % (with an average of 349,920 fibres entering the wetland day^{-1}).
2. Ingoldisthorpe ICW consistently retained 100 % of anthropogenic fibres over a 12-month period, although the wetland received low and intermittent anthropogenic fibre loads from WWTP effluent (averaging 9504 fibres day^{-1}).
3. There was no evidence of seasonality in anthropogenic fibre removal performance at either wetland.
4. Microplastic fragments and anthropogenic fibres were prevalent in the fine bed sediment of Northrepps ICW: averaging 10,090 (SD = 8519) items kg^{-1} dry sediment in the first cell. No microplastic fragments or fibres were detected in sediment samples in cells 2 and 3 at Northrepps ICW.
5. Approximately 54 % of anthropogenic fibres entering the ICWs were plastic, dominated by polyester. Of the suspected microplastic fragments in sediment samples from the Northrepps ICW, 73 % were confidently identified as plastic (mostly polystyrene, polyethylene, or polypropylene).

6. Future ICW design may include a smaller first cell to retain most of the sewage effluent derived microplastics and anthropogenic fibres to improve long-term management prospects.

Chapter 5

High resolution monitoring of the temporal variability in microplastic fragment and anthropogenic fibre retention at the Northrepps integrated constructed wetland

5.1 Introduction

Previous studies have highlighted the importance of high-frequency monitoring in capturing the temporal variability of pollutants in WWTP effluent (Rode *et al*., 2016). These patterns often result from variations in human activity and the operational schedules of WWTPs. During typical dry-weather conditions, WWTPs exhibit distinct diurnal patterns in the concentrations of organic matter, ammonia, phosphate, and overall wastewater flow (Figure 5.1). These patterns are characterized by a pronounced morning peak, followed by smaller fluctuations throughout the day, and significantly lower concentration overnight (Almeida *et al*.,1999). The morning peak is primarily driven by increased domestic activities, such as showering, toilet flushing, and other water-intensive tasks that occur during the early hours as people start their day. Throughout the day, the flow and pollutant concentrations typically exhibit smaller variations, reflecting intermittent water use patterns from residential, commercial, and industrial sources. Overnight, when water use significantly decreases, the concentrations of organic matter, ammonia, phosphate, and wastewater flow decrease. This reduction in overnight effluent flow into WWTPs provides greater capacity to process and treat the incoming wastewater, often resulting in more stable effluent quality during these hours (Servais *et al*., 1999).

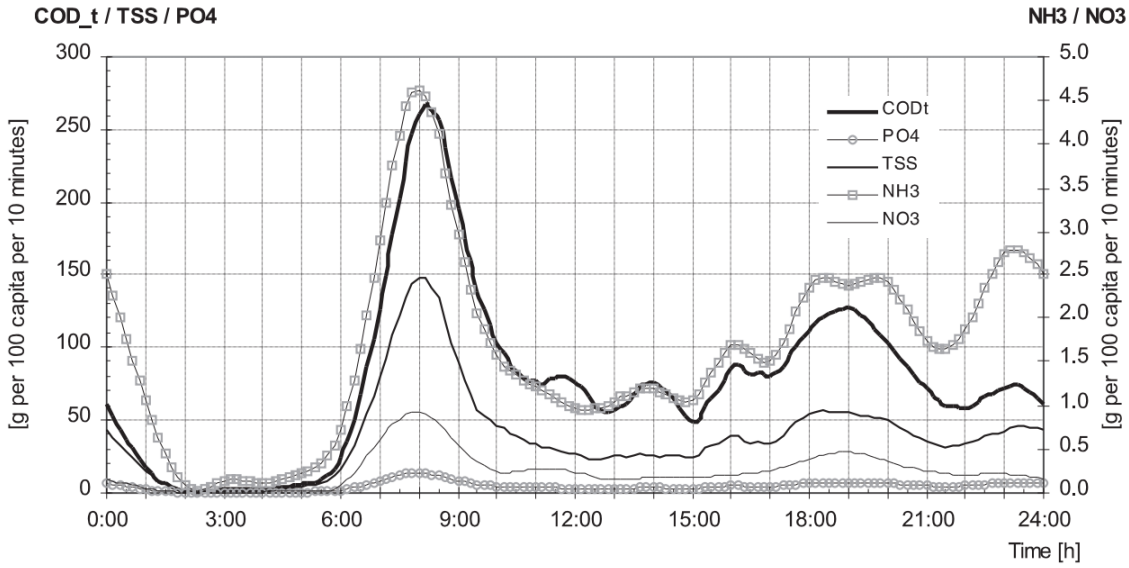


Figure 5.1 Diurnal pattern for load in wastewater for chemical oxygen demand (COD), phosphate (PO4), total suspended solids (TSS), ammonia (NH3), and nitrate (NO3). From Almeida *et al*. (1999).

WWTP effluent can also influence downstream diurnal pollutant patterns in rivers. Palmer-Felgate *et al*. (2008) found that the greatest concentration of total reactive phosphorous (TRP) generally occurred around 14:00 2 km downstream of a WWTP in the River Kennet, UK (Figure 5.2). A secondary peak occurred at around 02:00, reflecting patterns in domestic water usage with the morning and evening peak in usage (accounting for the time lag during WWTP processing). A close relationship was observed between the TRP response and discharge, with concentrations decreasing as flow increases, indicating dilution of the WWTP phosphorus input, although this was less pronounced during winter because of increased rainfall.

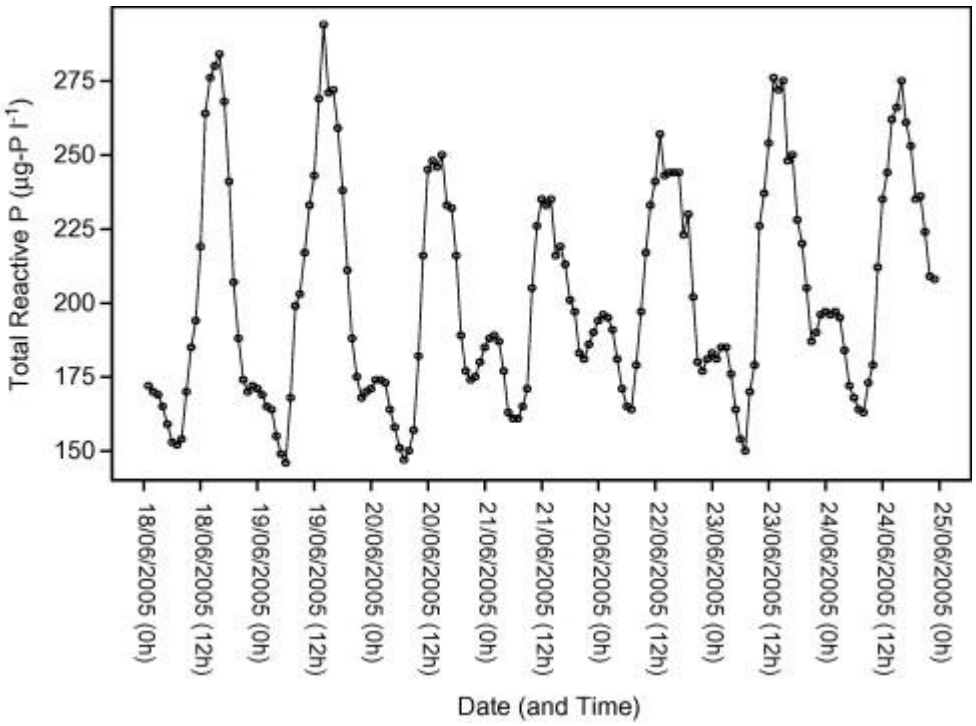


Figure 5.2 One-week time series for in situ hourly measurements of TRP in the River Kennet (18th–24th June 2005) (Palmer-Felgate *et al*., 2008).

While many studies have sampled WWTP effluent for microplastics, few have done high resolution sampling to detect changes in effluent concentrations throughout the day. Kukkola *et al*. (2024) assessed hourly trends in microplastic concentration in WWTP effluent, but they sampled 1 km downstream in a river (although there were no other upstream microplastic sources). The variation in hourly microplastic (MP) concentrations, based on three replicate measurements, was evaluated against the daily average concentrations. It was found that on only two samples had concentrations that were significantly different from the daily average (Kukkola *et al*., 2024). Based on their averages, a morning peak was observed at 9 am, followed by further peaks in the evening (6–8 pm), although concentrations were overall low (Figure 5.3).

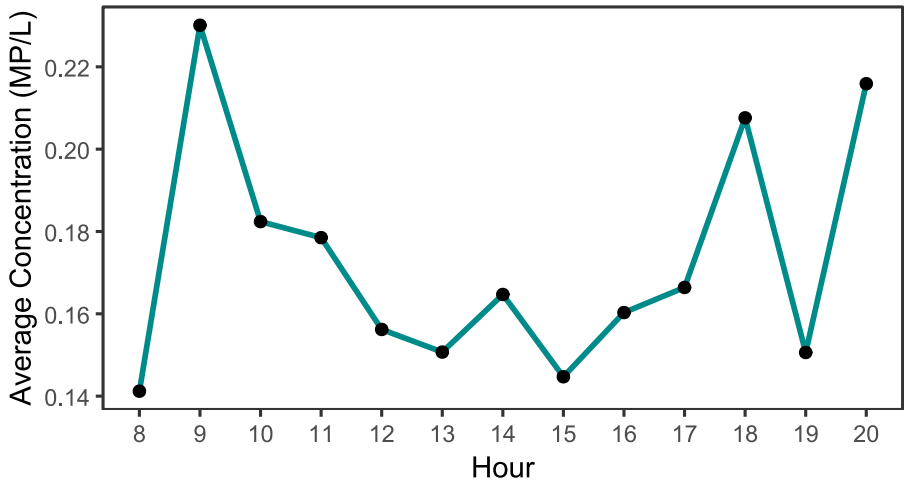


Figure 5.3 Calculated daily variation in average microplastics concentration 1 km downstream of a WWTP (Kukkola *et al*., 2024).

Similar temporal variability in microplastic/fibre concentration may be expected in WWTPs. The use of products containing microplastics, such as cosmetics, tends to be higher during morning and evening routines (Bikiaris *et al*., 2024). Emissions of anthropogenic fibres from washing machines may be high during usage hours (McIlwraith *et al*., 2019), which is evenly distributed throughout the day (Figure 5.4), leading to increased microplastic/fibre loads entering the WWTP during these times. Rainfall events may also dilute microplastic concentrations in WWTP effluent (Li *et al*., 2018).

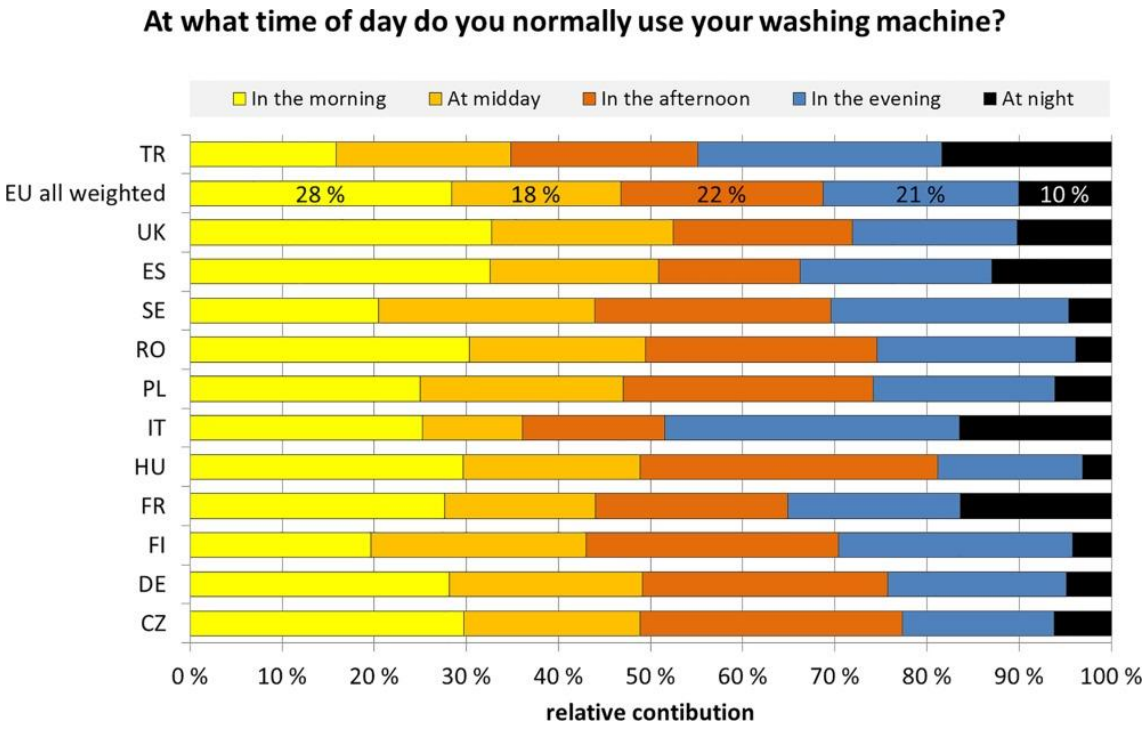


Figure 5.4 Average answers to the question ‘At which part of the day is the washing machine used?’ (Stamminger and Schmitz, 2016).

Most studies on microplastic pollution in wetlands have relied on low-resolution sampling strategies, often collecting samples over extended periods, such as days, weeks, or months (Liu *et al*., 2023). While these approaches provide valuable insights into the average concentrations of microplastics, they fail to capture the short-term fluctuations and peak events that may significantly influence the overall load and fate of microplastics in these systems.

The dense vegetation in ICWs acts as a natural filter of microplastics. The roots, stems, and leaves can physically trap and intercept microplastics as water flows through the system (McIlwraith *et al*., 2024). The microplastics become entangled in the root networks or adhere to the plant surfaces. Vegetation helps stabilize sediments, which can bind microplastics. The organic matter from decaying plants can also trap microplastics in biofilms that form around plant roots and sediments (Kalčíková, 2023). Some ICWs include physical barriers, baffles, or other structures that further slowdown water flow and create additional areas for sedimentation and microplastic retention. The sedimentation process is enhanced by the presence of organic matter and fine particles, which can aggregate with microplastics, making them larger and more prone to settling (Li *et al*., 2019).

Research Aim:

Investigate the temporal fluctuations in sewage-derived microplastic fragments and anthropogenic fibres entering an integrated constructed wetland and assess the variability in the wetland's ability to retain these particles throughout the day.

Objectives:

1. Quantify the inflow of microplastic fragments and anthropogenic fibres from the Northrepps WWTP hourly for 12 hours (06:30–18:30).
2. Quantify the outflow of microplastic fragments and anthropogenic fibres from the Northrepps ICW over the same 12-hour period.

5.2 Methods

5.2.1 Field sampling

Water samples were collected over a 12-hour period on 31st July 2024 from the inlet and outlet of the Northrepps ICW. The inlet was sampled every hour from 7:00 until 18:00, and the outlet was sampled every 3 hours from 6:30 until 18:30. Ideally the outlet would have been sampled hourly, but the walking distance from the inlet over rough, overgrown terrain and the large sample volume required meant this was logistically challenging. The weather was dry throughout the day.

Known volumes of water were collected (10 L for each inlet sample and 300 L for each outlet sample) and poured through a 38 µm stainless steel sieve (200 X 50 mm). At the

Northrepps ICW inlet, water samples (*n* = 12) were taken from an inspection point approximately halfway along the 150 m pipe supplying the wetland from the WWTP using a 1 L measuring cylinder. At the ICW outlet, water samples (*n* = 5) were collected by holding a metal bucket (12 L capacity) beneath the pipe, meaning the entire flow of the pipe was sampled.

After sampling, the sieves were rinsed in the field with MilliQ water into pre-cleaned 250 mL glass jars, that were then sealed with aluminium foil for transport back to the laboratory.

5.2.2 Laboratory analysis

Upon return to the laboratory, the samples within the glass jars were rinsed into glass beakers. MilliQ water was added to the 100 mL mark, and 50 mL of sodium hypochlorite (minimum 14 % free chlorine) was added. This beaker was then sealed with a glass petri dish and placed in a shaker incubator (Orbital Shaker Incubator ES-80) at 40 °C and 90 rpm for 3 hours to remove the organic matter. The resulting solution was then filtered through a 38 µm stainless steel sieve (200 x 50 mm), and then carefully rinsed back into the same glass beaker. The sample was then vacuum filtered onto 47 mm diameter cellulose nitrate filters (pore size 3 µm).

Each filter was transferred to a microscope (Leica CMA) and both fibres and suspected microplastic fragments were identified by visual inspection (with 4 x objective). Suspected microplastic fragments were identified by the following criteria:

- Fragment appearing artificially coloured or shiny (resembling glitter).
- Fragment dark in colour with sharp edges and smooth surface.

5.2.3 Justification of changes to laboratory analysis

Some methodological changes were made to the collection and processing of water samples during this high-resolution monitoring compared to that used in the 12-month field sampling campaign (Chapter 4, Warren *et al*., 2024).

Firstly, a lower sample volume (10 L) was collected at the Northrepps wetland inlet because, based on the previous 12-month campaign, it was expected that anthropogenic fibres would be found at concentrations above the limit of detection (LOD) in the inlet water samples, meaning the sample volume could be lowered to reduce the amount of time spent counting items in each sample (whilst still remaining at concentrations above the LOD).

Additionally, a consistent sample volume was collected during the high-resolution sampling, as opposed to the 12-month campaign when the final sample volume collected was based on the point at which the 38 µm sieve clogged. At the Northrepps ICW outlet, it was decided that a 300 L water sample would be taken each time as this was similar to the average taken during the 12-month campaign at this location. Ideally, the sample volume would have

been increased because many fibre concentrations found here previously were below the LOD, however this was logistically challenging due to the physically demanding nature of the sample collection. The 38 µm sieve may also have clogged beyond a 300 L water sample, meaning a second sieve may have been necessary. While this is feasible, it introduces another source of potential contamination.

Another change was made to the concentration of sodium hypochlorite in the chemical organic matter removal step: it was reduced from 50 % (v/v) to ~33 % (v/v). The reaction time was also reduced to 3 hours. In a test sample, the lower sodium hypochlorite concentration and reaction time remained effective at removing organic material, although small leaves of duckweed were not fully removed by the chemical digestion and were manually taken out (few were found in samples). Microplastic fragments also needed to be analysed in these water samples, which was not the case during the 12-month campaign. This meant that the final 47 mm filters that the samples were filtered on prior to analysis had to be cleaner than they were during the 12-month campaign to reduce the possibility of false positives and residual material covering small microplastic fragments. To do so, after the chemical digestion step, the solution was filtered through a 38 µm sieve prior to vacuum filtration. This meant that small partially degraded organic material was removed.

5.2.4 ATR-FTIR

Selected fibres and suspected microplastic fragments were extracted with either tweezers or a 33-gauge syringe needle into a glass beaker containing water. The samples were then vacuum filtered onto 25 mm silver coated filters (0.45 µm pore size). All particles and fibres found on each of these filters were analysed by a micro-ATR-FTIR microscope (Bruker Hyperion 2000, 20 X ATR objective, resolution = 4.0 cm^{-1}, 64 scans $sample^{-1}$). The spectra acquired were analysed using Open Specy (Cowger *et al*., 2021) to determine the best library match. Default pre-processing settings were used for the signal-noise threshold, smoothing, intensity adjustment, baseline correction and flatten region (removing CO2 peaks) options. Wavenumber range selection of 0–3500 cm^{-1} was applied. Identification was performed using the 'Cor: FTIR Deriv' option. A spectral hit quality score (Pearson correlation coefficient) of 0.7 was set as the threshold, below which all samples were considered unknown to avoid bias in spectral interpretation. In total, the material composition of 40 fibres and 40 suspected microplastic fragments were validated by ATR-FTIR (2 % and 7.6 % of total identified in all samples, respectively).

5.2.5 Quality control

During sampling a closed weave shirt was worn to reduce contamination from clothing fibres. All sieves and glass jars used to collect samples were pre-cleaned in the laboratory with MilliQ water and sealed (either with a sieve lid or aluminium foil) prior to sampling. Sampling equipment was rinsed with MilliQ water between samples.

Unless otherwise stated, all solutions used in sample processing were pre-filtered through 0.45 µm polycarbonate filters. Laboratory work was undertaken within a laminar flow cabinet that was vacuumed and wiped down with paper towel before use. Microscope analysis was done in a room with managed airflow to minimise airborne contamination and was regularly cleaned.

5.2.6 Positive controls

Because changes were made to the method from that used in the 12-month sampling campaign, recovery rate experiments were performed for the new method. To assess the recovery rate of the water sampling method, 30 individual pink polyester fibres were peeled from a sewing thread and cut to approximately 2–5 mm in length. These fibres were stored in a glass beaker in water and then poured through a 38 µm sieve, following which the standard water sampling method was followed. This was performed three times to achieve a more reliable average recovery rate. In addition, 30 blue PVC microplastic fragments were generated by filing macroplastic items to generate small fragments that were then sieved to 250–750 µm for use in recovery experiments, following the standard water sampling methodology. This was performed three times. Because a density separation was not used, it was deemed unnecessary to test low density microplastic recovery. An average recovery rate of 81 % of polyester fibres (2–5 mm) and 96 % of PVC fragments (250–750 µm) was achieved. The recovery rate of fibres was slightly lower (by ~5 %) than for the water sample processing method used in the 12-month campaign, which may be explained by the addition of a sieving step after digestion, where fibres may have passed through the 38 µm mesh. The results are not corrected based on recovery experiments because too few types of plastic were assessed.

5.2.7 Negative controls

A total of three procedural blank samples were taken. For each of these, an empty sieve was left exposed beside the outlet sampling location for the same amount of time it took to collect a sample there (particularly to assess airborne fibre contamination while sampling). The outlet was chosen because a larger sample volume was taken there, meaning the sampling time and subsequent contamination risk was greater. This sieve was then sealed, transported back to the laboratory, processed, and analysed following the standard method.

Anthropogenic fibres were found in every procedural blank: averaging 3 (SD=1) fibres sample^{-1}. A total of nine fibres were found in the procedural blanks: most were dark blue (67 %), clear (22 %), or dark (11 %). The dark blue fibres found in the blank samples are likely from the shirt worn during sampling, which was a similar colour and was 65 % polyester and 35 % cotton. The LOD was calculated as 6 fibres sample^{-1}, and this value was therefore subtracted from each sample fibre count (as done by Dawson *et al*. (2023)).

A suspected microplastic fragment was found in one of the procedural blanks: a blue PVC fragment. This likely originated from the blue PVC gloves worn during sampling. The LOD was calculated as 2 fragments sample^{-1}, and this value was subtracted from each sample fragment count.

5.2.8 Data analysis

The limit of detection (LOD) was calculated for both fibres and fragments separately (for sediment samples) and subtracted from the total value of each sample.

$$\text{LOD} = \text{Mean blank} + (3 \times \text{standard deviation blank})$$

This correction method was chosen based on the findings by Dawson *et al*. (2023), where LOD methods were recommended for microplastic studies.

Error propagation was applied to fibre loading rate (*Z*) calculations using the equation below (Fantner, 2013):

$$\frac{\sigma_Z}{Z} = \sqrt{\left(\frac{\sigma_x}{x}\right)^2 + \left(\frac{\sigma_y}{y}\right)^2}$$

where *x* is fibre concentration (fibres L^{-1}) and *y* is discharge (L s^{-1}). The propagated error is denoted with '±' in parenthesis. Standard deviation (SD) and coefficient of variation (CV) are also presented in parentheses.

5.3 Results

5.3.1 ICW Inlet / WWTP effluent samples

Anthropogenic fibres and microplastic fragments were found in every wetland inlet water sample. Across the entire 12-hour sampling period mean concentrations of 16.3 (SD=9.4, CV=0.57) anthropogenic fibres L^{-1} and 4 (SD=2, CV=0.51) suspected microplastic fragments L^{-1} were found (Table 5.1). Taking the mean discharge from the WWTP entering the wetland from April 2022 to June 2023 of 0.83 (SD=0.92) L s^{-1}, this equates to an average loading rate of 13.5 (± 16.9) anthropogenic fibres s^{-1} and 3.3 (±4.0) microplastic fragments s^{-1}. Scaled up, this amounts to 425,736,000 (±531,498,240) anthropogenic fibres year^{-1} and 104,068,800 (±126,144,000) microplastic fragments year^{-1}.

Table 5.1 Temporal variability in the concentration of anthropogenic fibres and suspected microplastic fragments emitted from the Northrepps wastewater treatment works into the top of the Northrepps ICW on 31st July 2024.

Time collected	Anthropogenic fibres L^{-1}	Microplastic fragments L^{-1}
07:00	25.7	7.5
08:00	15.1	1.7
09:00	12.3	3.4
10:00	28.8	5.4
11:00	34	5.7
12:00	7.0	2.2
13:00	16.1	5.8
14:00	7.0	1.8
15:00	3.8	2.0
16:00	19.5	3.9
17:00	17.2	6.0
18:00	9.2	2.1

Over the 12-hour sampling period, anthropogenic fibre concentration was variable (Figure 5.5), with the highest concentration (34 fibres L^{-1}) recorded at 11:00, and the lowest concentration (3.8 fibres L^{-1}) recorded at both 12:00 and 14:00. These results indicate that concentrations fluctuated significantly, by as much as a factor of 8.9, depending on when the sample is taken. Anthropogenic fibre concentrations were high in the early morning (07:00 sample) but declined from 08:00 to 09:00, then increased to the highest concentrations in the late morning (10:00 and 11:00). The concentration then declined sharply at midday, followed by a slight increase (back to mid-morning levels) at 13:00. Concentrations declined again to their lowest midafternoon (14:00 and 15:00), followed by a rise in the late afternoon.

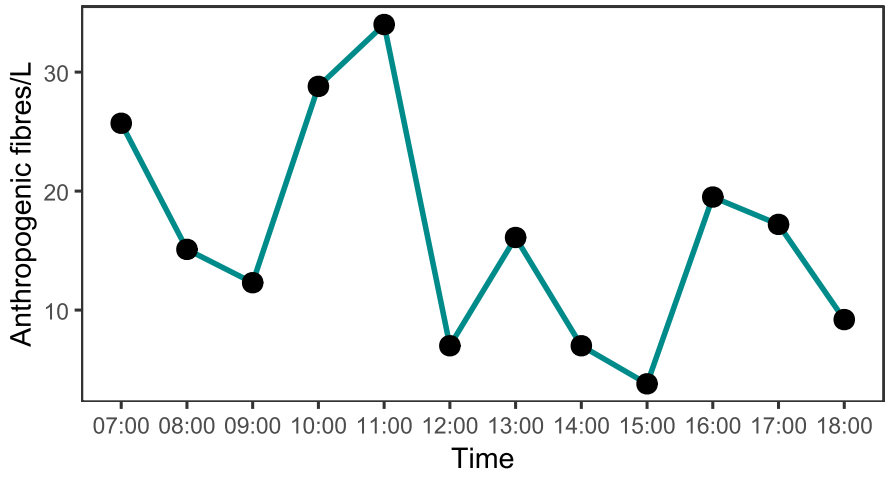


Figure 5.5 Temporal variability in anthropogenic fibre concentration in the Northrepps WWTP effluent over a 12-hour period on 31st July 2024.

Concentrations of microplastic fragments were also variable, although to a lesser degree than anthropogenic fibres (Figure 5.6). The highest concentration (7.5 fragments L^{-1}) was recorded at 07:00, and the lowest concentration (1.7 fragments L^{-1}) at 08:00. Microplastic fragment concentrations varied by approximately a factor of 4.4, depending on when the sample was taken. The concentrations of microplastic fragments displayed similar diurnal variability to anthropogenic fibres, although concentrations were highest in the early morning (07:00), and the late morning peak was not as pronounced.

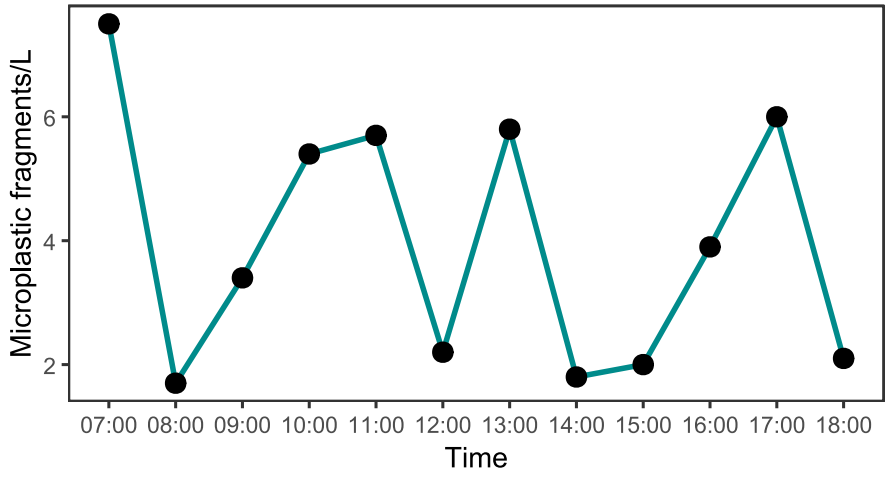


Figure 5.6 Temporal variability in suspected microplastic fragment concentration in the Northrepps WWTP effluent over a 12-hour period on 31st July 2024.

The combined concentration of both anthropogenic fibres and microplastic fragments was highest (39.7 items L^{-1}) at 11:00, and lowest (5.8 items L^{-1}) at 15:00 (Figure 5.7). Combined concentrations varied by a factor of 6.8, depending on when the sample was taken. The diurnal patterns resembled those of anthropogenic fibres, which is expected

because concentrations of anthropogenic fibres were higher than of microplastic fragments.

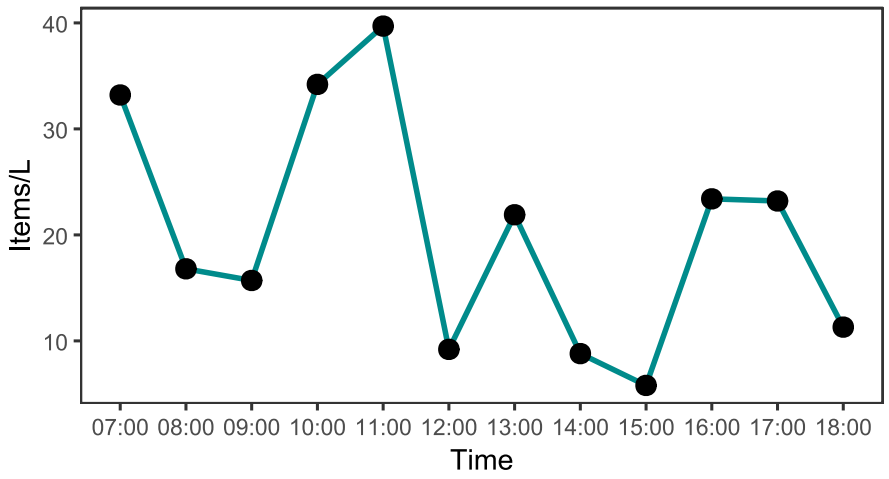


Figure 5.7 Temporal variability in the combined concentration of anthropogenic fibres and microplastic fragments in Northrepps WWTP effluent over a 12-hour period on 31st July 2024.

Discharge data (from the Northrepps WWTP) was not available on the sampling day, meaning no direct correlations between discharge and microplastic/fibre concentration could be made. However, some insights can be made by observing discharge data from April 2022 to June 2023, which reveals diurnal variability (Figure 5.8). The lowest average discharge occurred at 04:00 (0.27 L s^{-1}) and remained relatively low between 01:00 and 06:00. There was a noticeable increase in discharge starting from 06:00 and peaking at 10:00 (1.37 L s^{-1}). Discharge then declined throughout the afternoon (though remaining moderately high), followed by another peak at 20:00 (1.14 L s^{-1}), before declining later in the evening. Taking the average daily discharge from April 2022 to June 2023, discharge is not a good predictor of combined microplastic fragment and anthropogenic fibre concentration (Figure 5.9). Only about 1 % of the variability in concentration is explained by discharge.

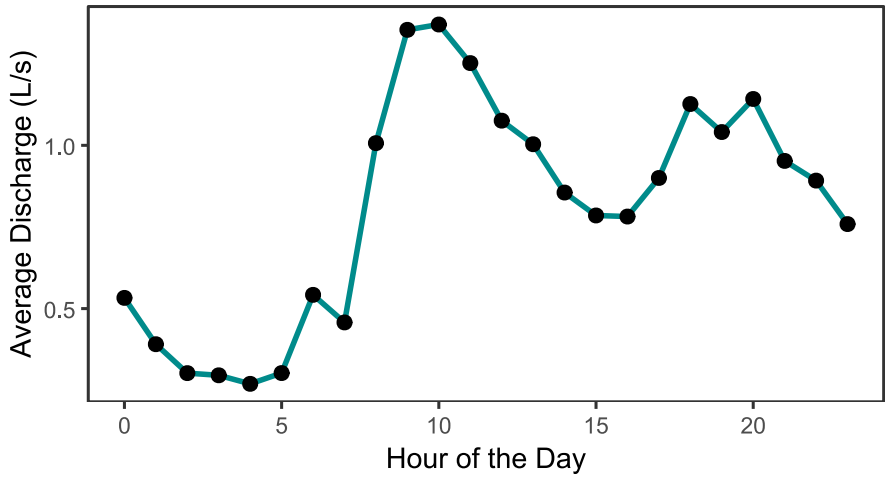


Figure 5.8 Average daily discharge from the Northrepps WWTP into the Northrepps ICW from April 2022 to June 2023. Data supplied by Anglian Water.

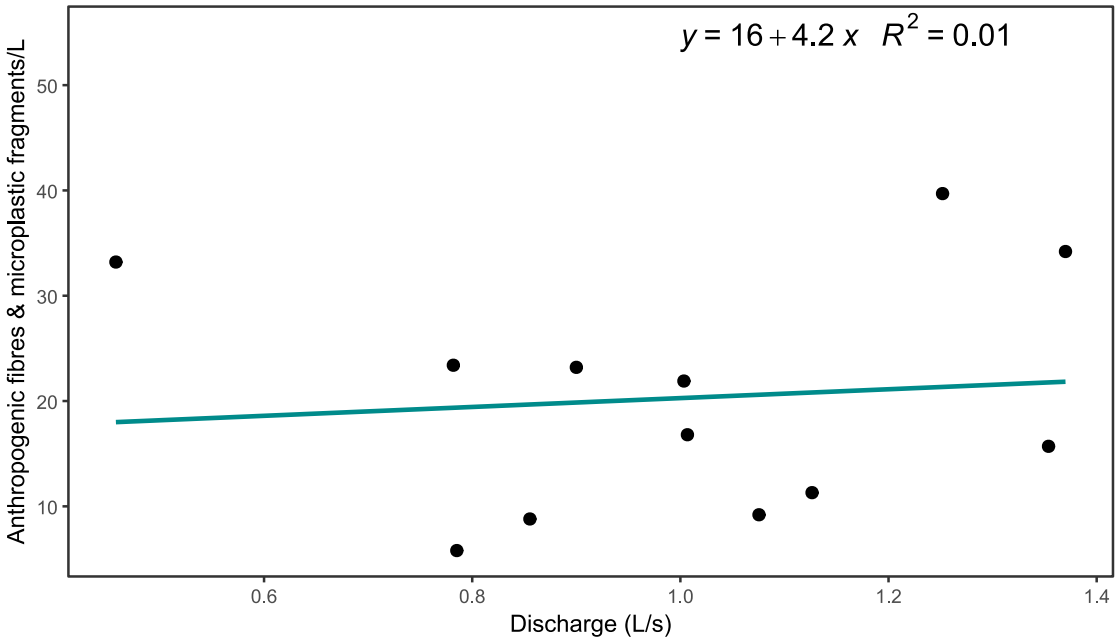


Figure 5.9 Relationship between average discharge (from April 2022–June 2023) and combined anthropogenic fibre and microplastic fragment concentration in Northrepps' WWTP effluent.

Figure 5.10 illustrates a positive linear relationship between the concentration of anthropogenic fibres and microplastic fragments. The regression analysis suggests that for each additional fibre per litre, there is an associated increase of approximately 0.168 microplastic fragments per litre. The coefficient of determination value ($R^2 = 0.6$) suggests a moderate to strong correlation, implying that sampling anthropogenic fibres alone could provide a reasonable indication of the likely presence of microplastic fragments. However, it is important to note that 40 % of the variance in microplastic fragment concentration is not

explained by the concentration of fibres, which could affect the consistency of this proportional relationship across different samples. Consequently, to achieve more accurate predictions of fibre and fragment concentrations, additional sampling and analysis would be advisable if only one variable is measured.

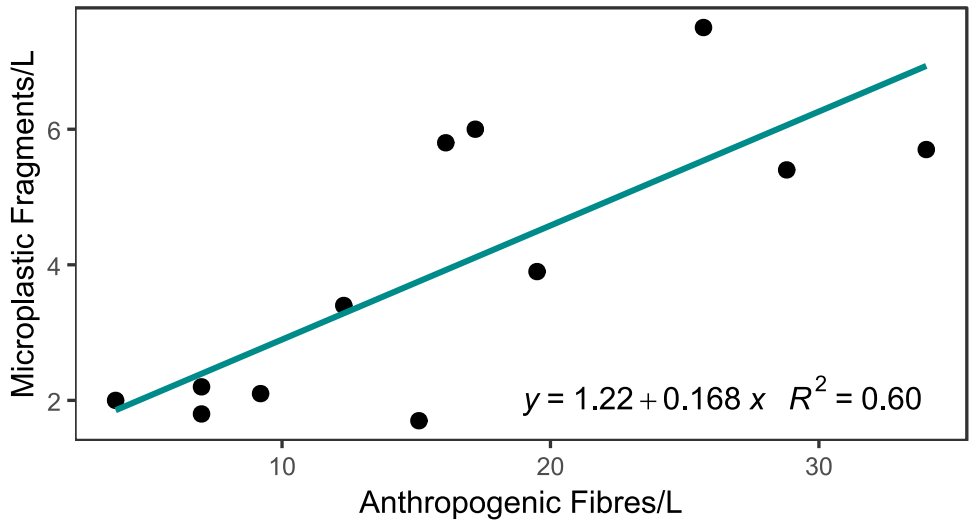


Figure 5.10 Relationship between the concentration of suspected microplastic fragments and anthropogenic fibres in the Northrepps WWTP effluent entering the Mun ICW.

5.3.2 ICW outlet water samples

In the wetland outlet water samples, anthropogenic fibres were found (above the LOD) in just two out of the five (40 %) samples collected, with a mean concentration of 0.01 (SD=0.02) fibres L^{-1} (Table 5.2). Suspected microplastic fragments were found (above the LOD) in four out of five (80 %) of the samples, with an average concentration of 0.01 (SD=0.01) fragments L^{-1}. Compared to the wetland inlet concentrations, the Northrepps ICW retained approximately 99.8 % of incoming anthropogenic fibres and microplastic fragments. The concentrations of anthropogenic fibres and microplastic fragments are generally very low, and there was no significant fluctuation in concentrations over the sampling period. Based on this finding, sampling at the wetland outlet at any time of the day should be reliable in capturing an accurate picture of the anthropogenic fibre and microplastic fragment concentrations in the outfall. However, for long term monitoring, periodically confirming that concentrations remain low and stable would ensure that grab sampling continues to be a suitable method.

Table 5.2 Temporal variability in the concentration of anthropogenic fibres and suspected microplastic fragments at the outlet of the Northrepps ICW on 31st July 2024.

Time collected	Anthropogenic fibres L^{-1}	Microplastic fragments L^{-1}
06:30	<LOD	0.007
09:30	0.03	0.010
12:30	0.02	0.017
15:30	<LOD	0.017
18:30	<LOD	<LOD

5.3.3 Microplastic and anthropogenic fibre characteristics

Most of the suspected microplastic fragments were blue, green, or red (Figure 5.11). However, this does not reflect the actual colours of microplastics within the wetland because clear and white microplastics were not identifiable with the method applied here. Additionally, only dark fragments that were obviously suspected microplastic were counted: those with sharp edges and a smooth texture. Therefore, tyre wear particles dark in colour could have been missed, although the catchment for both WWTPs was rural with generally low speed traffic so these were not likely to occur in high concentration in the Northrepps WWTP. No spherical microplastics resembling microbeads were found. The vast majority of suspected microplastic fragments found were <100 µm in their longest dimension (Figure 5.12).

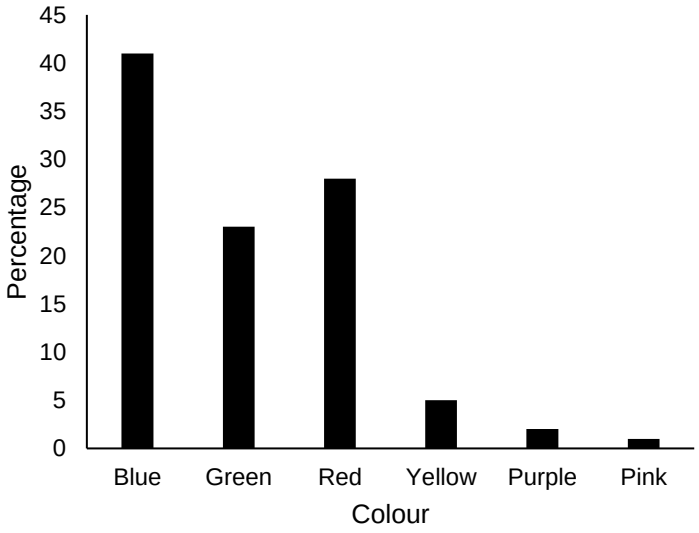


Figure 5.11 Colours of microplastic fragments found in the Northrepps WWTP effluent samples during the high frequency monitoring on 31st July 2024.

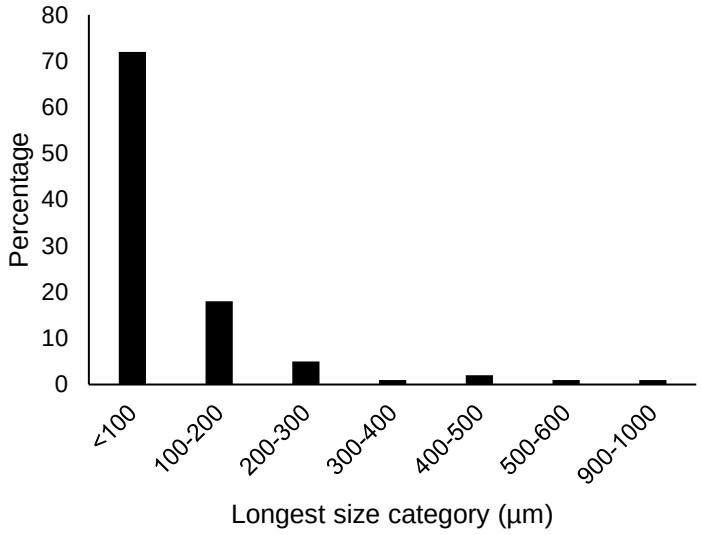


Figure 5.12 Longest size category of suspected microplastic fragments found in the Northrepps WWTP effluent samples during the high frequency monitoring on 31st July 2024.

Most of the anthropogenic fibres found were clear (84 %) or dark (12 %), while 20 % were approximately >800 µm, 56 % were 800–250 µm, and 24 % were 38–250 µm.

Of the 40 suspected microplastic fragments verified by ATR-FTIR, 87 % were confirmed as plastic material. The particles that were not confirmed plastic were mostly black particles, that were in fact mineral material. The method used therefore appears most reliable at identifying obviously coloured microplastic fragments. Of the 40 anthropogenic fibres verified by ATR-FTIR, 75 % were plastic (91 % of which were polyester), 22 % were cellulosic, and 3 % were non-identifiable due to poor spectra quality.

5.4 Discussion

5.41 Wetland microplastic retention

The results suggest that there was no significant diurnal variability in the anthropogenic fibre and microplastic fragment removal performance of the Northrepps ICW. This finding was expected for a number of reasons. Most microplastics are chemically inert and physically stable over short periods, including daily cycles. While sunlight can cause some degradation of plastics, this process is very slow (Dai *et al*., 2023). The time frame of a single day is generally too short to expect significant changes in microplastic properties that would impact their retention in an ICW due to photodegradation. The daily temperature changes in a wetland are generally mild and not extreme enough to alter the density, buoyancy, or settling characteristics of microplastics within a single day. In many ICWs, the inflow and outflow of water are designed to be steady and consistent to maximize treatment efficiency. This stability in hydraulic conditions would minimize any diurnal fluctuations in the transport and retention of microplastics. Biofilms, which can trap microplastics (Chen *et al*., 2019), tend

to develop gradually and maintain their trapping efficiency over longer periods (Ding *et al*., 2017). The formation and activity of biofilms are not likely to vary significantly within a 24-hour cycle (Blanken *et al*., 2017). While photosynthesis and respiration in plants do vary diurnally, these changes are unlikely to have a direct and immediate impact on microplastic retention. The physical structure of vegetation that traps microplastics remains constant throughout the day, providing continuous filtering capacity (McIlwraith *et al*., 2024; Helcoski *et al*., 2020).

Bioturbation is also unlikely to have a significant effect on microplastic retention efficiency of ICWs. Microplastics, once settled in the sediments, might be less prone to re-suspension compared to finer, organic particles because their size, shape, and density could mean they are less likely to be dislodged by the relatively gentle movements caused by small bioturbating organisms (Li *et al*., 2022). It is therefore not expected that nocturnal bioturbation will cause changes in the microplastic/fibre removal efficiency.

5.4.2 Implication of diurnal variability

Whilst there was no significant temporal variability in microplastic retention performance within the ICW, the results do reveal significant daytime variability in microplastic and anthropogenic fibre concentrations in WWTP effluent. This variability has important implications for future research and monitoring. Grab samples taken at most times of the day will not necessarily be representative of the average effluent concentration, meaning a one-point-in-time sampling approach to estimate daily–annual microplastic/fibre loads from secondary treatment WWTPs could therefore result in large uncertainties, in agreement with Kukkola *et al*. (2024). If the aim of sampling is to get a general picture of daily concentrations, composite sampling might be more appropriate to smooth out this variability. It is proposed to add this as a quality control criterion when evaluating studies that monitor microplastic fragment and anthropogenic fibre concentrations in WWTP effluent that has undergone primary and secondary treatment. However, there are logistical limitations to composite sampling, which often requires continuous or frequent manual collection of samples over a set period, which can be labour intensive. Automated samplers can mitigate this but add to the complexity and cost. For example, Carr *et al*. (2016) used PVC line splices to intercept tertiary WWTP effluent, and the calibrated flow was filtered through sieve stacks. This enabled sample volumes of 189,000 L to be collected. The diurnal variability in microplastic and anthropogenic discharge form WWTPs also highlights the importance of collecting samples at a consistent time, when sampling occurs on different days if comparisons between days/seasons/years are to be made.

Despite the benefits of composite sampling, it may not always be appropriate. In some cases, regulatory agencies require individual sample data to ensure that pollution levels do not exceed certain thresholds at any given time. Composite sampling may not meet these

requirements because it could hide exceedances. Although there are currently no regulatory WWTP discharge limits for microplastics and anthropogenic fibres in the UK, this research indicates that the highest concentrations in WWTP effluent occur in the late morning. Exceedance of potential regulatory limits may therefore be most likely at this time, and future research and monitoring may consider this in their sampling designs. This may also be true for plasticizers, such as DEHP (a WFD priority substance), since microplastic concentrations have been correlated with phthalate concentrations in rivers (Wang *et al*., 2023).

5.4.3 Can discharge be used as a predictor of MP concentration at hourly resolution?

No statistically significant relationship was found between discharge and combined microplastic fragment and anthropogenic fibre concentration. However, monitoring could be repeated over several days to further investigate the relationship between WWTP discharge and microplastic/fibre concentrations, improving reliability. It is therefore recommended that future research to investigate this further. Using WWTP effluent discharge as a predictor of microplastic concentration offers significant benefits for monitoring and managing microplastic pollution (Watkins, Sullivan and Walter, 2019). This method could streamline monitoring efforts, as continuous effluent data is often readily available, reducing the need for costly and time-intensive field sampling. However, the level of treatment in WWTPs significantly complicates the use of effluent discharge as a predictor of microplastic concentration. Secondary treatment, which primarily removes organic matter and suspended solids through biological processes, often fails to capture a substantial portion of microplastics, allowing them to pass into the effluent (Krishnan *et al*., 2023). Tertiary treatment, however, involves additional filtration or advanced processes like membrane filtration, which can more effectively remove smaller particles, including microplastics (Krishnan *et al*., 2023). This variation in treatment levels can lead to significant differences in microplastic concentrations between plants and possibly within the same plant over time, depending on operational conditions. Therefore, using WWTP effluent as a predictor would need to account for the specific treatment technologies in place.

5.4.4 Correlations between anthropogenic fibres and microplastic fragments

The idea of using one type of microplastic (e.g., fibres) as a proxy for another type (e.g., fragments) in environmental studies is a concept that has been discussed but is not established in the literature.

There is a general consensus that fibres are the dominant type of microplastic found in treated domestic wastewater (Gies *et al*., 2018; Long *et al*., 2019; Dris *et al*., 2015; Ziajahromi *et al*., 2017; Ngo *et al*., 2019; Cristaldi *et al*., 2020). Fibres are considered the most challenging morphology to remove during the WWTP process because of their mostly smooth surface and high length to width ratio (Talvitie *et al*., 2017). Fibres with a high length

to width ratio tend to have lower settling velocities because they have a large surface area relative to their volume, increasing the frictional resistance (drag), reducing the terminal velocity. Additionally, long and thin objects can change orientation as they descend in water, reducing their downward velocity. Fibres are therefore more likely to pass through primary treatment, which relies on gravity to separate out solids (Talvitie *et al*., 2017). In the secondary treatment stage, where flocculation is used to aggregate smaller particles into larger clumps (flocs), fibres may not integrate as effectively into these flocs due to their shape. Their elongated form can prevent them from adhering to other particles, making them more difficult to remove during filtration. The fibres' tendency to align with water flow further increases the likelihood of their passage through filtration systems. In contrast, fragments and other shapes are more easily eliminated by sedimentation process and biofouling system in WWTPs because they have angular, bifurcate, and twisted characteristics which are conducive to the colonization of microbes and increase their resistance in the wastewater column (Long *et al*., 2019).

In the context of WWTP samples, it is suggested to consider whether it is necessary to sample both microplastic fragments and anthropogenic fibres, depending on the specific research question. This approach would be context-dependent and not universally applicable. The main reason for suggesting this is that the analysis time that is required to identify microplastic fragments is generally much higher than for anthropogenic fibres. When visually scanning filters for suspected items, fibres are much easier to identify due to their unique shape, whereas fragments can be more camouflaged amongst natural detritus. This is particularly true for clear or white microplastic fragments, where there may be so many false positives on a filter that identifying these coloured fragments is not possible, as was the case for this study. To reduce the prevalence of false positives (e.g. partially degraded organic material, crystals etc.) generally will require a more complicated sample processing method (such as density separation, centrifugation, more concentrated chemical digestion) which is likely to reduce the accuracy of results by either reducing the recovery rate, by generating new fragments, or by increasing external contamination risk. While identifying coloured particles is relatively straightforward, confirming their material composition can be more complicated. This is particularly so if the particle must be removed from the filter to be analysed (this may be required because re-locating the particle on a large filter with an FTIR/Raman may be impossible). Fibres are much easier to extract from filters than small fragments, although fibres can be blown away easier than fragments, which can be frustrating. Laboratories that employ automatic FTIR/Raman scanning of parts, or the entire sample filter, may see less of a time/cost benefit from only analysing fibres. Even still, particle characteristics and instrumental parameters can significantly influence the identification success of microplastic/fibres (De Frond *et al*., 2023).

The decision to sample merely anthropogenic fibres instead of microplastic fragments depends on the specific research question. For routine monitoring assessing the treatment performance of a WWTP, sampling only anthropogenic fibres may be adequate. From a regulatory perspective, it could be more practical to establish discharge limits for anthropogenic fibres rather than microplastic fragments, given the greater simplicity and reliability of fibre sampling and analysis methods, which make monitoring more feasible (Coffin, 2023). At present, it is argued that laboratory methods are not precise enough to identify microplastics in environmental samples because they are such a broad contaminant class (Rochman *et al*., 2019), whereas existing methods to identify anthropogenic fibres in environmental samples are more accurate. For legislation to set microplastics limits, the definition of microplastic must be very well defined so that methods can be demonstrated to be highly effective at identifying those particle types (Lusher *et al*., 2020).

The benefits of a simpler, faster analysis method are also significant. In the case of the present research, it is estimated that also analysing microplastic fragments in these samples increased the sample processing/analysis time by 2–3 times compared to only analysing anthropogenic fibres. If a WWTP effectively removes anthropogenic fibres, the evidence suggest that it will be more effective at removing microplastic fragments, at least above approximately 20 µm (Iyare *et al*., 2020). Figure 5.10 also demonstrates that a proportional increase in microplastic fragment concentration can be expected as anthropogenic fibre concentration increases. At present however, anthropogenic fibre concentrations cannot be used as a proxy for microplastic fragment concentrations. Fibres often originate from the degradation of textiles, while fragments can come from the breakdown of larger plastic items or secondary fragmentation of other microplastics. This difference in origin means that the presence of fibres does not always equate to the presence of fragments, and vice versa.

It is also questionable whether the insights generated by identifying microplastic fragments in these WWTP samples (at Northrepps) are valuable enough to justify the time spent doing so. Yes, it is shown that microplastic fragments are present in WWTP effluent, but this was expected already given that the Northrepps WWTP only uses secondary treatment. It has also shown that microplastic fragments do not generally display the same diurnal variability as fibres and seem to be discharged more randomly, which is interesting to know, and may promote further research to do with modelling microplastic discharges from WWTPs. However, in terms of the bigger picture, what has been shown is that loading rates (into the Northrepps ICW) of anthropogenic fibres and microplastic fragments from WWTP effluent are high. What may have been more valuable, in hindsight, is to do a more extensive study into how well the wetland actually retains them.

While sampling only anthropogenic fibres in WWTP effluent may be a reasonable approach, it is not recommend to limit sampling to merely these at the outfall of ICWs. Currently, there is insufficient evidence to suggest that ICWs, including surface flow constructed wetlands, selectively retain microplastic fragments or anthropogenic fibres. The Northrepps ICW presents an ideal case to investigate this further. High concentrations of microplastic fragments and anthropogenic fibres in the first cell of the Northrepps ICW have already been found. By conducting additional sampling at the end of this first cell, it is possible to evaluate its removal performance and determine which types of microplastics, and fibres are most effectively retained. The fundamental structure of the wetland (e.g., plant species, density, substrate) is similar across all cells, with the only major difference being the size of the first cell. This makes Northrepps an ideal site for studying the retention behaviour of these pollutants in ICWs.

5.4.5 Evaluating own work

The results of the current study provide a basis for re-evaluating the reliability of the previous 12-month sampling campaign. The large window of sampling times (08:00 to 12:45) in the earlier study may have affected the comparability of anthropogenic fibre concentrations across samples. Despite this, the earlier campaign consistently showed high concentrations of anthropogenic fibres entering the Northrepps ICW. The sediment sampling further demonstrated extensive contamination of microplastic fragments, macroplastics, and anthropogenic fibres.

The highest concentration reported in Northrepps WWTP effluent was on 13th March 2023, with 39 anthropogenic fibres L^{-1}. This value was reported as being abnormally high. In the 12-month campaign, the average anthropogenic fibre concentration was 5.48 (SD = 9.70) fibres L^{-1}, compared to 16.3 (SD=9.4) fibres L^{-1} in the present study. The observed increase in microplastic and fibre concentrations compared to the previous 12-month sampling campaign is notable. However, the reasons for this rise are not fully understood. Based on the recovery rate experiments, there is no reason to believe that the minor changes in the sample processing methods explain the increase in reported concentrations. It is also unlikely that anything within the WWTP has changed, since the Northrepps WWTP is so simple. The WWTP effluent was also not all sampled at the same time of day during the 12-month campaign, meaning this difference cannot be attributed to the sampling time. Given the small size of the WWTP population served (<1000), variability in anthropogenic fibre export is likely more pronounced as total loads could be influenced more strongly by specific contaminating events. For example, studies have shown that there is considerable variability in fibre export from domestic laundry, thus it is not unreasonable to assume that clothe washing habits have significant influence on fibre discharge from the Northrepps WWTP (Stanton *et al*., 2023). The individuals living in the area that are served by the WWTP

may benefit from being informed that their habits may have a more direct impact on environmental contamination than they may expect because the WWTP receiving their wastewater is not advanced. The village of Northrepps may therefore be a suitable site for future studies to assess the effect of behavioural changes on water quality parameters (e.g. microplastic/fibre concentration) in WWTP effluent, such as done by Erdle *et al*. (2021).

5.5 Conclusions

1. Anthropogenic fibre concentrations in secondary treated WWTP effluent ranged from a low of 3.8 to a high of 34.0 fibres per litre over a 12-hour sampling period (06:30-18:30) - varying by a factor of 8.9.
2. Microplastic fragment concentrations in secondary treated WWTP effluent ranged from a low of 1.7 to a high of 7.5 fragments per litre over the same 12-hour period - varying by a factor of 4.4, although this did not include clear and white microplastics.
3. Diurnal patterns in anthropogenic fibre concentration were more pronounced than microplastic fragments, with a clear late morning peak around 11:00, likely linked to an increase in early morning domestic water use.
4. No correlation between WWTP discharge and MP concentration was found, implying discharge should not be used as a predictor of microplastic concentration where monitoring is not taking place, although further research is recommended to explore this further.
5. A positive correlation was also found between microplastic fragment and anthropogenic fibre concentrations in WWTP effluent, potentially having implications for future monitoring.
6. The Northrepps ICW effectively retained over 99.8 % of microplastic fragments and anthropogenic fibres, with no evidence of daytime variability in retention performance.
7. To maximise the effectiveness of subsequent sampling campaigns, it is recommended to:
    a) Collect composite samples from WWTP effluent throughout the day
    b) Collect WWTP effluent samples at the same time of day when sampling over multiple days (if composite sampling is not appropriate)

A limitation of this study is that the reliability of the diurnal pattern from one day to the next has not been assessed. Further research should explore this.

Chapter 6

Evaluating riverine phthalate pollution and the mitigation potential of integrated constructed wetlands

6.1 Introduction

6.1.1 Introduction to phthalates

Phthalates are esters of phthalic acid that are widely used as plasticizes in the manufacture of plastics. Plasticizers are organic compounds with low volatility that are incorporated into plastic formulations to enhance their flexibility, extensibility, and processability. Phthalates were first introduced as plasticizers in 1920 and remain the largest class of plasticizers in modern times (Rahman and Brazel, 2004). Phthalates generally work by reducing the viscosity of polymer melts, the glass transition temperature, the melting temperature, and the elasticity modulus of the final products. This enhances the flow and thermoplasticity of the plastic materials (Chanda and Roy, 2007). Phthalates can typically leach from plastics because, if the plasticizer were chemically bonded to the polymer, it would no longer enhance the plastic's flexibility. Not all phthalates are solely used as plasticizers, for example C1 to C3 phthalates (i.e., DMP, DEP, DBP) are also used as solvents in cosmetics, fragrances, candles, and shampoos (Godwin, 2010).

In Europe, the most widely used phthalates, in order, were DEHP, DIDP, DINP, and DBP (Peijnenburg, 2008). No exact production numbers of phthalates were identifiable, although production was estimated at 4.3 million tonnes year^{-1}, 90 % of which were used in plasticizers (Peijnenburg, 2008). Data from Statista (2022) also show that DEHP remains the most popular plasticizer, followed by DINP/DIDP. DEHP was first introduced in the 1930s and has maintained its prominence due to its favourable chemical properties, cost-effectiveness, and compatibility with PVC (Jagarlapudi *et al*., 2023).

Phthalates are a significant component of the global plasticizer market, which was valued at approximately USD 17.99 billion in 2023 and is expected to reach USD 23.88 billion by 2030 (Fortune Business Insights, 2024). However, in recent years, there has been a notable move towards using non-phthalate-based plasticizers due to growing awareness of the adverse health and environmental impacts associated with phthalates, particularly in North America and Europe (Fortune Business Insights, 2024). Phthalates accounted for approximately 70 % of global plasticizer consumption in 2014, down from ~88 % in 2005 (Plastics Technology, 2016). Non-phthalate plasticizers are perceived as safer and more

eco-friendly alternatives to traditional phthalate-based plasticizers (Krauskopf, 2003). This shift is driven by several factors, including stricter government regulations (ECHA, 2024), rising consumer demand for safer products (Lubowiecki-Vikuk *et al*., 2021), and corporate commitments to sustainability (Meuer *et al*., 2019). Despite the declining share of phthalates being used as plasticizers, production is still increasing, with approximately 5.5 million tonnes being made in 2015 (Holland, 2018). China also accounts for 45 % of global phthalate use (Holland, 2018).

The most frequently plasticized polymers include PVC, polyvinyl acetate (PVA), polyvinyl butyral (PVB), cellulose moulding compounds, acrylics, and polyamides (Rahman and Brazel, 2004). Globally on average, 80% of all plasticizers are used for PVC (Rahman and Brazel, 2004). Several phthalates, including DEHP, are commonly used in PVC and copolymers of vinyl chloride and vinyl acetate because they have an affinity for these polymers, and impart flexibility and ductility to them, particularly at low temperatures (Chanda and Roy, 2007). DBP is not ideal for PVC plasticization due to its relatively high volatility. However, it is an effective gelling agent for PVC and PVCA and is sometimes used as a secondary plasticizer to enhance solvation. DBP is mainly used in cellulose-based varnishes and adhesives due to its high dissolving capacity for cellulose nitrate. DMP also has a high dissolving capacity for cellulose nitrate and good compatibility with cellulose esters. It is used in celluloid made from cellulose nitrate and in plastic compounds or films made from other cellulosic polymers, such as cellulose acetate. Although it is light-stable, it is highly volatile. DEP shares similar properties with DMP but is slightly less volatile (Chanda and Roy, 2007).

The desirable properties phthalates can exert on various plastics mean they are widely used in consumer products. Phthalates, particularly DEHP, are used as plasticizers in a variety of medical devices, including blood bags intravenous tubing, and catheters, due to their ability to impart flexibility and durability to the plastic materials (Sampson and de Korte, 2010). Phthalates like DEP are used in personal care products such as perfumes, lotions, and hair sprays to help dissolve and stabilize other ingredients and to impart a desirable texture. Certain phthalates are used in the production of soft, flexible plastic toys and childcare articles. However, regulatory restrictions limit the use of specific phthalates in these products due to health concerns. Phthalates are used in various building materials, including vinyl flooring, wall coverings, and roofing membranes, to enhance flexibility, durability, and weather resistance (Godwin, 2010). In the automotive industry, phthalates are used in the manufacture of interior materials such as upholstery, dashboards, and trim, as well as in exterior coatings to improve flexibility and durability. Phthalates are used in certain types of food packaging materials to provide flexibility and strength, although their use is subject to regulatory limitations due to potential health risks (Fierens *et al*., 2012).

Phthalates are used as plasticizers in some textile applications to provide a soft feel and enhance the durability of synthetic fabrics and coatings (Eales *et al*., 2022) (Godwin, 2010).

6.1.2 Human and aquatic impacts of phthalate exposure

Phthalates are known endocrine disruptors and can be toxic to humans. Phthalate exposure can cause reproductive system anomalies, early puberty in females, neurodevelopmental issues, obesity and metabolic disorders, hormone sensitive cancers, and pregnancy complications (Table 6.1).

Table 6.1 Impacts of phthalate exposure on humans, including mechanisms of action and associated references.

Clinical Condition (Impact)	Mechanisms of Action	Reference
Reproductive System Anomalies	Receptor Binding: Antagonism of androgen receptors (ARs), reducing testosterone action	Diamanti-Kandarakis *et al*., 2009
	Steroidogenesis Disruption: Inhibition of enzymes such as 3β-HSD and 17β-HSD	Martinez-Arguelles & Papadopoulos, 2015
	Downregulation of StAR Protein: Reducing cholesterol transport necessary for steroid hormone synthesis	Martinez-Arguelles & Papadopoulos, 2015
	Epigenetic Modifications: Altered DNA methylation and histone modifications affecting gene expression	Schug *et al*., 2011
	Prenatal Effects: Reduced anogenital distance in males due to prenatal exposure	Swan *et al*., 2005
Early Puberty in Females	Receptor Binding: Mimicking estrogen by binding to estrogen receptors (ERs)	Diamanti-Kandarakis *et al*., 2009
	Steroidogenesis Disruption: Inhibition of aromatase, affecting estrogen synthesis	Martinez-Arguelles & Papadopoulos, 2015
	Epigenetic Modifications: Altered gene expression through changes in DNA methylation	Martinez-Arguelles & Papadopoulos, 2015
Neurodevelopmental Issues	Receptor Binding: Disruption of ERs and ARs affecting brain development	Schug *et al*., 2011
	Hormone Signaling Pathways: Alteration of cAMP and MAPK/ERK pathways impacting neurodevelopment	Schug *et al*., 2011
	Epigenetic Modifications: Changes in DNA methylation and histone acetylation affecting neurodevelopmental genes	Martinez-Arguelles & Papadopoulos, 2015

	Prenatal Effects: Association with lower IQ and attention disorders due to prenatal exposure	Swan *et al*., 2005
Obesity and Metabolic Disorders	Receptor Activation: Activation of PPARα and PPARγ affecting lipid metabolism and adipogenesis	Schug *et al*., 2011
	Hormone Signaling Pathways: Alteration of insulin signaling pathways	Schug *et al*., 2011
	Epigenetic Modifications: Alterations in gene expression related to metabolism	Schug *et al*., 2011
Hormone-Sensitive Cancers	Receptor Binding: Mimicking estrogen and antagonizing androgen action	Diamanti-Kandarakis *et al*., 2009
	Steroidogenesis Disruption: Affecting the synthesis of steroid hormones involved in cancer progression	Martinez-Arguelles & Papadopoulos, 2015
	Epigenetic Modifications: Epigenetic changes leading to altered expression of oncogenes and tumor suppressor genes	Schug *et al*., 2011
Pregnancy Complications	Steroidogenesis Disruption: Altered synthesis of progesterone and other hormones essential for pregnancy maintenance	Martinez-Arguelles & Papadopoulos, 2015
	Epigenetic Modifications: Changes in DNA methylation affecting genes involved in pregnancy duration	Latini *et al*., 2003

Human exposure to phthalates is a significant public health concern, particularly because of their widespread presence in the environment and consumer products. Detectable concentrations of phthalate metabolites (indicative of phthalate exposure) have been found in over 95 % of the US population (Calafat *et al*., 2008). Phthalate exposure has shown temporal trends. Urinary concentrations of DEHP, DBP, DEP, and BBP metabolites have shown a significant decline (20–50 %) over time in the population of the USA, while DINP exposure has doubled (Zota *et al*., 2014). Children often have higher concentrations of phthalate metabolites compared to adults due to higher intake relative to body weight, behaviours such as hand-to-mouth activity, and greater exposure to phthalate containing toys (Becker *et al*., 2009). Phthalate metabolite concentrations have also been shown to vary depending on gender, with females generally showing higher concentrations of metabolites associated with personal care products (Petra Stuchlík Fišerová *et al*., 2022).

The extensive manufacturing and application of phthalates, combined with their ability to leach from plastic, make their environmental fate and impacts of interest. The European Chemicals Agency (ECHA) lists 12 phthalates as substances of very high concern, with toxic for reproduction and endocrine disrupting properties given as their reason for inclusion.

The US Environment Protection Agency's (EPA) phthalate action plan addresses eight phthalates for "their toxicity and the evidence of pervasive human and environmental exposure to them" (U.S. Environment Protection Agency, 2012, page 1). Included in both is DBP and DEHP. DEHP is the only phthalate currently on the EU Water Framework Directive (WFD) list of priority substances, with an annual allowable concentration of 1.3 µg L^{-1} for inland surface waters.

Phthalate toxicological impacts on aquatic organisms is thoroughly presented in Zhang *et al*. (2021), though most evidence focuses on the impacts on fish. The documented impacts include disruptions to the immune system, endocrine system, metabolic functions, and developmental processes, along with observable behavioural changes (Figure 6.1).

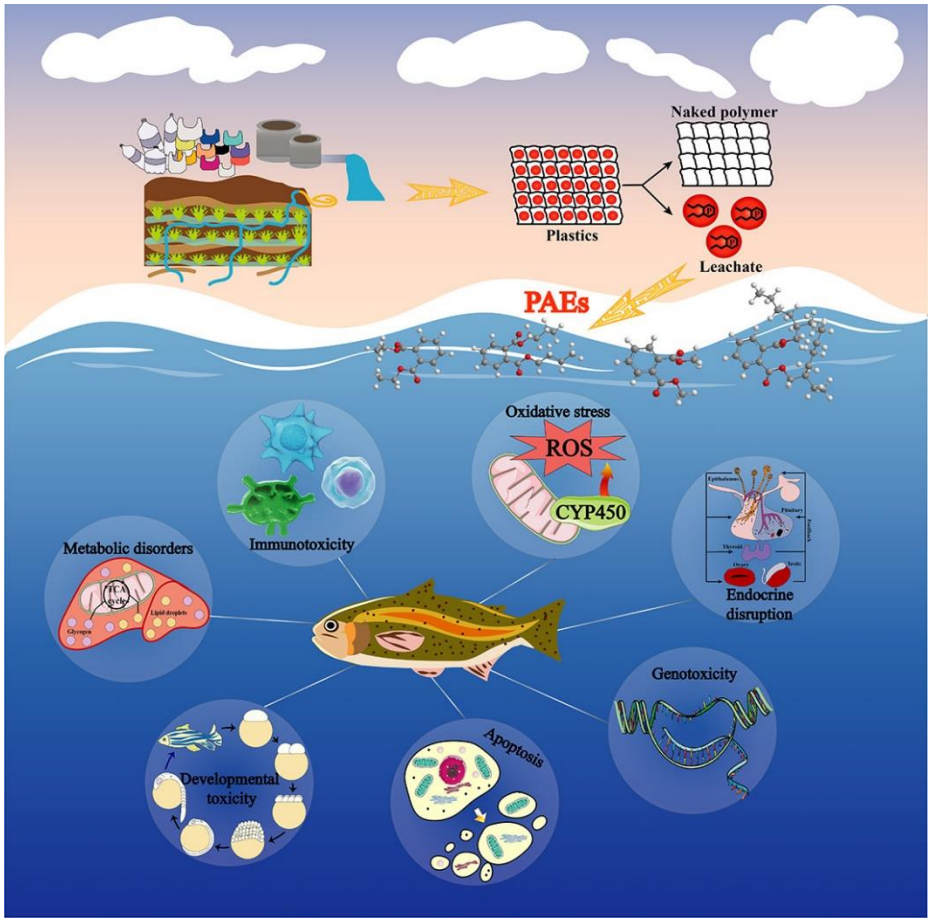


Figure 6.1 Phthalate exposure impacts on fish (Zhang *et al*., 2021).

Studies have also demonstrated that DEHP and DBP can reduce the growth rate and chlorophyll content in various algal species, such as *Chlorella pyrenoidosa* and *Chaetoceros* species (Li *et al*., 2019; M'rabet *et al*., 2019). The toxic effects of phthalates on algae are primarily attributed to oxidative stress and the disruption of cellular processes. Phthalates can induce the generation of reactive oxygen species within algal cells, leading to oxidative damage to lipids, proteins, and DNA (Shen *et al*., 2019a). This oxidative stress can impair cellular functions and ultimately inhibit growth. Phthalates like DBP and DEHP

have been also shown to cause significant adverse effects in crustaceans (Shen *et al*., 2019b).

6.1.3 Phthalates in rivers

Phthalates can enter rivers through multiple pathways, including industrial discharges, urban runoff, and wastewater from sewage treatment plants (Dargnat *et al*., 2009). Additionally, plastic waste that breaks down in the environment can release phthalates into nearby water bodies, where they may pose significant ecological risks.

Net *et al*. (2015) conducted a global survey of phthalate contamination in rivers. DEHP and DBP were the most frequently detected phthalates, with concentrations reaching up to 50 µg/L in some cases (Figure 6.2).

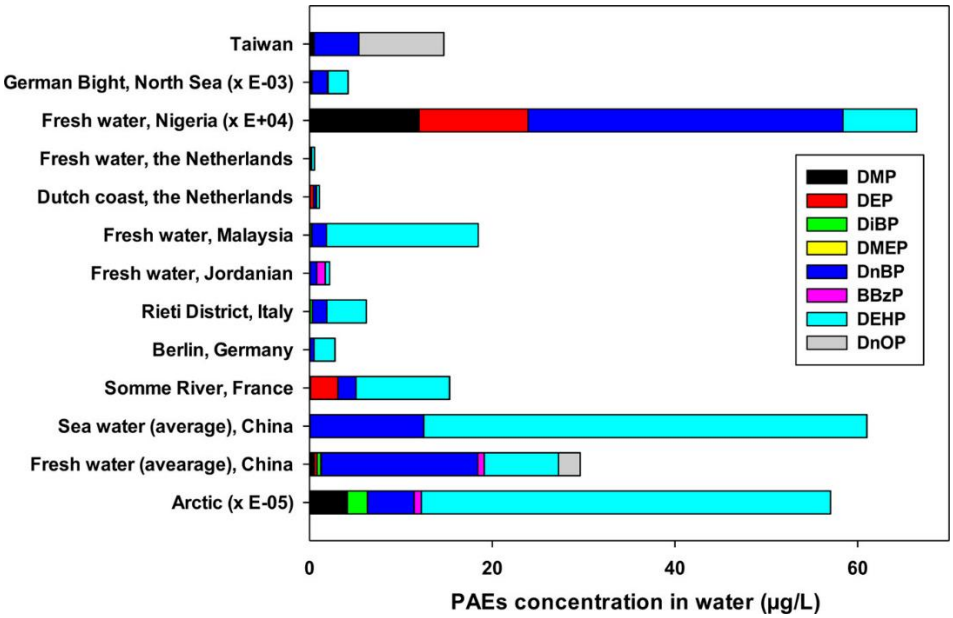


Figure 6.2 Composition of phthalates in different types of fresh and marine water (Net *et al*., 2015).

Fatoki and Vernon (1990) studied phthalate concentrations from rivers in Manchester (rivers Irwell and Etherow) and revealed significant concentrations of phthalates in them, with an average value of 6.39 (±10.72) µg L^{-1} and a range from 0.2 to 33.5 µg L^{-1}. The five phthalates recorded in the rivers were DEP (1.4 %), BMP (14.6 %), DBP (79.4 %), DEHP (1.6 %) and DIOP (3.0 %). The concentration of DBP (average, 25.38 + 10.70 µg L^{-1}) was very high in both rivers and was a major component in all samples analysed. The high concentrations found here are not unexpected because there is a lot of plastic industry in the area, including plastic producing factories located near the banks of these rivers that potentially discharge their waste waters directly into them. Few recent studies have explored the scale of phthalate contamination in UK rivers.

6.1.4 Phthalates in Wastewater treatment plant (WWTP) effluent

WWTPs can be a significant source of phthalates to rivers (Tran *et al*., 2015), with global average concentrations ranging from 0.12–30.99 µg L^{-1} in WWTP effluent (Bai *et al*. 2022). The persistence of phthalates in WWTP effluents is attributed to their widespread use in consumer products and the limitations of conventional wastewater treatment technologies in fully degrading these compounds. In the WWTP process, most phthalates are removed during primary treatment by adsorbing to solids, followed by secondary treatment (biodegradation and adsorption), while tertiary treatment is generally the least effective (Bai *et al*., 2022). Average global removal in WWTPs was 69.5 % for DMP, 70.6 % for DEP, 82.2 % for DBP, 81.6 % for BBP, 67.9 % for DEHP, and 73 % for DNOP (Bai *et al*., 2022). The lowest phthalate removal rate recorded was 14.2 % for DEHP in Nigeria, and the highest was 99.82 % for DMP in China. DBP and DEHP are frequently identified as the most commonly detected and prevalent phthalates in various environmental settings, including wastewater (Xiaoyan *et al*. 2015; Gao and Wen 2016).

6.1.5 Potential for Integrated Constructed Wetlands (ICWs) to retain phthalates

ICWs may be well suited to provide a cost-effective treatment option for the removal of phthalates from WWTP effluent. The most significant removal mechanism of phthalates in aquatic, sediment, and soil environments is biodegradation (Staples *et al*., 1997), when phthalates are used as a source of carbon and energy for anaerobic and aerobic microbes (Lertsirisopon *et al*., 2009). Phthalate biodegradation rates are higher in eutrophic waters (Rubin *et al*., 1982) and under aerobic conditions (Lertsirisopon *et al*., 2009). DBP has been shown to degrade in nutrient rich constructed wetland sediment, with a half-life of 1.4 days in surface (0–5 cm) soil and 4.0 days in subsurface (20–25 cm) soils (Zhou *et al*., 2005). Favourable conditions may be present within ICWs for the rapid biodegradation of phthalates because they receive a continuous supply of nutrient rich WWTP effluent (Cooper *et al*., 2020). Thus, assuming the hydraulic residence times is high enough, ICWs will effectively remove phthalates by biodegradation. Additionally, most phthalates have relatively high Log K_{OW} values, meaning they will readily adsorb to sediments and suspended material. ICWs contain a large amount of internally generated suspended material that high Log K_{OW} phthalates will adsorb to and therefore be retained within the ICW.

Indeed, evidence has been presented to show that constructed wetlands can successfully retain WWTP effluent derived phthalates. Diepenheim *et al*. (2020) calculated a 68 % reduction in sum of 15 phthalate concentrations across a surface flow constructed wetland (SFCW) in Oregon USA, receiving 11,356 m^3 of WWTP effluent per day (from 40,000 people). Lab scale SFCWs have also been shown to retain phthalates, with removal efficiencies of 45–83 % (Xiaoyan *et al*., 2015).

Phthalate retention in constructed wetlands is, however, complicated by the leaching of phthalates from accumulated plastic material, since constructed wetlands effectively retain microplastics in sediment. For example, Henkel *et al*. (2023) calculated that the leaching half-life of DEHP from PVC plastic was 122 years under average river conditions, and >900 years in slow flow conditions. Some studies have reported higher phthalate concentrations at the outlet of constructed wetlands than at the inlet, although they did not directly attribute this to accumulated microplastic leaching phthalates (Xu *et al*., 2019; Nas *et al*., 2022). In the present study at the Northrepps ICW, significant microplastic accumulation occurs. For example, in the first cell, concentrations of microplastic fragments were a minimum of 1938 (SD = 991) fragments kg^{-1} dry fine bed sediment. Based on ATR-FTIR verification, it can be estimated that approximately 12 % of microplastics were PVC in Northrepps sediment, which may contain a high phthalate additive content. There is a clear gap in the science: that phthalate removal by surface flow constructed wetlands containing high concentrations of microplastics has not been addressed.

6.1.6 Aims and objectives

Aim: This study aims to ascertain the extent of phthalate contamination in English Rivers and then assess whether integrated constructed wetlands can be an effective mitigation solution.

Objectives:

1. Ascertain the extent of phthalate pollution in English rivers and WWTP effluent using secondary water quality data from the Environment Agency over the period 2003–2023.
2. Quantify the spatial and temporal phthalate removal performance of the Ingol and Mun ICWs over a 6-month period (January-June 2024).
3. Investigate the partitioning of phthalate between water, plant, and sediment phases within the Northrepps ICW.
4. Compare the removal efficiency of different phthalates within ICWs and determine compliance with environmental standards for DEHP.

This study was intended to be novel by being the first to associate the microplastic content of constructed wetlands with their phthalate removal efficiency.

6.2 Methods

6.2.1 Environment Agency Data

The Environment Agency Water Quality Archive (https://environment.data.gov.uk/water-quality/view/landing) offers comprehensive data on water quality metrics collected from various sampling locations across England, dating back to 2000. These sites include coastal

and estuarine areas, rivers, lakes, ponds, canals, and groundwater sources. Samples are collected for multiple purposes, such as monitoring compliance with discharge permits, investigating pollution events, and conducting environmental assessments. The Water Quality Archive contains data on DEHP concentrations in rivers and in WWTP effluent (DEFRA, 2024). No other phthalates were monitored in this dataset. DEHP data for each year were extracted using python and combined into a single CSV file.

In the rivers dataset DEHP concentrations were recorded 9132 times from 2003 to 2023 from 553 river sampling locations (Figure 6.3). Across the entire sampling period, the number of samples taken from each location varied from one to 96 (Figure 6.4).

DEHP concentrations were also recorded 140 times from 2007 to 2014 from 27 WWTPs in England (DEFRA, 2024).

Locations of all river water quality monitoring sites in England where DEHP was recorded from 2003–2023.

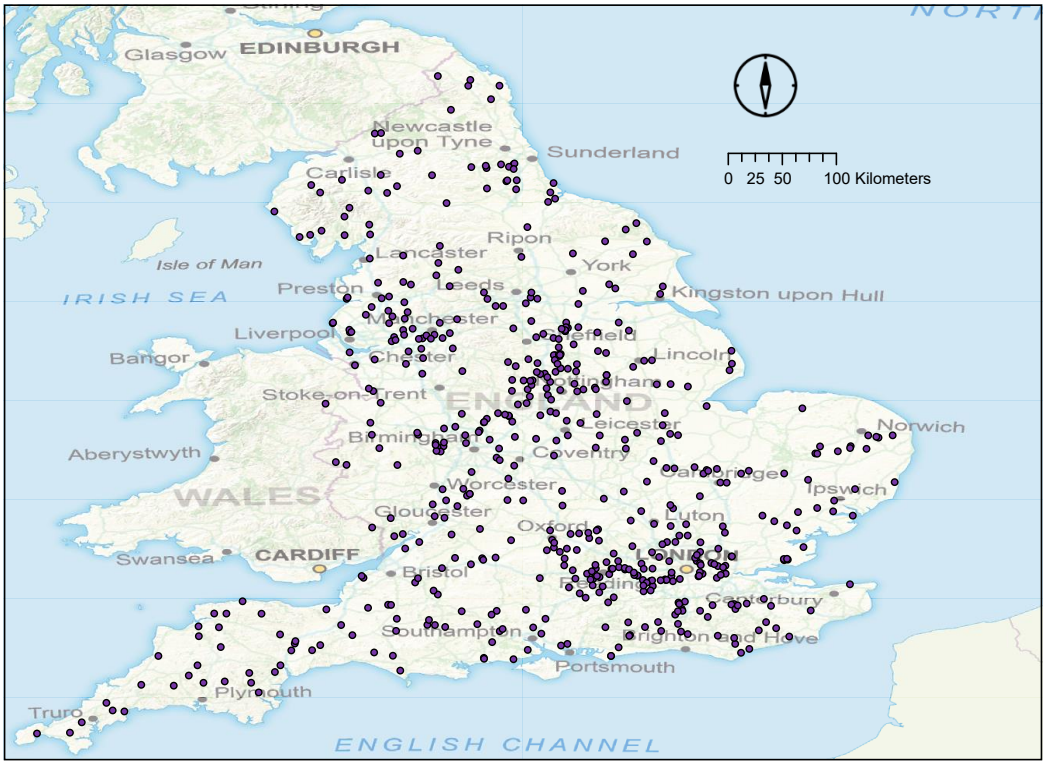


Figure 6.3 Locations of all river water quality monitoring sites in England where DEHP was recorded from 2003–2023. Basemap from Ordnance Survey.

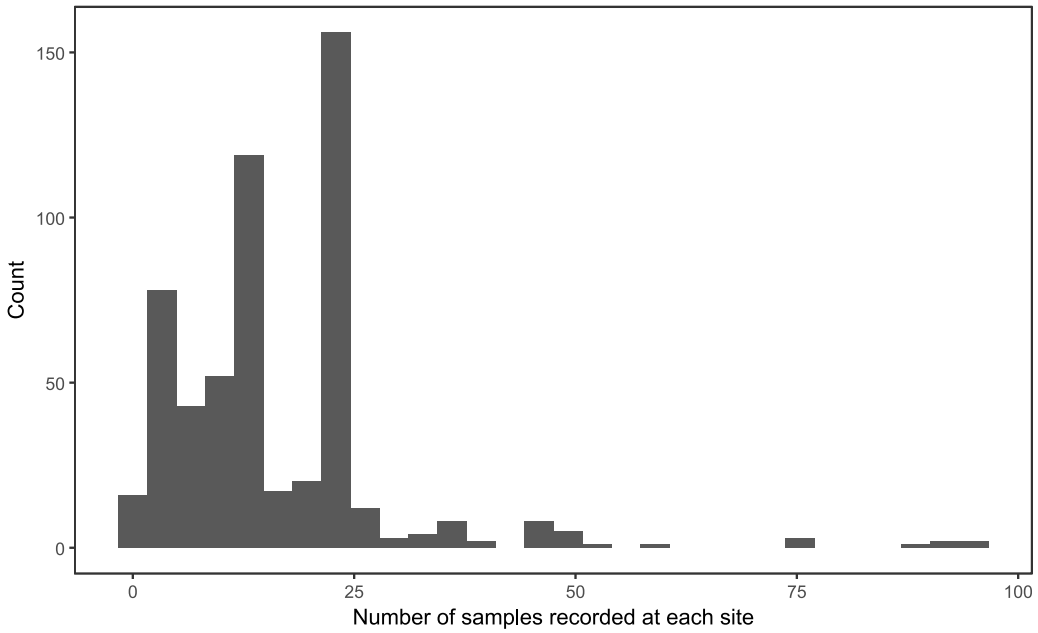


Figure 6.4 Histogram showing the number of samples where DEHP was recorded at each river water quality monitoring site from 2003–2023. Data from DEFRA (2024).

6.2.2 Field campaigns

Water samples were collected between January to June 2024, sampling at approximately monthly intervals at the Northrepps ICW. Water samples were collected from the wetland inlet (taken from an inspection point approximately halfway along the 150 m pipe supplying the wetland from the WWTP), approximately 250 m upstream of the wetland outlet, 10 m downstream of the wetland outlet, and at the end of cells 1,2 and 3. During the first sampling event, three subsamples were taken from each location to better gauge the precision of the method. Thereafter, a single sample was collected from each location, replicated in time. Water samples were taken at Ingoldisthorpe ICW in March 2024. To collect water samples, a 2 L glass bottle was lowered such that approximately the top 2 cm of water was sampled. The bottles were refrigerated within 2 hours after collection.

Plant and sediment samples were collected on 11th June 2024 from within 5 m of the inlet in the first cell at the Northrepps ICW. Plant samples were collected by cutting off the above water stems of linear leaved emergent plants. In order to distinguish plant uptake from root or rhizome adsorption, only the shoot was collected. These were then freeze dried and ground down with a pestle and mortar before storage in a freezer. Fine bed sediment (FBS) samples were collected using an isolation agitation method. A stainless-steel cylinder (300 x 900 mm) was pushed down as firmly as possible approximately 5 cm into the sediment, sometimes requiring vegetation to be carefully pulled apart. The FBS samples were agitated into suspension using a stainless-steel saucepan for 30–60 seconds. Turbid water samples were poured (with the stainless-steel saucepan) through a sieve stack of 2 mm and 38 µm

until enough sediment could be extracted from the 38 µm sieve to approximately fill a glass jar (240 mL). These samples were then freeze dried before storage in a freezer.

6.2.3 Laboratory analysis

Oasis HLB solid phase extraction (SPE) cartridges were used to clean-up the sample prior to LCMS analysis. Wolecki *et al*. (2021) tested the extraction efficiencies of three SPE cartridges: Strata C19-ec, Strata X, and Oasis HLB (Figure 6.5). The most effective of these was the Oasis HLB, achieving absolute recovery rates of 114 % for DBP and 101 % for DEHP. For more polar phthalates, such as DMP and DEP, the absolute recoveries were lower, although these were not targeted for analysis in the present study.

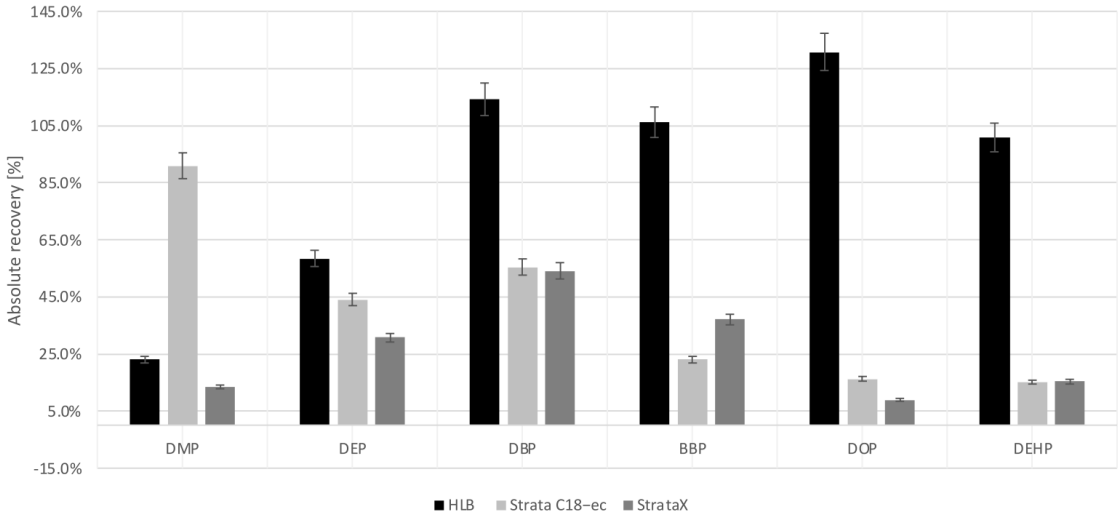


Figure 6.5 Absolute recovery of six phthalates using various SPE cartridges (Wolecki *et al*., 2021).

The cartridge was conditioned with ~25 mL of methanol (HPLC LC-MS grade), followed by ~25 mL of ultrapure water. Approximately 500 mL of water was loaded into the cartridge using a peristaltic pump (filtered through a 0.2 µm filter). The volume loaded onto each cartridge was measured and recorded. Internal standards were added to each cartridge (0.1 µg each of DEHP-d_4 and DBP-d_4 in methanol). The cartridge was then stored in a refrigerator to prevent phthalate degradation before elution. The samples were eluted with 10 mL of methanol and re-constituted in acetonitrile for analysis with the LCMS. Only the samples collected in January were eluted, the others remained in storage (in SPE cartridges).

6.2.4 LCMS analysis – UEA laboratory

The instrument used was a Synapt XS QTOF high resolution mass spectrometer (Waters, UK, Wilmslow, UK). A Mass Spectrometry elevated energy (MSe) continuum with ion

mobility data acquisition technique was used. MSe is a data-independent acquisition method where all ions (up to a mass of 600 m/z) in a sample are fragmented across the entire mass range without pre-selection of precursor ions. This generates comprehensive fragmentation data, making it possible to identify and quantify all ions present in the sample, including those of low abundance. Unlike traditional data-dependent acquisition, MSe does not require prior knowledge of the sample and can capture data for all detectable ions in a single analysis. Traveling Wave Ion Mobility Spectrometry (TWIMS) is a technique that separates ions based on their size, shape, and charge as they travel through a drift gas under the influence of an electric field.

A Waters C18 BEH column (100 mm length x 2.1 mm internal diameter) with a 1.7 µm particle size was utilized for chromatographic separation. This type of column is commonly used for its high efficiency in separating a wide range of compounds due to its small particle size and reversed-phase characteristics. The solvents used for the gradient elution were 100 % water with 0.1 % formic acid (solvent A), and 100 % methanol with 0.1 % formic acid (solvent B). Formic acid enhances the ionization of analytes in electrospray ionization. The flow rate was maintained at 0.5 mL min^{-1} throughout the run. The total duration of the gradient was 12 minutes, segmented into 4 minutes for the initial phase (98% water), 2 minutes for the transition phase, and 6 minutes for the final phase (98% methanol with 0.1% formic acid). A scan time of 0.2 seconds was employed, allowing for rapid data acquisition and sufficient resolution across the mass range. The collision energy ramp was set between 20 to 50 volts, providing the necessary energy to fragment the ions for detailed mass spectral analysis. Samples were ionised via electrospray ionisation (ESI) with a capillary voltage of 3 kV.

Mixed standards were prepared for DBP, DEP, DEHP, BBP and DMP (1 µg L^{-1}). Deuterated DEHP was added at 0.1 µg L^{-1} to each sample during preparation as an internal standard. All standards were purchased from Sigma-Aldrich, UK.

6.2.5 Quality control

Efforts were taken to reduce potential phthalate contamination throughout sample collection and processing. Sampling bottles were rinsed with acetone and dried at 100 °C for 24 hours before usage, and aluminium foil was placed under the HDPE bottle lids to prevent direct contact of water samples with plastic. A peristaltic pump with Tygon E-3603 (phthalate free) tubing was used to prevent phthalate contamination during preparation.

6.2.6 Sample analysis at UEA

While the sample processing (onto SPE cartridges) went according to plan, there were significant problems with getting useable results with the LCMS. Phthalates (protonated

molecules and sodium adducts) were found in the samples that were ran, but they were not quantifiable (Table 6.2).

The mass accuracy of the instrument kept drifting, meaning it could not reliably identify the correct molecular ions. The software, UNIFI, could also not identify the internal standards in the samples, meaning it was not able to correctly calculate the concentrations. The software generally was buggy and could not generate re-produceable results.

Table 6.2 Mass to charge ratio and retention time of phthalates identified with the LCMS.

Phthalate	**Mass to charge (m/z)**	**Retention time (minutes)**
DEHP	393.19 & 413.15	5.4–5.6
DBP	281 & 301	5.5–5.7
BBP	313 & 335	4.5–4.65
DMP	195 & 217	2.25–2.45

After six months of trying to generate useable results without success, the decision was taken to send the remaining samples (41 water samples, in the form of SPE cartridges, 5 plant samples, and 5 sediment samples) to an external laboratory (Cawood Scientific) for analysis. Twelve samples (including one procedural blank) remained in the UEA laboratory that had been previously eluted. After nine months of trying to get results from these with the LCMS, useable data were eventually generated.

6.2.7 Sample analysis – External laboratory

The SPE cartridges were eluted with 5 mL of methanol and 1mL was transferred into an autosampler vial and run by GCMS. The GCMS was calibrated using a 3-point calibration with standards at 5, 25 and 50 µg (equivalent per cartridge) the calibration was linear within this range.

Two empty SPE cartridges were spiked with 25 µL of a stock solution of DBP and DEHP and two were eluted as method blanks. The spiked sample recovery was 87% and 93% for the two samples. One of the blanks had zero detectable phthalates and one had 7.5 µg of DEHP.

For the solid (plant and sediment) samples, 0.1g was extracted in 5 mL of methanol using an ultrasonic bath for 15 minutes. The extract was filtered using a 0.2 µm syringe filter and run against the same calibration.

6.3 Results and discussion

6.3.1 Environment Agency data

In all river water samples where DEHP was recorded, 6408 (70.2 %) were <LOD (0.2 µg L^{-1}). For samples that were below the detection limit, a value of ½ LOD (0.1) has been substituted, as recommended by Antweiler and Taylor (2008).

Across all samples, the average DEHP concentration was 0.32 (SD = 0.80) µg L^{-1} (Figures 6.6–6.8). The highest concentrations generally occurred in urban areas (Figure 6.7), although because the number of samples taken from each site varied considerably, it is not possible to reliably compare sites or establish nationwide trends. There is weak evidence of a slight decline in riverine DEHP concentrations from 2003 to 2023 (Figure 6.6).

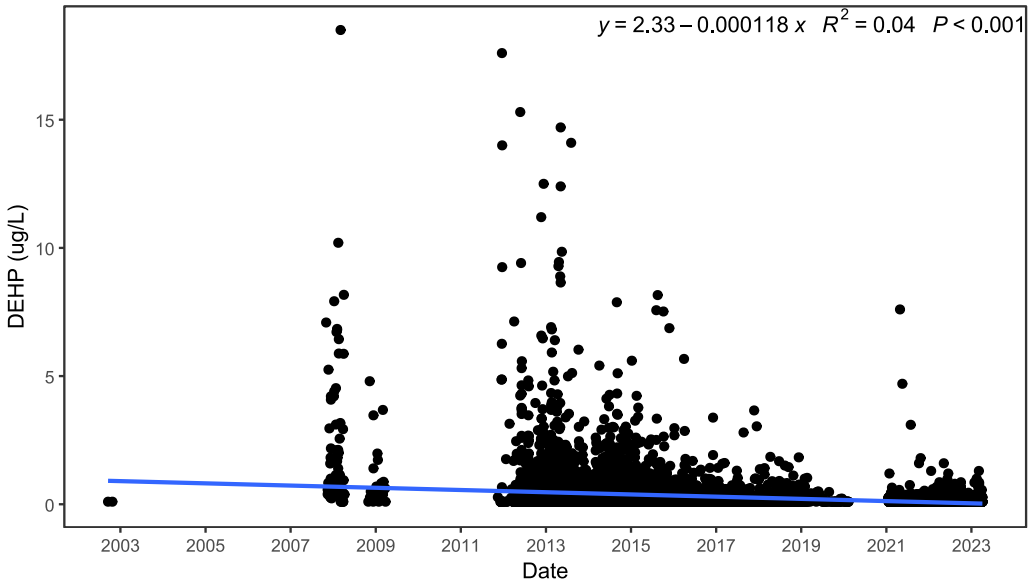


Figure 6.6 Concentrations of DEHP recorded across 553 river monitoring sites in England from 2003 to 2023.

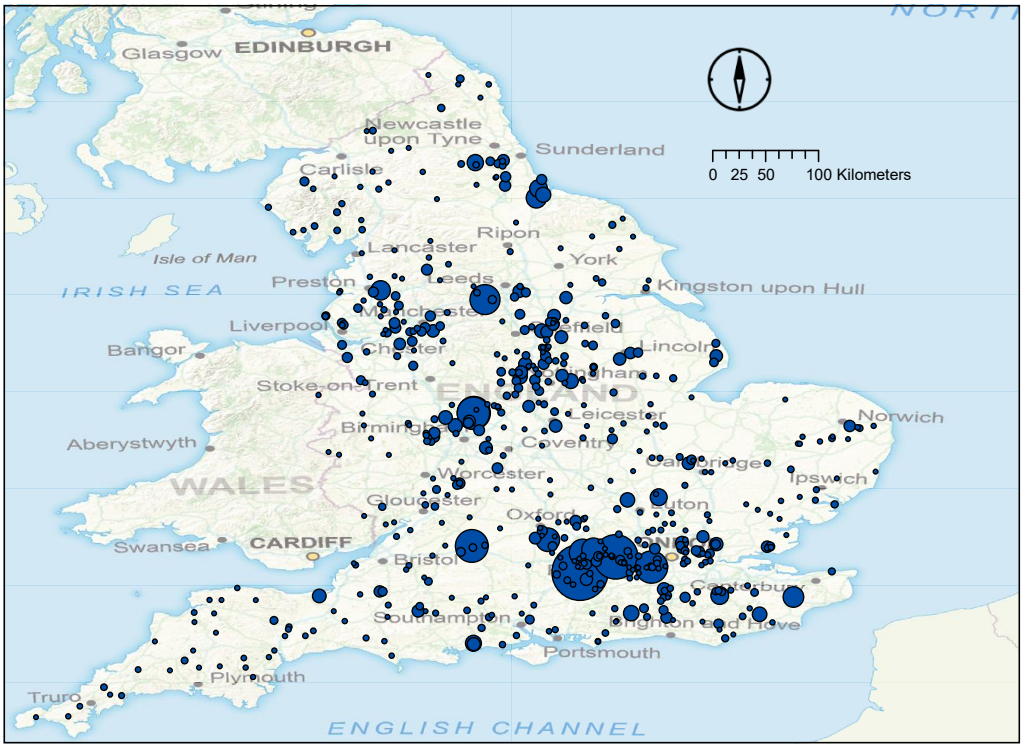


Figure 6.7 Locations of all river water quality monitoring sites in England where DEHP was recorded from 2003–2023. The size of the circles represents the proportional average DEHP concentration at each site (over the period 2003–2023). Basemap from Ordnance Survey.

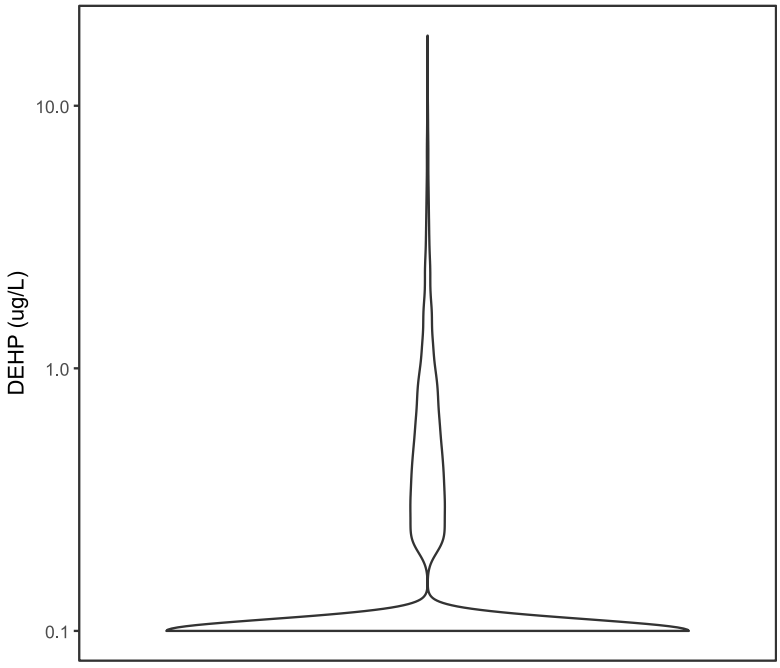


Figure 6.8 Violin plot of DEHP concentrations at all river sites from 2003–2023. The width of the plot at any given Y-value represents the density of the data points (how frequently that concentration of DEHP appears in the dataset).

There was little seasonal variation in reported riverine DEHP concentration, average concentrations were 0.35 µg L^{-1} in winter, 0.35 µg L^{-1} in spring, 0.31 µg L^{-1} in summer, and 0.30 µg L^{-1} in autumn (Figure 6.9). Excluding all samples that were below the LOD, average concentrations were 0.91 µg L^{-1} in winter, 0.99 µg L^{-1} in spring, 0.79 µg L^{-1} in summer, and 0.73 µg L^{-1} in autumn.

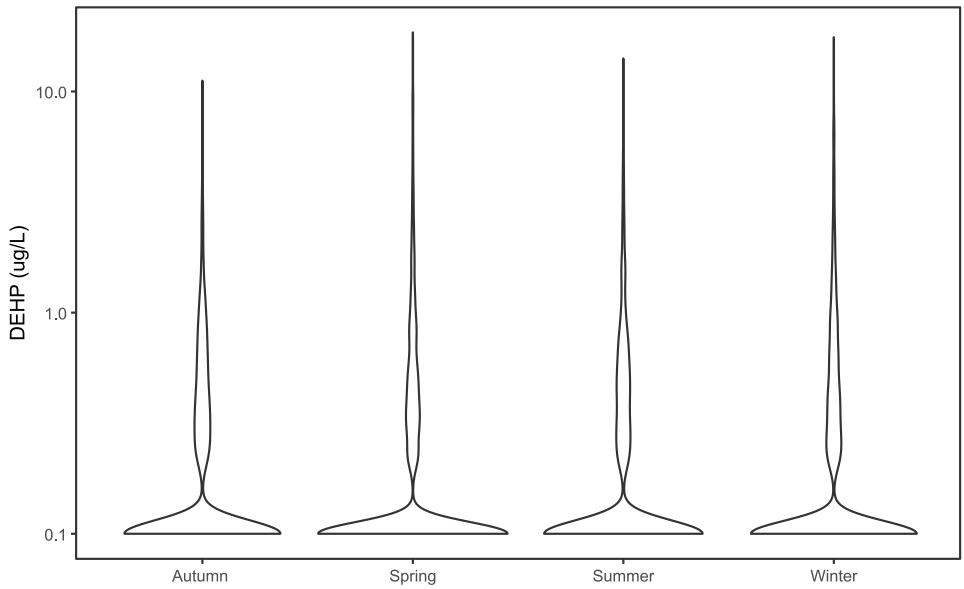


Figure 6.9 Violin plot showing average DEHP concentration of all 'river/running surface water' sites from 2003–2023, grouped by season.

The EU WFD quality standard for DEHP (1.3 µg L^{-1}) was exceeded in 369 samples (4 % of total) from 138 sampling locations (25 % of total) (Figure 6.10). WFD concentrations were exceeded most frequently in winter months (30.4 %), followed by summer (24.9 %), spring (24.4 %), and autumn (20.3 %) (Figure 6.11). Therefore, there appears to be little seasonal variation in WFD DEHP exceedance in English rivers (ANOVA, *P*=0.06).

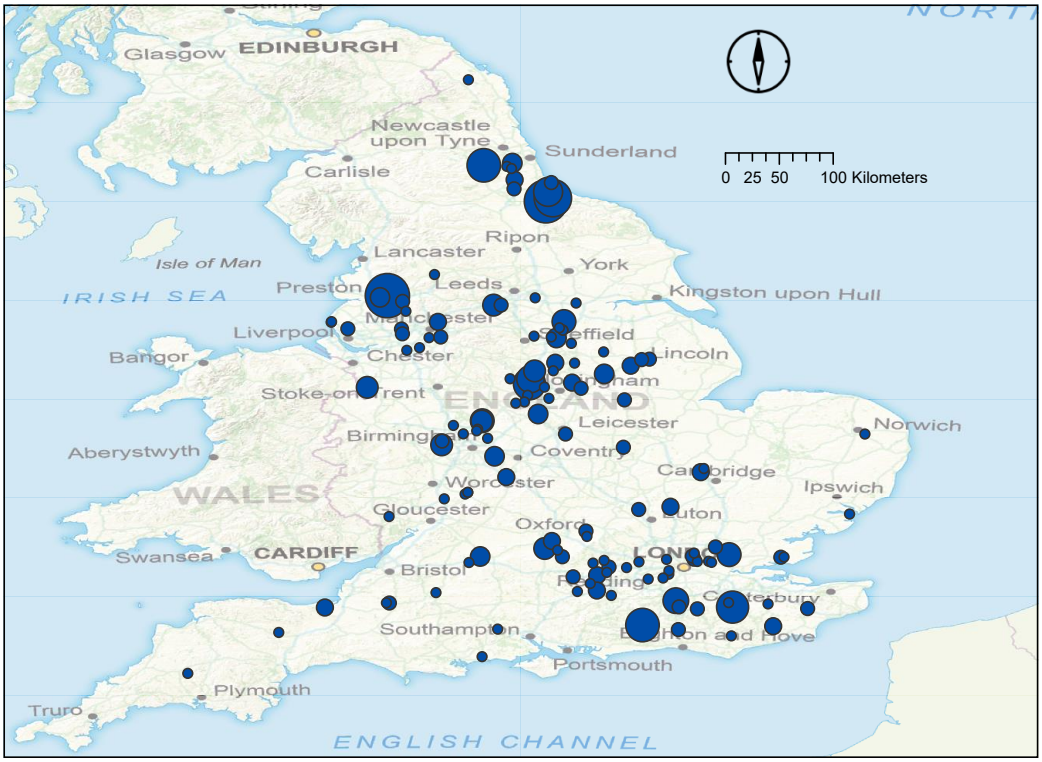


Figure 6.10 Locations where WFD standard of 1.3 µg L^{-1} DEHP was exceeded in all river sites from 2003–2023. Circles indicate proportional number of times exceedance occurred. Basemap from Ordnance Survey.

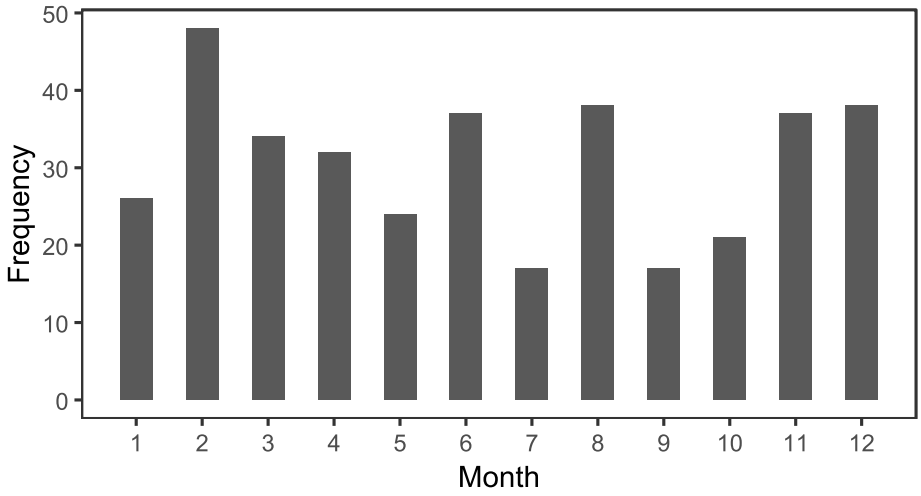


Figure 6.11 Times WFD standard of 1.3 µg L^{-1} DEHP was exceeded in each month in all river sites from 2003–2023. Data from DEFRA (2024).

In the Environment Agency Water Quality Archive dataset, DEHP concentrations were recorded 140 times from 2007 to 2014 from 27 WWTPs in England (DEFRA, 2024) (Figures 6.12 and 6.13). Of these, 22 (15.7 %) were <LOD (0.2 µg L^{-1}). Substituting a value of 0.1 for samples <LOD, the average concentration was 1.23 (SD = 1.69) µg L^{-1} across all the

sampling sites (Figure 6.14). There was no significant change in DEHP concentration over this period (Figure 6.12).

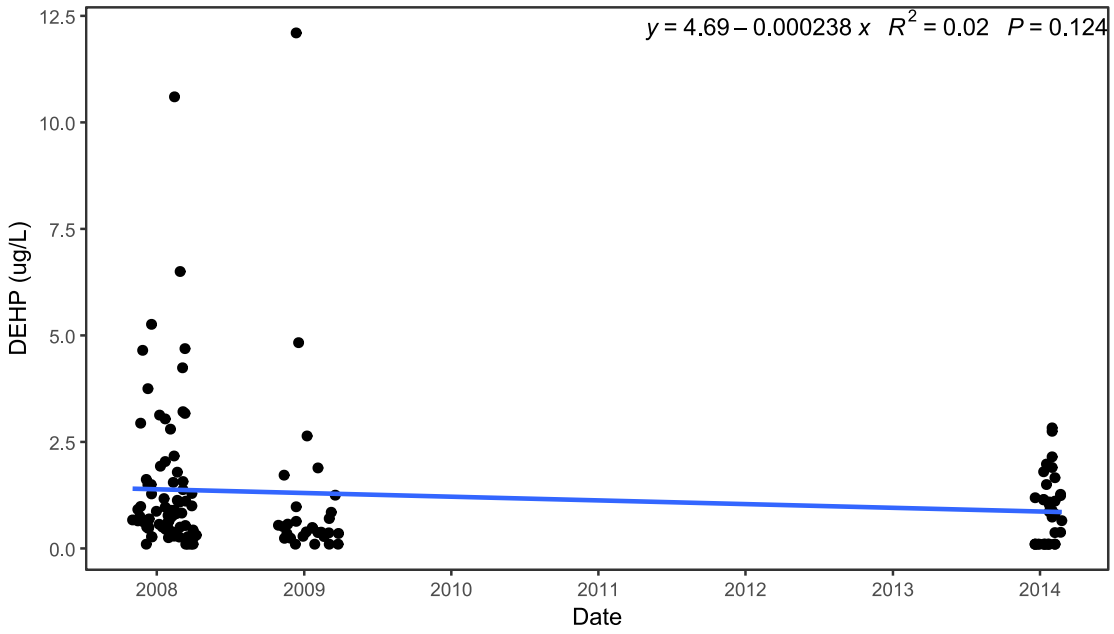


Figure 6.12 Concentrations of DEHP recorded at 27 WWTPs from 2007 to 2014.

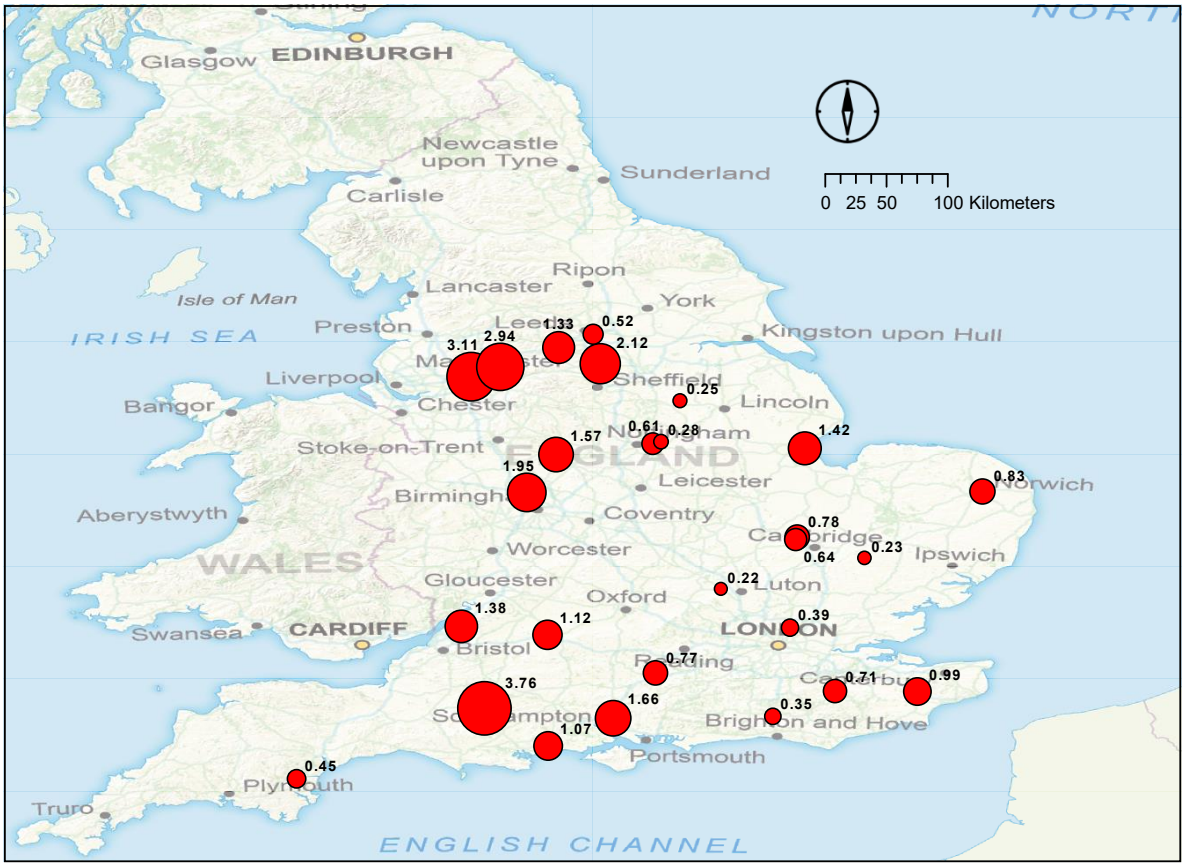


Figure 6.13 Locations of all WWTPs where DEHP was recorded from 2007–2014. The size of the circles represents the proportional average DEHP concentration at each site (over the period 2007–2014). Annotations show the average concentration of DEHP at each site. Basemap from Ordnance Survey.

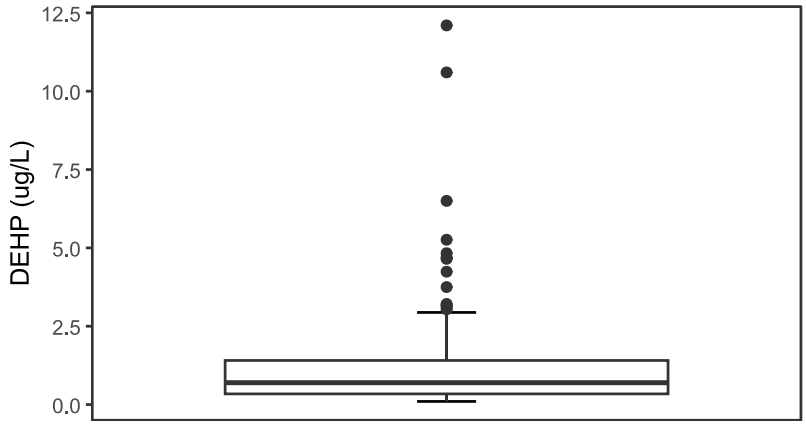


Figure 6.14 Boxplot of DEHP concentrations from 27 WWTPs from 2007–2014.

The EU WFD quality standard for DEHP (1.3 µg L^{-1}) was exceeded in 36 WWTP effluent samples (25.7 % of total), and at 16 of the 27 WWTPs (59 %) (Table 6.2). At WWTPs where WFD exceedance concentrations were not breached (although WFD standards apply to river water not WWTP effluent), the average DEHP concentration was 0.45 (SD = 0.98) µg L^{-1}. Table 6.3 shows that the total number of samples recorded was merely one for two of these WWTPs.

Table 6.3 demonstrates the variability in average DEHP concentration and WFD standard exceedance in WWTPs of various sizes. The worst performing WWTP was Gillingham, where WFD standards were exceeded in 83.3 % of samples and average DEHP concentrations were 3.76 µg L^{-1}. The Davyhulme WWTP, one of Europe's largest WWTPs, also performed poorly, with a WFD standard exceedance rate of 71.4 % and average DEHP concentrations of 3.11 µg L^{-1}.

Table 6.3 Names of each WWTP where DEHP was recorded from 2007–2014, as well as the total number of samples where data was collected, the number of times WFD standards were exceeded, and the average DEHP concentration for each WWTP over this period.

WWTP name	Approximate population (from HydroSheds (2022))	Total number of samples where DEHP was recorded	Times WFD AA-EQS exceeded	Times WFD exceeded as % of total	Average DEHP concentration (µg L^{-1})
Ashford STW	104,041	5	1	20.0	0.99
Basingstoke STW	117,000	4	1	25.0	0.77
Burgess Hill STW	51,177	5	0	0.0	0.35
Davyhulme WWTW	1,026,444	7	5	71.4	3.11
Deephams STW	989,000	5	0	0.0	0.39
Dunstable No.1 Chamber STW	50,677	6	0	0.0	0.22
Eastleigh Chickenhall STW	97,233	6	4	66.7	1.66
Gillingham STW	15,100	6	5	83.3	3.76
Goscote STW, Bloxwich, Walsall	120,806	6	4	66.7	1.95
Highpoint Prison Stradishall STW	N/A	6	0	0.0	0.23
HM Prison Channings Wood	N/A	6	1	16.7	0.45
HM Prison North Sea Camp Biodisc	N/A	6	1	16.7	1.42
Holdenhurst STW	169,595	5	2	40.0	1.07

Huddersfield STW - Colne Bridge Plant	281,479	6	1	16.7	1.33
Knostrop STW Humus Tank Final Effluent	811,683	4	0	0.0	0.52
Leyhill S/W	N/A	6	2	33.3	1.38
Longstanton Bk.Trib.Ouse D/S STW	N/A	1	0	0.0	0.78
Lundwood (Barnsley) WPC Works	84,412	4	2	50.0	2.12
Non-Tidal River Trent At Gunthorpe	N/A	1	0	0.0	0.28
Oldham STW	155,760	5	1	20.0	2.94
Rampton Hospital STP	N/A	6	0	0.0	0.25
Stoke Bardolph STW	620,000 (Water Projects, 2022)	7	0	0.0	0.61
Sudbury Prison STP	N/A	6	4	66.7	1.57
Swindon STW Outlet 'A'	209,000	6	1	16.7	1.12
Tunbridge Wells North STW	28,255	3	0	0.0	0.71
Uttons Drove STW	N/A	6	0	0.0	0.64
Whitlingham STW	265,000	6	1	16.7	0.83

6.3.2 Integrated constructed wetland water samples

Unfortunately, the results obtained from the external laboratory were too unreliable to provide meaningful insights into how well ICWs retain phthalates. The concentrations of DEHP in many samples were unexpectedly high, suggesting significant errors in the sample processing/analysis by the external laboratory (Table 6.4). The water samples that were sent off were labelled with a number from one to 41. It was found out after emailing the company that these samples were processed in the same order as the sample label numbers. Figure 6.15 shows an extremely large increase by three orders of magnitude at sample number 24, whilst Figure 6.16 also reveals an increase in reported DEHP concentration from samples 1 to 20, suggesting that there was some kind of analytical error.

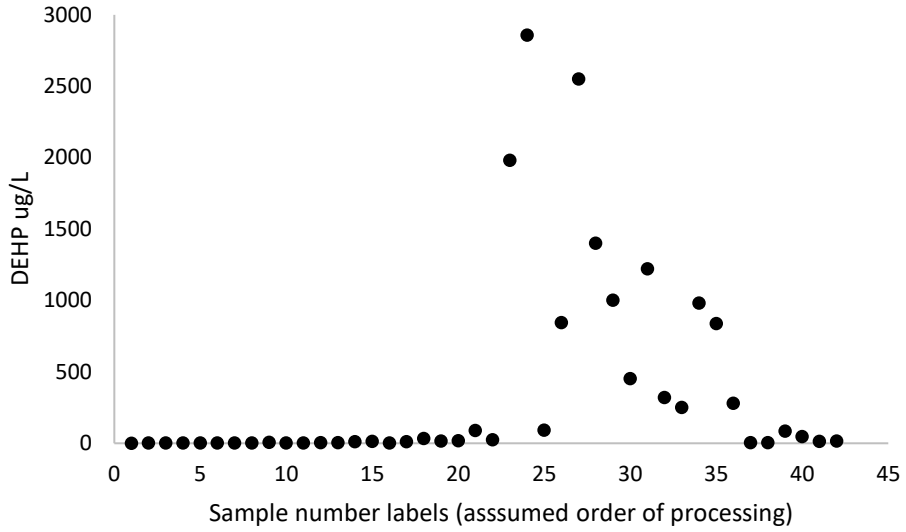


Figure 6.15 DEHP concentrations in water samples collected from the Northrepps and Ingoldisthorpe ICWs between January and June 2024.

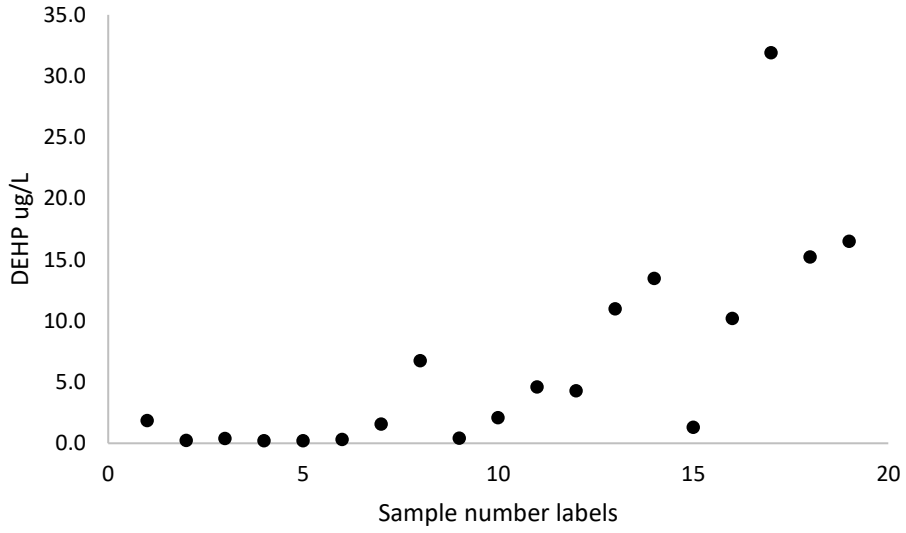


Figure 6.16 DEHP concentrations in the first 20 water samples collected from the Northrepps and Ingoldisthorpe ICWs between January and June 2024.

The results that appeared most reliable were those collected in February at the Northrepps ICW. Assuming these samples were reliable, DEHP concentrations declined by 87.5 % between the inlet and outlet, whilst there was an 18.8 % increase in DEHP concentration between the inlet and the end of cell 1, indicating that phthalates were leaching from accumulated plastic material in cell 1. This would provide additional evidence in support of the recommendation to install a smaller first cell in future ICWs, so that microplastics are retained as early as possible in a small as area as possible to ensure that leached phthalates from accumulated plastic can be removed by the proceeding cells.

Table 6.4 Concentrations of DEHP and DBP in water samples.

Sampling date	Wetland	Location	DEHP µg/L	DBP µg/L	Sample label number
01/02/2024	Northrepps	Inlet	1.6	<LOD	7
		Cell 1	1.9	<LOD	1
		Cell 2	0.2	<LOD	5
		Cell 3	0.2	<LOD	2
		Upstream	0.2	<LOD	4
		Downstream	0.4	<LOD	3
		Blank	0.3	<LOD	6
04/03/2024	Northrepps	Inlet	10.2	<LOD	16
		Cell 1	15.2	<LOD	18
		Cell 2	31.9	<LOD	17
		Cell 3	16.5	<LOD	19
		Upstream	88.2	0.2	20
		Downstream	23.9	<LOD	21
04/03/2024	Ingoldisthorpe	Inlet	0.4	<LOD	9
		Cell 1	2.1	<LOD	10
		Cell 2	4.6	<LOD	11
		Cell 3	4.3	<LOD	12
		Cell 4	11.0	0.2	13
		Upstream	6.7	<LOD	8
		Downstream	13.5	<LOD	14
		Blank	1.3	<LOD	15
02/04/2024	Northrepps	Inlet	46.9	<LOD	39
		Cell 1	12.2	<LOD	40
		Cell 2	4.0	<LOD	36
		Cell 3	14.2	<LOD	41
		Upstream	4.5	<LOD	37
		Downstream	83.7	<LOD	38
01/05/2024	Northrepps	Inlet	1979.6	0.5	22
		Cell 1	1400.0	0.5	27
		Cell 2	2549.0	0.7	26
		Cell 3	843.1	0.2	25
		Upstream	89.8	<LOD	24
		Downstream	2857.1	0.6	23
		Blank	1000	0.2	28
11/06/2024	Northrepps	Inlet	250.0	<LOD	32
		Cell 1	320.0	<LOD	31
		Cell 2	451.0	<LOD	29
		Cell 3	280.0	<LOD	35
		Upstream	1220.0	0.4	30
		Downstream	836.7	0.2	34
		Blank	980.0	0.2	33

Twelve samples were eventually successfully analysed in the UEA laboratory with LCMS: one procedural blank, three from the inlet, three from the end of cell 1, two from the end of cell 2, and three from the end of cell 3 (outlet) of the Northrepps ICW. Table 6.5 shows that there is variation in reported phthalate concentrations from the same location, particularly for DEHP. Given that the samples at each location represent subsamples (collected in the same glass bottle), a relatively low variance would be expected, assuming homogeneity in the distribution of the chemicals and uniform sample collection methods. Procedural inconsistencies should be minimal, particular as care was taken to avoid contaminating samples. It is possible that the phthalates are not uniformly distributed across the sample, which could occur due to uneven mixing, differential adsorption to surfaces and particulates, or varying degradation rates of phthalates. These samples were eluted 9 months prior to final analysis on the LCMS, meaning some degradation may have occurred, despite refrigeration.

Table 6.5 Concentrations of phthalates (µg L^{-1}) in water samples collected on 4th January 2024 at the Northrepps ICW.

Location	BBP (µg L^{-1})	DEHP (µg L^{-1})	DBP (µg L^{-1})	DEP (µg L^{-1})	DMP (µg L^{-1})
Inlet	0.63	1.96	0.13	0.02	0.05
Inlet	0.16	4.82	0.20	0.00	0.04
Inlet	0.15	2.40	0.27	0.03	0.04
Cell 1	0.52	1.62	0.15	0.02	0.04
Cell 1	0.45	1.78	0.15	0.01	0.04
Cell 1	0.14	1.25	0.22	0.04	0.00
Cell 2	0.49	2.31	0.18	0.01	0.05
Cell 2	0.09	1.33	0.05	0.02	0.02
Cell 3	0.10	4.54	0.14	0.01	0.03
Cell 3	0.08	1.39	0.05	0.02	0.05
Cell 3	0.11	0.05	1.31	0.04	0.01

A positive retention efficiency from the inlet to outlet was observed for four of the five phthalates analysed at the Northrepps ICW. Only DMP had a negative removal efficiency, with a 3.1 % increase in average concentration from the inlet to outlet (Table 6.6). Removal performance was highest for BBP and DBP, which is surprising because they are significantly more water soluble than DEHP.

It was previously hypothesized that phthalate leaching from accumulated plastic material may elevate concentrations at the end of cell 1 in the Northrepps ICW. This was observed for BBP and DEP only, which had an 18.4 % and 28.4 % (respectively) average increase in

concentration from the inlet to the end of cell 1 (Figure 6.17). DEHP, DMP, and DBP concentrations declined from the inlet to the end of the first cell.

Concentrations of BBP, DBP, and DEP declined from the end of cell 1 to the end of cell 2, and from the end of cell 2 to the end of cell 3 (Figure 6.17). DMP and DEHP concentrations both increased from the end of cell 1 to the end of cell 2, and from the end of cell 2 to the end of cell 3 (Figure 6.17). Because this data is limited and is of sub-optimum reliability (elution occurred 9-months prior to analysis), further investigation is required to make any meaningful conclusions as to phthalate removal performance across each cell at the Northrepps ICW. Despite this, there is some indication that ICWs can effectively mitigate sewage derived phthalate pollution.

Table 6.6 Average concentrations of phthalates (µg L^{-1}) in water samples collected on 4th January 2024 at the Northrepps ICW, and removal efficiency (%) for each phthalate from the wetland inlet to outlet.

Location	**BBP (µg L^{-1})**	**DEHP (µg L^{-1})**	**DBP (µg L^{-1})**	**DEP (µg L^{-1})**	**DMP (µg L^{-1})**
Inlet	0.31	3.06	0.20	0.02	0.04
Cell 1	0.37	1.55	0.17	0.02	0.03
Cell 2	0.29	1.82	0.11	0.01	0.04
Cell 3	0.10	2.41	0.08	0.01	0.04
Removal efficiency %	68.6	21.2	61.1	38.8	-3.1

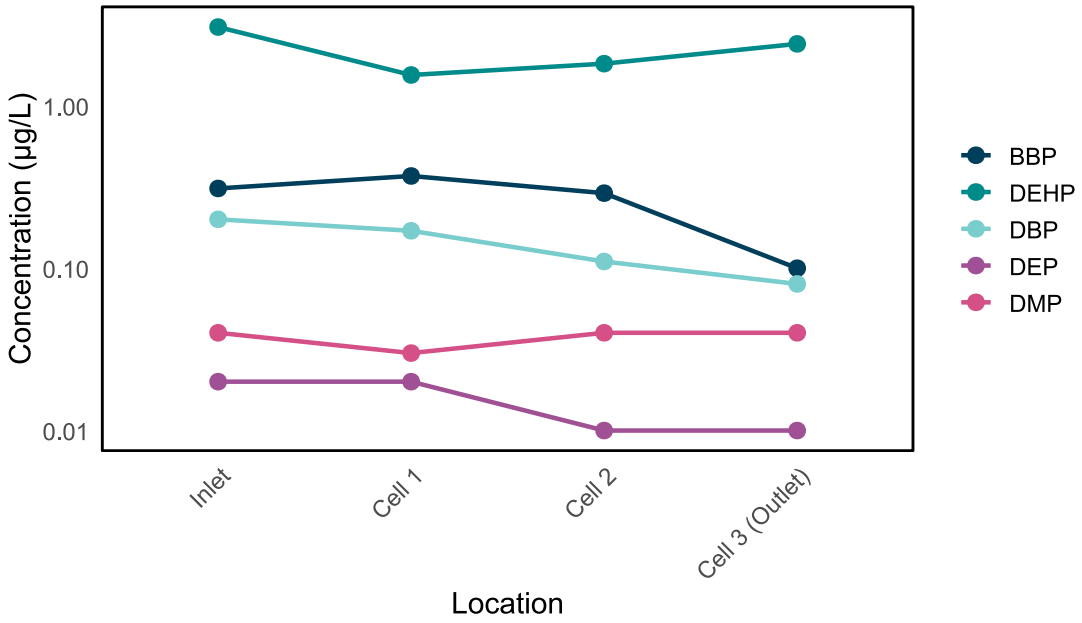


Figure 6.17 Change in concentration of phthalates across the Northrepps ICW. Samples collected on 4th January 2024.

6.3.3 Plant and sediment samples

Although the overall phthalate analysis was unreliable, the concentrations reported in sediment and plant samples were more realistic than the water sample results (Table 6.7). In fine bed sediment samples, the average concentration of DEHP was 0.51 (SD=0.13) µg g^{-1} dry weight and the average concentration of DBP was 0.02 (SD=0.04) µg g^{-1} dry weight. In another constructed wetland receiving WWTP effluent in Oregon, USA, Diepenheim *et al*. (2020) found concentrations of <LOD to 0.34 µg g^{-1} dry weight DEHP and <LOD to 1.87 µg g^{-1} dry weight DBP in sediment samples.

In plant samples from the Northrepps ICW, the average concentration of DEHP was 0.20 (SD=0.04) µg g^{-1} dry weight, while DBP was not detected in any samples. Diepenheim *et al*. (2020) found concentrations of 0.22–1.17ug g^{-1} dry weight DEHP and 0.088–2.02 µg g^{-1} dry weight DBP in *Typha* shoots. The constructed wetland studied by Diepenheim *et al*. (2020) occupies 364,00 m^2 and the WWTP supplying it serves around 40,000 people, although the level of treatment is not stated. Despite this being a much larger wetland than the Northrepps ICW, the results detected in the present study are therefore similar to expected concentrations, certainly more so than the water sample results.

DEHP was found in every sediment and plant sample, whereas DBP was only found in detectable concentrations in one sediment sample. This may be because DBP is more water soluble than DEHP. Microbial degradation is the dominant removal mechanism of DBP in constructed wetlands (Li *et al*., 2020). DEHP concentrations were higher (*t*-test, p=<0.05) in sediment than in plants, highlighting the importance of adsorption as a removal

mechanism. That being said, the presence of DEHP in plant shoots agrees with Diepenheim *et al*. (2020) that plant uptake of DEHP in constructed wetlands may be more important than previously thought, particularly because shoots are expected to contain fewer accumulated contaminants compared to the roots and rhizome (Dordio *et al*. 2011). Previous studies have suggested that microbial degradation is the dominant phthalate removal mechanism in constructed wetlands and that plant uptake is insignificant (Xiaoyan *et al*., 2015).

Table 6.7 DEHP and DBP concentrations in fine bed sediment and plant stems from within 5 m of the inlet of the Northrepps ICW.

Sample type	DEHP µg/g	DBP µg/g
Fine bed sediment	0.35	0
	0.45	0
	0.50	0
	0.70	0.1
	0.55	0
Plant (emergent stem)	0.20	0
	0.15	<LOD
	0.20	<LOD
	0.25	<LOD
	0.20	<LOD

6.4 Conclusions

1. The Environment Agency nationwide riverine monitoring data reveals that DEHP is widely detected in English rivers, with average concentrations of 0.32 (SD = 0.80) µg L^{-1}. EU WFD standards for DEHP were exceeded in 4 % of all samples and at 25 % of river sampling sites.
2. WWTPs are a clear source of DEHP to rivers, with average concentrations of 1.23 (SD = 1.69) µg L^{-1} in the effluent of 17 WWTPs across England.
3. In the Northrepps ICW, DEHP was found in fine bed sediment and plant (emergent stem) samples, indicating that ICWs may mitigate phthalate pollution from WWTP effluent.
4. Water samples show positive removal efficiency for four of five phthalates (from 21.2 to 68.6 %) at the Northrepps ICW, but further analysis is required to confirm the accuracy of this result.

Chapter 7 Conclusions

What new knowledge this research has contributed

Future research recommendations

1. ***Nile Red fluorescence dye staining methods are not capable of identifying a comprehensive range of microplastic types and colours, including anthropogenic fibres (high confidence). This method is also prone to producing false positives in organic-rich wastewater samples.***
    i. Future research should work to build a better profile of material types (including weathered particles), colours, and sizes that are identifiable with a Nile Red dye staining approach to enable better comparisons between studies that use different methods (this is achievable if it is known what each unique method can and cannot identify in environmental samples).
2. ***ICWs are effective at retaining high loads of sewage derived anthropogenic fibres (over 99 % efficiency), with no daily or seasonal variation in removal performance detected (high confidence).***
    i. Taking into consideration this result, future research should collect larger water samples at the outlet of ICWs, ideally considering an auto sampling approach because of the low concentrations expected. This may enable fibres to be quantified at concentrations above the limit of detection.
    ii. Long term monitoring at the Northrepps ICW may prove insightful to determine if there is any leakage of anthropogenic fibres from the wetland outlet. This ICW was operational in October 2014, and the present study sampled there from June 2021 to July 2024.
    iii. Future monitoring should be conducted across a wider range of ICWs of different ages, cell numbers, sizes, plant compositions, and residence times, receiving effluent from WWTPs with a wider range of prior treatment types and effluent loading rates. This would help to inform the design and maintenance of future ICWs, for example if certain configurations are found better at retaining microplastics/fibres.
3. ***ICWs are effective at retaining high loads of microplastic fragments (over 99 % efficiency), with no daily variation in removal performance (medium confidence).***
    i. It could be argued that the limitations of the analysis method used in the present study mean that a portion of microplastic fragments in the ICW outfall

may have been missed (particularly white and clear microplastic fragments), thus the removal efficiency may not be as high as suggested when considering all types of microplastics. Further investigation should be conducted to explore this, using different analytical methods that are capable of quantifying all white and clear microplastics in samples. Because of the uncertainties of the types of plastics Nile Red works for, and the high amount of organic material present in ICW derived samples, the Nile Red approach would not be recommended for this. An FTIR or Raman scanning method may be best suited to reliably identify white and clear particles in samples. There is no obvious reason why retention efficiency would be different for white and clear fragments, thus similar performance would be expected.

4. ***Microplastic fragments and anthropogenic fibres are retained quickly in vegetated ICWs, with most of these being stored close to the inlet (within 10 m) in the first cell (high confidence).***
    i. Future research should monitor the long-term spatial migration of microplastics and anthropogenic fibres through ICWs by collecting water samples at the end of each cell, to better inform management. It may be particularly interesting to explore whether there is a leakage of microplastics/fibres from the first cell at the Northrepps ICW during/after active management activities, such as vegetation clearance.
    ii. Collecting water samples at the end of each ICW cell would also allow calculations of areal removal rates per cell, which may enable better comparisons to be made between studies.
5. ***A smaller first cell may aid future ICW management by effectively retaining microplastics in a small as area as possible, simplifying sediment removal (if required in the long term) and reducing the risk of microplastics being discharged from ICWs into receiving water bodies (medium confidence).***
    i. Future research should investigate the minimum size first cell that is capable of retaining 'most' microplastic fragments and anthropogenic fibres (to enhance longer term management prospects), based on expected loading rates from WWTP effluent. A particle transport modelling approach may be used, such as Stride *et al*. (2023). It may also be possible to perform a study that traces microplastic movement in ICWs, therefore showing the dispersion of marked microplastics from a known location (e.g. the inlet). This could be done if selected microplastics that are pre stained with Nile Red are used (ensuring the plastic type and colour produces a high fluorescence). If using real microplastics is prohibited due to environmental concerns, solute tracers such as Rhodamine may be used (Cook *et al*., 2020). The historical deposition of microplastics and anthropogenic fibres into the Northrepps ICW

before the vegetation was fully developed mean that it is not possible to state that because microplastics/fibres were found e.g. 40 m from the inlet, that they are capable of penetrating 40 m into vegetation stands.

6. ***The Environment Agency nationwide riverine monitoring data reveals that WWTPs are a source of phthalates to rivers across England. Plant uptake and adsorption to fine bed sediment appear to be significant phthalate removal mechanisms in ICWs (medium confidence).***
    i. It is recommended that studies continue to investigate phthalate removal in ICWs. The Northrepps ICW is an ideal study site because it has been established that it continuously receives high loads of anthropogenic fibres and microplastic fragments from the WWTP, and that large concentrations are deposited in sediment there, including PVC particles that may contain a high phthalate content. Other ICWs that receive effluent that has undergone secondary treatment are also likely to receive high loads of microplastics and anthropogenic fibres.
    ii. It is recommended that the studies that do investigate phthalate retention by ICWs collect water samples from the wetland inlet and outlet, as well as at the end of each cell. This data from the end of each cell may provide valuable insights into whether or not deposited plastic material is leaching phthalates. For example, at the Northrepps ICW, cell 1 contains a higher concentration of microplastic in sediment than cells 2 and 3. Therefore, if phthalate concentrations are higher at the end of cell 1 than at the wetland inlet (i.e., WWTP effluent), then it can be deduced that accumulated plastics are leaching phthalates. This has significance because it may inform future design changes, for example if a smaller first cell is employed that effectively retains 'most' microplastics/fibres, leached phthalates may degrade in the proceeding cells before being discharged into potentially environmentally sensitive rivers.
    iii. The Ingoldisthorpe ICW would also be interesting to study because unlike Northrepps, this wetland does not receive significant loads of microplastics/fibres from the WWTP. The wetland therefore has little direct marketable benefit, in terms of microplastic retention (because there are few to retain), but the ICW may yet effectively retain WWTP derived phthalates.
    iv. Any future studies that monitor phthalate retention by ICWs would also ideally sample microplastics to determine the spatial distribution of them in the wetland, or at least be mindful of the WWTP treatment type (for example WWTPs with sand filters are more effective at retaining microplastics than secondary treatment only). This is because accumulated plastics are likely to leach plasticizers, which may influence the wetland removal performance.

v. A broader suite of plasticizers and endocrine disrupting hazardous chemicals should also be monitored at ICWs (e.g., PFAS, BPA, BPS, trisphosphates, nonylphenols). There is little prior research on how well constructed wetlands retain such emerging contaminants, particularly surface flow constructed wetlands.

Reflections on the original research question

The main aim of this PhD was to assess how well integrated constructed wetlands retain sewage derived microplastics, anthropogenic fibres, and phthalates. The 12-month field sampling campaign effectively showed how well two ICWs retain anthropogenic fibres, and there is no indication that collecting composite water samples at the outlet would have improved the reliability. Extensive sediment sampling also effectively showed that microplastic fragments and anthropogenic fibres are deposited close to the inlet in ICWs. In hindsight, it may have proved more valuable to reduce the amount of time spent analysing sediment samples and collect more water samples from the end of each cell to enable areal removal rate calculations. While clear and white microplastic fragments were largely omitted from analysis, I stand by the view that the results are made more reliable by completely omitting them than by attempting to identify an undefined portion of white and clear fragments that would misrepresent the microplastic composition in samples.

Micro ATR-FTIR is both time-consuming and labour-intensive; for example, acquiring a spectrum, converting it to CSV format, and analysing it with software like OpenSpeccy takes at least 15 minutes per particle. Even tripling the validation efforts would still result in a low percentage of verified particles, making it difficult to thoroughly assess the variety of plastic compositions. However, thoroughly assessing microplastic composition in WWTP effluent is a distinct research question that was not addressed in this study. Given the manual nature of ATR-FTIR, this method may not be suitable for such an inquiry unless significant resources are allocated. In the context of this study, focusing on additional sampling to further explore the retention of microplastics and fibres in ICWs is more valuable than assessing microplastic composition. Moreover, the practical utility of identifying the specific material composition of microplastics in WWTP effluent could be questioned, as the primary goal of this research is to evaluate the effectiveness of ICWs as a mitigation measure for sewage-derived microplastics and anthropogenic fibres. While the type of plastic might have ecological implications within the wetland, this area of study remains underdeveloped. It might be more beneficial to allocate resources to researching chemical concentrations, such as phthalates, where evidence of their environmental risks is better established in the literature.

An obvious omission is that the phthalate aspect of this research question was unfortunately not fully answered. However, there was not much more that could have been done

differently to enable us to answer it, taking into consideration the laboratory resources available and the misfortune of receiving unreliable results when the samples were sent to an external laboratory.

Final statement on the application of ICWs for microplastic and anthropogenic fibre removal

Integrated constructed wetlands are not a long-term solution to removing microplastics and anthropogenic fibres from wastewater and should not be seen as a replacement to conventional treatment. The accumulation of plastics in these systems complicates long term management, particularly by influencing disposal options and increasing the risk of sudden peaks of microplastic/fibre discharge into receiving rivers, if significant sediment disturbance occurs during maintenance. It is important to note that there is still a disposal issue with microplastics at conventional WWTPs because a large portion of it ends up in sewage sludge, which is commonly applied to agricultural land, leading to widespread soil contamination. The presence of plastics in ICWs also raises concerns about increased exposure of wildlife to microplastics and chemical leachates, potentially reducing the ecological benefits of ICWs that made them attractive compared to other wetland designs, although further research is needed to confirm this. It is essential that WWTP operators continue to prioritise improvements in microplastic and fibre retention at all treatment plants, including at smaller facilities, rather than relying on ICWs for this task. Despite this, ICWs are an effective polishing treatment step and can be responsible for a 99 % reduction of sewage derived microplastic and anthropogenic fibre contamination in environmentally sensitive receiving water bodies. ICWs should also be used to capture storm overflow effluent that would otherwise enter rivers untreated.

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Appendices

Appendix 1 Anthropogenic fibre and suspected microplastic fragments found in sediment samples in the first cell of the Northrepps ICW.

Distance from inlet (m)	Dry weight (g)	Total fragments	Total fibres	Fragments /kg	Fibres/ kg
2	84.6	326	630	3853	7447
6	31.3	104	431	3323	13770
10	28.4	37	158	1303	5563
14	71.7	105	716	1464	9986
34	137.2	12	58	87	423
30	34.6	13	42	376	1214
26	38.4	60	206	1563	5365
22	12.3	35	315	2846	25610
18	8.6	11	44	1279	5116
14	28.3	38	312	1343	11025
10	18.4	72	250	3913	13587
6	29.6	92	507	3108	17128
2	24.5	195	482	7959	19673
50	26.9	6	37	223	1375
38	41.8	86	267	2057	6388

Appendix 2 Sizes of anthropogenic fibres and distance from inlet in the first cell at Northrepps ICW.

Distance from inlet (m)	% Large	% Medium	% Small
2	44.5	51.9	3.6
6	41.3	43.9	14.8
10	31.0	45.6	23.4
14	76.2	22.3	1.5
34	41.4	41.4	17.2
22	47.0	42.5	10.5
18	18.2	75.0	6.8
30	23.8	21.4	54.8
26	40.3	43.7	16.0
14	53.2	33.0	13.8
10	29.2	55.2	15.6
6	64.9	29.0	6.1
2	63.7	32.0	4.4
38	65.9	31.5	2.6
50	51.4	10.8	37.8

Appendix 3 Colour of suspected microplastic fragments identified in sediment samples from the Northrepps ICW.

Colour	Count
Blue	503
Green	376
Red	164
Dark	84
Yellow	22
Pink	17
Purple	16
Light	10
Orange	8

Appendix 4 Size category (longest dimension) of suspected microplastic fragments identified in sediment samples from the Northrepps ICW.

Size category (µm)	Count
<100	420
100-200	444
200-300	198
300-400	53
400-500	29
500-600	18
600-700	13
700-800	4
800-900	5
900-1000	1
>1000	20

Appendix 5 Material composition of the 369 anthropogenic fibres analysed by ATR-FTIR.

Composition	Count
Plastic	202
PET	*182*
Nylon	*2*
HDPE	*4*
Acrylic	*7*
Polyamide	*1*
Polypropylene	*6*
Anthropogenic cellulosic	14
Unknown cellulosic	117
Anthropogenic Unknown	1
Unknown	11
Glue	24

Appendix 6 Colour of suspected microplastic fragments found in wetland sediment and analysed by ATR-FTIR.

Colour	Count
Blue	66
Dark	18
Green	17
Light	5
Orange	2
Pink	3
Purple	3
Red	18
Shiny	3
Yellow	5

Appendix 7 Size category (longest dimension) of suspected microplastic fragments found in wetland sediment and analysed by ATR-FTIR.

Size (µm)	Count
<100	29
100-200	40
200-300	17
300-400	8
400-500	12
500-600	12
600-700	5
700-800	1
1000+	16

Appendix 8 Material composition of the 140 suspected microplastic fragments found in wetland sediment and analysed by ATR-FTIR

Composition	Count
Non-plastic	8
PVC	12
Polyethylene	21
Polyethylene terephthalate	3
Polybuthylene terephthalate	2
Polypropylene	24
Polystyrene	35
Polyurethane	2
Silicon	4
Unknown	29

Appendix 9 Average hourly discharge from the Northrepps ICW from April 2022 to June 2023.

Hour	Discharge ($L\ S^{-1}$)
0	0.53
1	0.39
2	0.30
3	0.30
4	0.27
5	0.30
6	0.54
7	0.46
8	1.01
9	1.35
10	1.37
11	1.25
12	1.08
13	1.00
14	0.86
15	0.79
16	0.78
17	0.90
18	1.13
19	1.04
20	1.14
21	0.95
22	0.89
23	0.76

Appendix 10 Colour of suspected microplastic fragments found in the Northrepps WWTP effluent samples during the high frequency monitoring on 31st July 2024.

Colour	Percentage
Blue	41
Green	23
Red	28
Yellow	5
Purple	2
Pink	1

Appendix 11 Size category of suspected microplastic fragments found in the Northrepps WWTP effluent samples during the high frequency monitoring on 31st July 2024.

Size (µm)	Percentage
<100	72
100-200	18
200-300	5
300-400	1
400-500	2
500-600	1
900-1000	1