

A Review of Current Knowledge

**Microplastics in the
Freshwater Environment**

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Microplastics in the Freshwater Environment



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1 The plastic problem

As a human race, we have been enormously successful in populating this planet, adapting the environment and its resources to suit our increasingly demanding lifestyle. Plastic is one of the many results of our innovative society, having been developed and hailed as a ‘miracle product’ only a few decades ago. Although plastic has only been mass-produced since the 1950s, it has since been produced in such high quantities that it is now ubiquitous worldwide. In the year 2015, 322 million tonnes of plastic were produced and this figure continues to rise year on year (fig. 1). It is estimated that since the first production of plastic, 8300 million metric tonnes have been produced (Geyer et al., 2017). Some of the plastic produced over the last few decades is still in use for its manufactured purpose, however the vast majority is residing as waste within landfills, on land (including waterways) or within the oceans. The trouble with plastic as a pollutant is that, unlike most chemicals, once it is in the environment it can take hundreds of years to degrade. For this reason, plastic is continually accumulating within the environment, with one estimate suggesting that by 2050 the amount of plastic in the ocean may outweigh the number of fish (Neufeld et al., 2016).

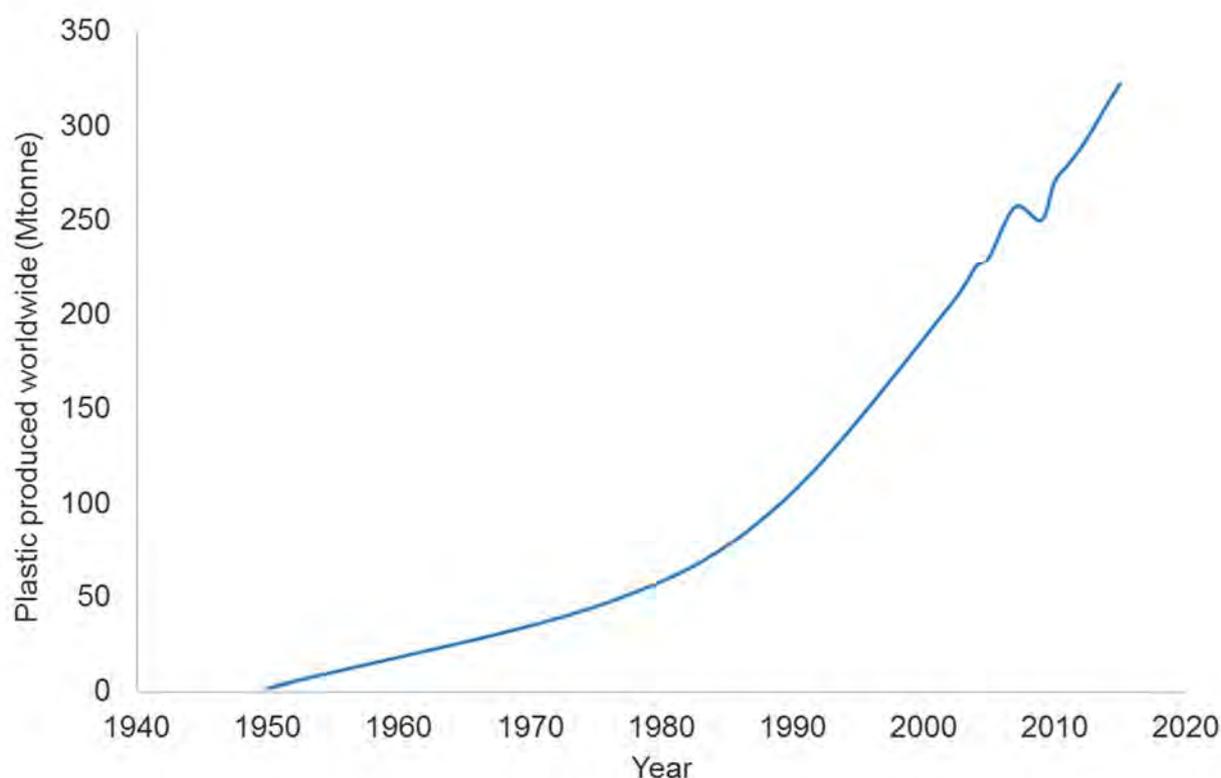


Figure 1. Plastic production data. Original data from PlasticsEurope (PlasticsEurope, 2012, 2016).

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2 What are microplastics?

Microplastics are classified as plastic particles less than 5mm in size, ranging in size down to 100 nanometres (particles smaller than this are classed as ‘nanoplastics’). The term ‘microplastics’ covers a very wide range of types, shapes and sizes of plastics. Particles can be spherical beads, fragments, fibres or films and can be made of a variety of polymers. The most commonly-used polymers, and those found often within the environment, are polyethylene, polypropylene, polyester, PVC and nylon.

Microplastics are generally classified into two categories: primary and secondary. Primary microplastics are those that are specifically manufactured to be of a small size for a specific application. This includes pre-production pellets (‘nurdles’) that are used in the plastic industry to manufacture larger plastic items and ‘microbeads’ – tiny spheres or granules added to products such as toothpastes and face scrubs for their exfoliating properties, and to cosmetics for their light-reflecting properties.

Secondary microplastics are those that form from the breakdown of larger plastic items and are therefore an unintentional consequence of the degradation of manufactured products. Plastics are complex composites of polymer compounds in addition to dyes and plasticiser chemicals. It is plasticisers that give plastics their specific properties – flexibility, durability and heat resistance. Over time, these plasticisers leach out, rendering the plastic brittle and susceptible to damage. This can lead to fragmentation, leading one plastic item to form hundreds or thousands of resulting microplastic particles. Examples of secondary microplastics are wide-ranging and include plastic fragments from litter degradation, tyre debris, microfibrils from textiles (e.g. nylon, polyester) and degradation products of road-marking paints (thermoplastic composites, fig. 2), among others.

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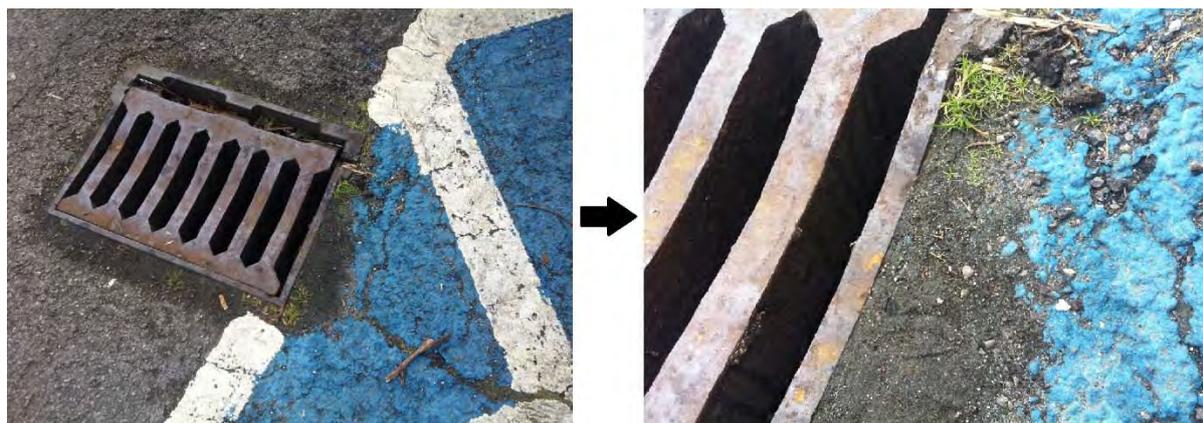


Figure 2. Painted surface next to an urban drain, giving an indication of how easily paint particles can enter freshwater systems (*Images: Alice Horton*).

3 Microplastics within the freshwater environment

3.1. Sources and transport of microplastics to freshwater systems

Despite the fact that the majority of microplastic studies have been carried out within the marine environment, most plastic products are manufactured, used and disposed of on land. It is estimated that anywhere between 4.8 and 12.7 million metric tonnes of plastic enter the oceans every year (Jambeck et al., 2015), with at least four times more than this being released onto land (Horton et al., 2017). With rivers acting as the primary conduit through which plastic will travel from the land to the sea, it is therefore likely that a large proportion of the plastic in the ocean will have passed through river systems at some point. In fact, an estimated 1.2 – 2.4 million tonnes of plastic waste enters oceans from rivers each year, with 75% of this input occurring between May and October. This correlates with monsoon season in Asian countries, where the most polluting rivers are found (fig. 3) (Miller et al., 2017). Additionally, rivers are in close proximity to many point sources of microplastic inputs, with plastic on land likely to reach freshwater systems at some point. It is probable that throughout this journey some of this plastic will be retained, either temporarily or indefinitely, within the freshwater environment.

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Figure 3. Plastic litter covering a stream and adjacent banks in Haridwar, India. Animals can be seen rummaging for food amongst the litter (*Image: Alice Horton*).

This likelihood that microplastics will be present in high numbers within freshwater systems has been recognised by researchers who are developing an understanding of sources, behaviour and retention of microplastics within rivers and lakes. Little is understood about the processes that govern the transport of microplastics throughout freshwater aquatic environments. This understanding is hindered further by the heterogeneity of particle types, sizes and shapes, all coming under the umbrella term of ‘microplastics’, that will influence their behaviour. Given the huge diversity in origins, types and characteristics of particles, it is extremely difficult to pinpoint sources and determine behaviours of microplastics once in the environment.

There are a variety of ways in which microplastics can enter the freshwater environment. The most significant input is runoff from road surfaces due to the breakdown of road-marking paints and tyre wear debris (fig. 2 and fig. 4). Another widely recognised input is that of sewage effluent: microplastics will enter the

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sewage system via domestic wastewater (e.g. in the form of microbeads from bathing products or microfibres released from textiles during laundering). Sewage treatment works (STWs) are reasonably efficient at removing these small-scale particles from the wastewater stream, anywhere between 96 – 99.9% depending on the treatment process (Ziajahromi et al., 2016). However, the small remaining percentage that is not effectively removed will be released to the environment in effluent. Due to the large volume of water and the high number of particles entering the STWs, this still represents a significant release, equivalent to anywhere between 900,000 to nearly 4000 million particles per day for a large STW (Carr et al., 2016; Ziajahromi et al., 2016). This input of microplastics to waterways via effluent shows periodic fluctuations throughout the day relating to trends in household activities e.g. showering.

Of the particles that are removed during the water treatment process, many will be retained within the sewage sludge. In Europe, sewage sludge is often applied to agricultural land as a fertiliser. Microplastic particles derived from sewage sludge, in addition to degradation products of agricultural plastics such as those used for plastic mulching (fig. 5), can enter watercourses due to direct land runoff or drainage via purpose-built ditches (Nizzetto et al., 2016). There is little information available on application rates of sewage sludge to land, therefore the magnitude of this input is extremely difficult to quantify.

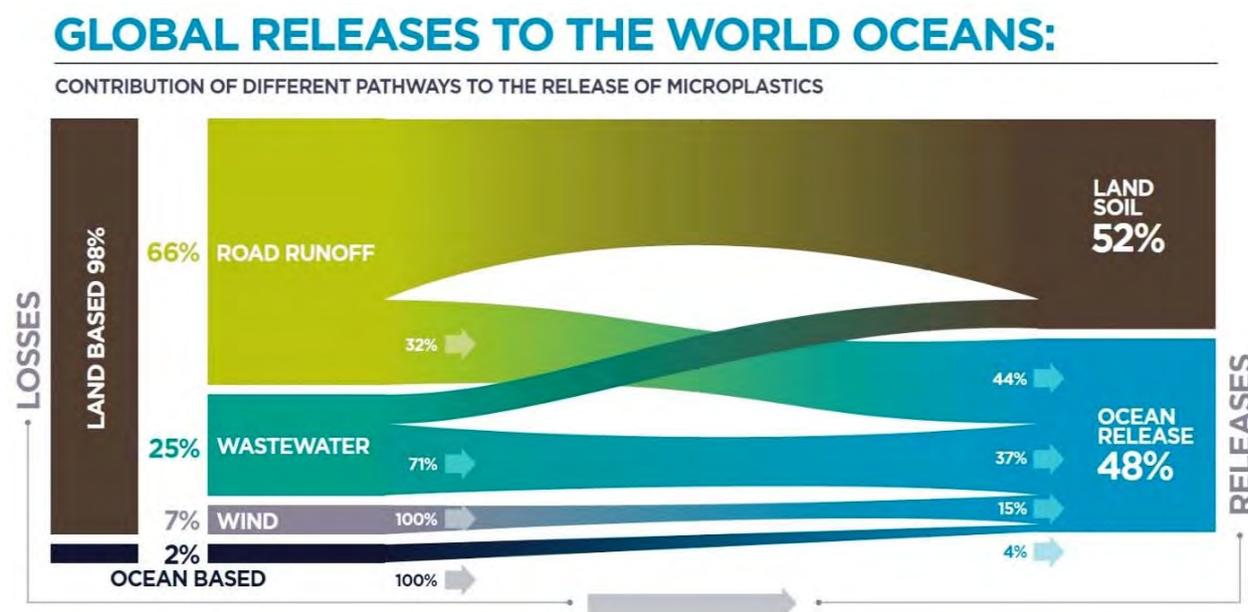


Figure 4. Sources, transport pathways and sinks of microplastic pollution within the environment (Boucher and Friot, 2017)

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Figure 5. Agricultural plastic mulching (Image: Alice Horton).

Additional routes of entry for microplastics to rivers include systems designed to cope with storm events and periods of high rainfall. These include storm drains (fig. 6) which allow runoff from roads and urban areas to go directly to rivers to prevent flooding within populated areas, often bypassing treatment systems. Combined sewage overflows (CSOs) are also a release pathway to rivers, as they are designed to discharge untreated sewage directly to rivers at times when sewage pipes are unable to handle the volume of flow (for example, where surface water inputs as a result of heavy rainfall events lead to excessive flow within the pipes). CSOs then automatically overflow to rivers, reducing the likelihood of flooding to land.

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Figure 6. Storm drain outfall near Bracknell, UK (Image: James Miller).

Litter is another significant source of (micro)plastics to the freshwater environment, either directly into the water or onto the bank or surrounding land where it will later be washed in. In areas where waste is badly managed, plastic may be released into the environment unintentionally, e.g. landfill sites that do not employ effective containment measures. This transport can be facilitated by wind, blowing plastic items long distances. Usually litter will be in the form of ‘macroplastics’ i.e. large plastic items; however, various factors lead to the breakdown of these items within the environment to form secondary microplastics. These include mechanical degradation, e.g. breakdown by traffic or footfall on land, friction on rocks underwater, shredding in vegetation due to wind, biological degradation due to organism interactions with plastics, and UV degradation leading to oxidative reactions within the polymer, causing embrittlement and chemical degradation, e.g. due to changes in pH (Göpferich, 1996; Lambert et al., 2014).

A recently identified means of transport of microplastics to the environment is that of airborne transport and subsequent deposition. This shows the potential for

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microplastics as ‘urban dust’, containing synthetic fibres and industrial particles, to be transported from their origins within homes and on streets to the wider environment via wind transport (fig. 6). It has been found that deposition of these particles is higher during rainfall events, implying that the particles become incorporated into the water droplets before hitting the ground and being washed into watercourses via runoff or drainage systems (Dris et al., 2017).

3.2. Transport of microplastics within freshwater systems

Within the oceans, microplastics will be widely dispersed by currents and wind, in addition to vertical depth transport. Although these processes are also applicable to freshwater systems, unlike the marine environment, freshwater environments are comparatively enclosed and likely to be in closer proximity to microplastic sources. This means that particles entering these environments may be dispersed less readily and can accumulate, resulting in higher concentrations. The research conducted to date suggests that microplastics are as abundant within freshwater environments as they are within the oceans, with studies in both freshwater and marine sediments finding very high concentrations of thousands of particles per kilogram of sediment (Leslie et al., 2017; Mathalon and Hill, 2014).

Environmental factors will have a significant bearing on the fate and transport of microplastics within the environment. Biological interactions can lead to settling of otherwise buoyant particles (more detail in section 4.1.), while physical structures such as dams can also intercept particles. Heavy rainfall and flood conditions can lead to turbulence within the water column and remobilisation of particles that had previously settled, while simultaneously introducing particles via runoff from land. Similarly, seasonal low flow conditions or hydrological factors such as changes in water depth or velocity can lead to the deposition of particles (Horton et al., 2017). Once deposited, it is possible that particles will be buried by organic detritus and deposited sediment, increasing the likelihood of retention. Organisms can also contribute to this burial by incorporating particles deeper into sediment due to bioturbation, i.e. the alteration of the sediment structure by organisms that burrow or move through the sediment (Näkki et al., 2017).

Although microplastics are easily transported away from the source once within the environment, concentrations within sediments tend to correlate with urbanisation and human activity. The greatest concentrations of microplastics within freshwater environments are often found in close proximity to large cities, or downstream of areas that are highly urbanised (Yonkos et al., 2014). Sewage effluent is also seen to influence the number of particles present within the water column, with frequently higher concentrations seen downstream of STWs

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compared to upstream (Estahbanati and Fahrenfeld, 2016). In studies quantifying particles released from sewage treatment facilities, it is more common to measure concentrations within the water column than the sediment. However, it is likely that a proportion of sewage-derived microplastics will be retained within sediments. As such, it is important that future studies look to quantify this settling and retention in relation to environmental and biological interactions.

3.3. Types of freshwater environment

The term ‘freshwater environment’ relates to a hugely diverse and highly variable range of conditions. The term ‘freshwater’ relates simply to the water characteristics, i.e. low concentrations of dissolved salts. This definition can therefore represent many different types of water body of different sizes, with different surrounding land uses, topographical and hydrological features. This can include huge lakes such as the Laurentian Great Lakes (USA), with a volume of around 23 million m³, down to a small pond with only a small volume. Additionally it may refer to a river with significant volume (e.g. the Amazon River with a discharge of 209,000 m³/s), a small natural stream or an artificially constructed drainage ditch. These environments will all be subject to a wide range of influencing factors. It must therefore be noted that simply because studies are identified as ‘freshwater’ they may not necessarily be comparable and waterbody characteristics must be taken into account.

4 Biological associations with plastics

4.1. Biofilm formation

Plastic within the environment can provide a substrate for organisms to live on, e.g. algae, bacteria and diatoms. This coating of a surface by organisms and associated organic material is known as ‘biofouling’. This is a natural occurrence which happens on all underwater surfaces and substrates; however, communities forming on plastics may be much more diverse due to the variation in the physical and chemical nature of plastics compared to natural substances. The degree of colonisation and species present will depend on the location and environmental conditions, in addition to polymer and particle-specific properties (Oberbeckmann et al., 2015). Microplastics can also act as an oviposition site for organisms which require hard substrates on which to attach their eggs. Due to the buoyant and easily dispersed nature of (micro)plastics, particles that become colonised may transport invasive or pathogenic organisms, including sewage-derived bacteria and harmful microalgae to regions where they wouldn’t otherwise have been found (Masó et al., 2003).

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Although the inherent characteristics of plastics may lead to vertical transport due to density, biofilms have the capacity to alter the way these plastic particles behave. Biofilms can lead to aggregation and increased density of particles, leading to sinking and possible incorporation into the sediment. This means that particles that would be expected to be buoyant may not behave as expected (Lagarde et al., 2016). This association with algae and bacteria also has another consequence: it has been observed that an organic biofilm coating can lead fish, such as the anchovy, to mistake microplastics for food as the biofilm releases olfactory signals indicative of the anchovy's normal food, leading the fish to believe this is a nutritious food source (Savoca et al., 2017). It is likely that this will be the case for a range of aquatic organisms where plastic particles are masked by an organic coating, especially in the case where biofilms cover the surface of particles such that the plastic underneath is not visible.

4.2. Biodegradation

Although it is widely believed that plastic is resistant to biodegradation (unless marketed otherwise), this is not strictly true. There are organisms capable of breaking 'non-degradable' polymers down to their constituent carbon molecules. One example is the mealworm, which can digest and degrade polystyrene, primarily due to bacteria within the gut, and is in fact able to use the resultant carbon as a source of nutrition on which to thrive (Yang et al., 2015). Similarly, the waxworm (also known as Indian mealmoth) has been shown to have the capability of degrading polyethylene in a similar manner (Yang et al., 2014). There are also bacteria and fungi capable of degrading polymers within the environment, although many rely on the polymer already being degraded to some extent due to thermo or UV degradation (oxidisation). It must be noted that for biodegradation to take place, the correct biological and environmental conditions must occur and that with the current volumes of plastic within the environment, biodegradation is unlikely to provide a large-scale solution. Even 'degradable' polymers are unlikely to provide the solution to reducing plastic waste within the environment as these often require the conditions of an industrial composter (high temperature, humidity and presence of specific microbes) to fully and effectively degrade. These are therefore unlikely to completely degrade under natural environmental conditions, especially if not within a composting system.

There is also the issue of 'biodegradable' plastics. Biodegradable plastics are often synthetic polymers that are manufactured to contain chemical bonds that can be easily degraded (esters, ethers and amides). However, polymers manufactured in this way still have a non-degradable hydrocarbon base (as with other plastic products) and the resultant pieces are non-degradable fragments, i.e. microplastics

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(Shah et al., 2008). The only plastics that are currently fully degradable under natural conditions (in the absence of the organisms mentioned above) are bioplastics, or compostable plastics, made of natural organic substances such as starch or cellulose and designed to break down naturally within the environment. However, it is understood that the plastic industry are working to rapidly advance technological progress within this area, developing and refining additives that may allow conventional synthetic polymers to fully degrade. Given the overwhelming array of options and terminology, it can be confusing for members of the public to determine whether the plastics they are using truly are ‘environmentally friendly’.

5 Ingestion and physiological harm

It is widely recognised that organisms will ingest microplastics (fig. 7), and many studies have shown a variety of harmful effects of microplastics to organisms following ingestion. Effects include blockage of the gut, leading to reduced ingestion of food items, inflammatory responses, reduced mobility, altered behaviour, reduced or delayed reproduction, increased stress, reduced immunity and, ultimately, mortality (Greven et al., 2016; Lwanga et al., 2016; Ogonowski et al., 2016; Rist et al., 2017).

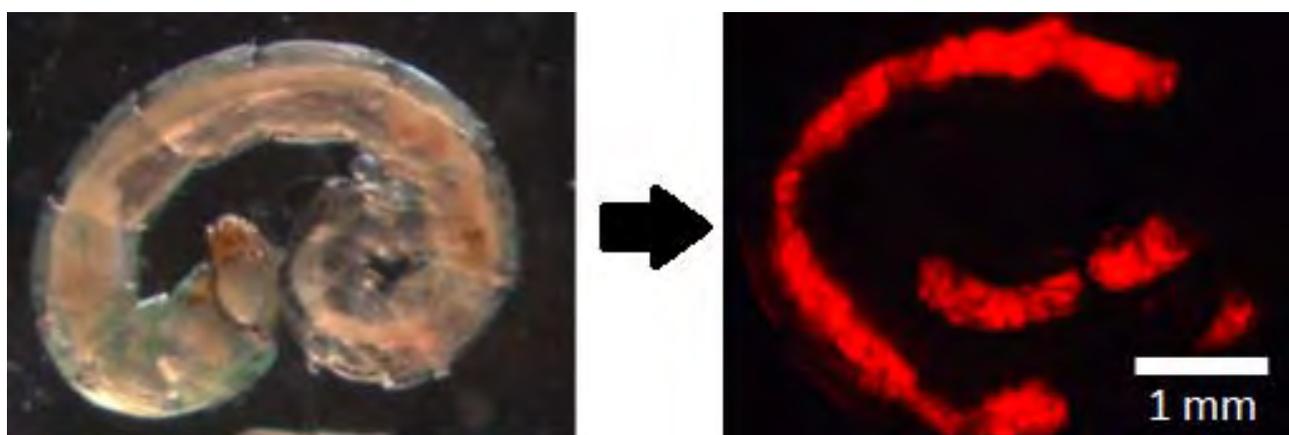


Figure 7. Chironomid (midge) larvae under normal feeding conditions (left) and having consumed fluorescent microplastics (right, image taken using fluorescence microscope) (Images: Alice Horton/Rodrigo Disner).

Due to ethical and legal constraints surrounding biological testing, the majority of toxicological effect studies to date have been conducted on invertebrates. This can give us an understanding of the effects of microplastics on organisms at the bottom of the food chain and how these effects might have knock-on implications for ecosystems. However, we have insufficient understanding of the direct effects of microplastics on higher trophic (predatory) organisms such as large fish, birds and mammals. Predators have two possible routes of uptake: direct ingestion from the

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environment or ingestion via prey that have ingested microplastics (trophic transfer). A recent study by Mattsson et al. (2017) found that nanoplastic particles were not only transferred up the food chain from algae to *Daphnia* (water fleas) to Crucian carp, but that following ingestion, the particles were transported within the fish to brain tissue, leading to behavioural changes and brain damage. Other fish studies suggest effects including stress, inflammation and altered metabolism (Greven et al., 2016; Lu et al., 2016).

To date, the majority of studies have run toxicity tests using microbeads, the main reason being that these are uniform in size and shape so can be easily characterised, plus they are widely available for purchase. However, it is understood that in order to observe realistic effects of microplastics, it will be necessary to use those that most closely resemble those found within the environment – often fibres or angular fragments, rather than spherical, homogeneously-shaped beads. Effects of microplastics appear to be particle-specific (polymer, shape and size). Size and shape can affect whether particles are egested or retained, and whereabouts retained particles are internalised within the organism tissues (e.g. whether particles will pass through membranes into the circulatory system, whether they are incorporated into tissues, whether they can cause internal lacerations due to sharp edges or whether they cause blockages within the gut). Particles that are too large to be ingested will not have the potential to cause any internal physical harm, although may cause external damage such as abrasion, entanglement or blockage of gills (Wright et al., 2013).

Microfibres are the most common type of microplastic found within environmental samples and thus there is a high likelihood that these particles will be encountered and ingested by organisms within the aquatic environment. For this reason, in addition to the more commonly studied microbeads, researchers have recently begun studying the impacts of microfibres on organisms. Studies on marine crustacea have shown that fibres appear to tangle and aggregate within the gut, leading to reduced food consumption and therefore reduced energy available for growth. Blarer and Burkhardt-Holm (2016) found reduced assimilation efficiency (conversion of ingested food into utilisable energy) in a freshwater amphipod following ingestion of microfibres. Au et al. (2015) saw reduced growth, also in a freshwater amphipod, following exposure to microfibres, presumably due to the reduced assimilation efficiency identified above. Additionally, they found microfibres to be significantly more toxic than microbeads, causing mortality at comparably much lower concentrations after 10 days.

Few studies observe microplastics directly causing mortality to organisms, rather death may occur following a combination of damaging effects as highlighted

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above. The most concerning effects are those that may not be possible to observe over the duration of acute laboratory tests; for example, reproductive or transgenerational effects. There are a number of studies that have observed effects of delayed or impaired reproduction following exposure to microplastics, including to freshwater crustacea and impaired growth of freshwater algae (Besseling et al., 2014). One study by Lee et al. (2013) saw no effects on survival of the marine copepod *Tigriopus japonicus* during an acute 96 hour test. However, during a chronic exposure they saw reproductive effects, with delayed time to reproduction and impaired development of offspring compared to controls.

Further, they found that second-generation individuals were more susceptible to the effects of microplastics than their parents had been, with detrimental effects on survival and development seen at lower concentrations, suggesting an increased sensitivity in offspring from exposed adults. This study highlights that the effects of microplastics are very complex and difficult to unravel, and that potentially critical consequences of exposure are unlikely to be observed in the majority of (acute) laboratory tests. It is also the case that effects will vary between species. Given the longevity and accumulation of plastic within the environment, the chronic low-level exposure of the current environmental scenario may have significant and long-lasting effects for organisms and ecosystems.

6 Chemical toxicity and associations

There are two ways in which microplastics may be associated with chemicals: (1) due to the addition of plasticiser chemicals during manufacture (2) by adsorption of chemicals from the environment.

6.1. Plasticisers

Plastics are synthetic composites derived from hydrocarbons. During their manufacture, many ingredients are added to give plastics their specific properties, e.g. dyes (including metals) for colour, phthalates for flexibility and longevity, bisphenol A for strength, and flame-retardant chemicals. These compounds are often not chemically bound to the polymer itself, and over time can leach out into the surrounding environment. Lithner et al. (2012) have carried out studies looking at the toxicity of different plastic leachates to the water flea *Daphnia magna*, finding that not only do different polymers have different toxicities based on variation in the plasticisers added but products made of the same polymer can also have differing toxicities depending on the chemicals and techniques used during the manufacturing process.

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PVC is known to be one of the most toxic polymers due to the high proportion of plasticisers added to give it flexibility, heavy metals added as stabilisers, and also because PVC itself is comprised of carcinogenic vinyl chloride monomers (Lithner et al., 2009). The newer and more pristine the polymer, the more highly toxic it is likely to be with respect to plasticisers, as aged polymers will have leached plasticisers over time and will therefore have a lower plasticiser concentration. However, aging also allows particles time to associate with externally-derived chemicals and thus estimations of toxicity depend on a complex balance of inherent or acquired characteristics.

6.2. Associations of microplastics with persistent organic pollutants

Persistent organic pollutants (POPs) include many chemicals used as pesticides, flame retardant chemicals and coolant fluids. Many POPs have been banned due to their endocrine-disrupting properties, among other negative effects (DDT being the best-known example). However, due to their persistence many of those that have been banned can still be detected within the environment.

It is understood that microplastics will adsorb and accumulate chemicals within the environment – the hydrophobic (water-repellent) nature of many persistent chemicals means they will preferentially bind to particulate matter rather than remaining dissolved within the water. As microplastics are also hydrophobic, these substances will bind together and form an association (fig. 8). The smaller the particle, the larger the surface area and the greater the affinity for adsorption of chemicals to the surface (Velzeboer et al., 2014). This binding is especially likely in freshwater environments where point sources of microplastics and POPs are likely to be close together and converge across a small area, compared to marine systems which are further from sources and dispersed across a wider area. An example is that of pesticides from agricultural fields which can wash into watercourses along with agricultural plastic particles, in addition to encountering plastic particles already present in the watercourse from other upstream inputs.

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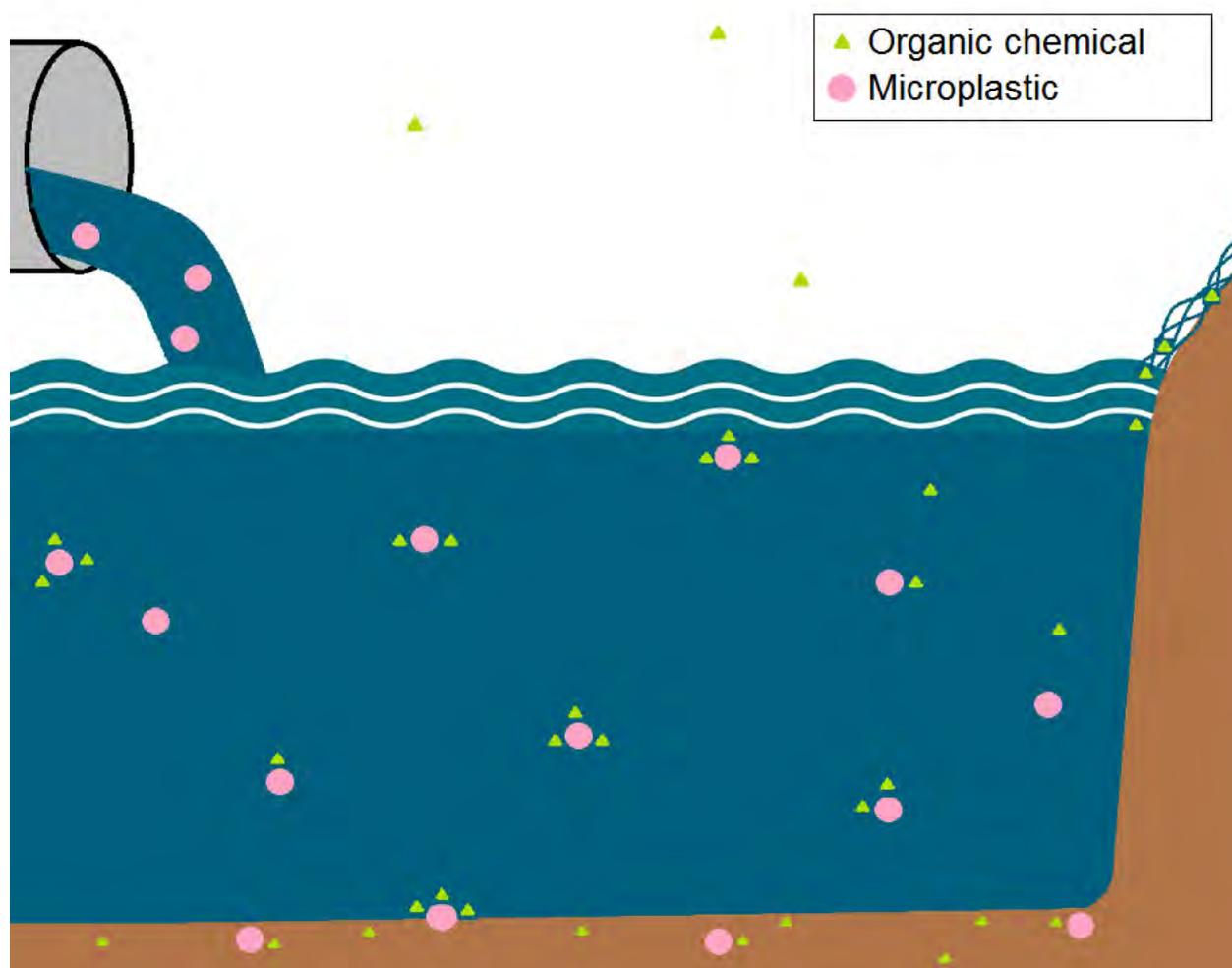


Figure 8. Simple conceptual diagram showing associations of microplastics with organic chemicals within an aquatic environment.

There is some debate over the role of microplastics as a vector for the transport and uptake of POPs. It has been suggested that this association can lead to the bioaccumulation of these chemicals by organisms that ingest contaminated microplastics, with gut conditions favouring desorption of associated chemicals from the surface of plastics after ingestion. However, recent evidence suggests that although microplastics do accumulate these chemicals, and may indeed release these once within the gut, this route of uptake into organisms is negligible compared to the uptake and accumulation that would normally occur from the environment (e.g. via sediment particles or natural organic matter (Koelmans et al., 2016)). In fact, it might even be the case that due to the high affinity for binding, microplastics reduce the availability of bound chemicals, as these chemicals will remain strongly bound to plastics and will not dissociate, even within the organism. As there is evidence for all of these scenarios across various studies, there is currently insufficient information available to determine which of these is the most likely under realistic environmental conditions. It is probable that the

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dynamics of association/dissociation leading to bioaccumulation will vary significantly dependent on constantly changing environmental conditions, polymer type, chemical type and the organism in question. More research is therefore needed to determine the extent to which microplastics may act as a vector for the uptake and bioaccumulation of a range of chemicals within the environment.

6.3. Associations of microplastics with metals

It has been shown that in addition to POPs, microplastics will also adsorb metals. Concentrations of metals on microplastics taken from coastal environmental samples have been found to be higher than those within the surrounding sediment, suggesting an accumulation on the particles (Holmes et al., 2012). Within the freshwater environment the interactions of microplastics with metals vary depending on the metal, based on reactivity, although a trend of attachment is seen in aged and beached pellets due to altered surface properties such as weathering (increasing surface area) and organic coatings giving the particles a surface charge (Turner and Holmes, 2015).

Regarding microplastics as a vector for metal accumulation within organisms, few studies have been carried out. One notable study by Khan et al. (2015) found that zebrafish larvae exposed simultaneously to microplastics and silver showed no difference in accumulation of silver compared to the treatments containing silver alone. However, when the microplastics were pre-exposed to the same concentration of silver as before and then fed to the fish, the accumulation of silver within the body was reduced. This suggests that once bound to a plastic particle, silver becomes less bioavailable to the organism even if the particle is ingested.

6.4. Microplastics as a multiple stressor

The studies detailed above highlight the potential for microplastics to alter interactions between organisms and pollutants, leading to results that can be difficult to anticipate if considering the effects of one pollutant alone. Given that microplastics can have detrimental physical effects, in addition to the toxicity of incorporated plasticiser chemicals (both of which are independent again from adsorbed chemical effects), this implies that microplastics may act as multiple stressors (Rochman, 2013; Wang et al., 2016). This may be an especially significant consideration in the face of a changing climate with associated changes in environmental conditions. For example, increased water temperatures and changes in pH may lead to reduced fitness, making organisms more susceptible to harm as a result of physical or chemical stress.

7 Complications with microplastics research

7.1. Environmental studies

A recognised problem with microplastics research is the lack of standardised methods for sampling, extraction, quantification and identification of microplastic particles from environmental media. This can lead to a lack of consistency and difficulty making comparisons of particle abundances between studies. For example, some studies give the concentration of microplastics as a number of particles per kg or L, whereas others give a mass (e.g. mg/kg). Lack of sufficient data to allow for unit conversion means that studies using these different units often cannot be accurately compared. The broad definition of microplastics (incorporating many different polymer types and particle sizes) also adds a layer of complexity to identification of particles from environmental samples, especially where composites are concerned. It is important for future studies that researchers standardise, or at least harmonise, techniques to allow for accurate comparison between studies (Rochman et al., 2017).

7.1.1. Sampling

The main issue with sampling is that there are various different types of sampling equipment that are used to collect samples. For water samples, researchers either tend to collect bottles of water which are later filtered in the laboratory or, more commonly, a net (fig. 9). Nets can effectively sample and filter many litres of water on site so are generally a preferred method of sampling. Various types of nets used include plankton nets, neuston nets, manta trawls, bongo nets and epibenthic sleds. These are all designed to sit within different depths of the water column, and mesh sizes vary from around 53 μ m up to around 3mm, therefore two different sampling regimes at the same location could produce very different results based on the equipment used. Sediment sampling is more standardised in terms of sampling as a bulk sample is normally taken, either by grab, core or simply a scoop taken by hand. However, differences are introduced in the sorting of these samples when samples are sieved to give different size fractions, with a proportion of the sample often discarded. As the lower limit of the definition of a 'microplastic' is 100nm (a size which would be impossible to manually detect within a sample without the assistance of analytical or spectroscopic equipment), no standard lower size for sample analysis has been agreed.

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Figure 9. Sampling microplastics in river water using a plankton net.
(Image: Elma Lahive).

Due to high variation in microplastic concentrations throughout the environment, even within a relatively small area, it is possible that the selection of the sampling location could inadvertently influence the results of the study (e.g. sampling from one side of a lake may give a different result to the other side of the lake). This must be taken into consideration when selecting sampling sites.

7.1.2. Microplastic particle extraction

Density separation (flotation) is the most common way of separating microplastics from sediments. The principle behind flotation is that microplastics are less dense than sediment so will float in an aquatic solution, while the sediment will not, allowing for separation of the two fractions. As many polymers are denser than water, this flotation media usually consists of a saturated salt solution. Solutions of differing densities are used within different studies, from saturated NaCl at a density of 1.2 kg/L up to NaI and ZnCl₂ at 1.8 kg/L. Again, this difference between studies will lead to a variation in the number and dominant types of microplastics

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extracted, leading two studies to potentially give very different results in samples taken from the same location due to differences in methodology. None of these flotation media are capable of floating all particles, especially in the case of the commonly used NaCl which is less dense than the common polymers, polyvinyl chloride (PVC, up to 1.58 g/cm^3) and polyethylene terephthalate (PET, up to 1.4 g/cm^3). Even the densest salt solutions used are not capable of floating the densest polymer, polytetrafluoroethylene (PTFE also known as Teflon), which has a density of 2.2 g/cm^3 . This will naturally lead to an underestimate of particles within a sample.

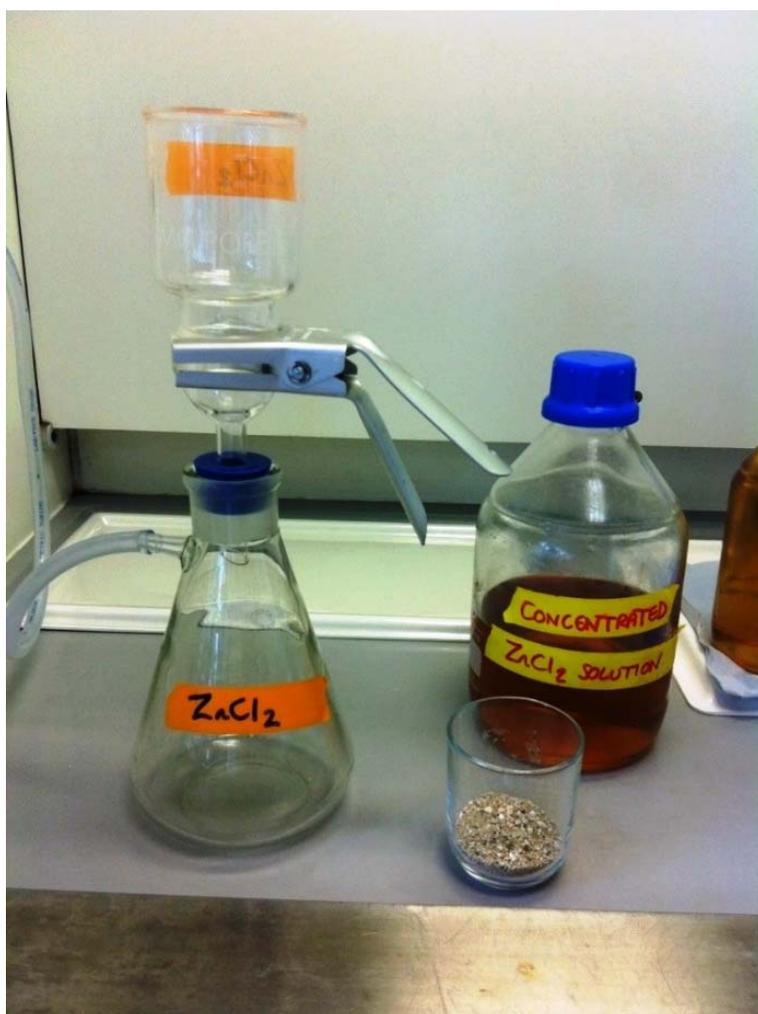


Figure 10. Laboratory equipment for ZnCl_2 flotation followed by vacuum filtration (*Image: Alice Horton*).

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The simplest methods used for separating particles from sediments include shaking or stirring with a salt solution, followed by filtration of floated matter for manual sorting (fig. 10). Some researchers have developed or adapted equipment to aid with the separation of particles from sediments. An example of custom-built equipment based on the principle of density separation for particle extraction is a flow column, which acts by pushing a high-pressure flow of water or air bubbles through sediment from underneath so that buoyant particles float out. These methods are widely applicable as they are non-technical and inexpensive to implement, although can be difficult to standardise between studies. More technical methods eliminate the need for manual sorting by using fluorescence microscopy or spectroscopy for quantification and identification of microplastics (Maes et al., 2017; Tagg et al., 2015). These methods are likely to form the basis of future microplastic research as they eliminate the issue of subjectivity when it comes to particle identification (section 7.1.2.) and hold great promise for improving the accuracy, speed and efficiency of sample analysis. However, these methods require a high level of expertise and specialised laboratory equipment and so may not be widely accessible to all researchers. Therefore current manual sorting methods are unlikely to be completely disregarded.

When considering techniques for extracting microplastics from organisms, these are similarly varied. For water and organic (e.g. plankton) samples it is common to filter the water and then apply a digestion step to remove organic material before manually sorting for microplastics. The method of digestion varies across studies and may include acids, alkalis or enzymes. Concern has been raised that some methods of acid and alkaline digestion could damage or destroy microplastic particles although these methods have been used and published (Desforges et al., 2015; Van Cauwenberghe et al., 2015). If considering large organisms such as fish and marine mussels, this may include manual dissection, sample homogenisation, whole gut digestion or dissection followed by content digestion (Avio et al., 2015; Van Cauwenberghe and Janssen, 2014). All methods hold risks for underestimation of particles, for example, due to inefficient dissection or destruction of plastic particles during digestion. In the case of some studies where simply manual sorting is deployed, it may be the case that particles are missed as they are incorporated within organic matter.

7.1.3. Quantification

One of the biggest problems with microplastics research is that the majority of studies rely on a manual sorting step for identification and extraction of microplastics from environmental matrices, identifying particles by eye. This lends itself to a high chance of bias and subjective selection, with differing

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interpretations depending on the analyst. Additionally, it is difficult to visually identify particles of a very small size (e.g. $<500\ \mu\text{m}$) and therefore manual counting could give a vast underestimate of the number of particles present, especially as it is estimated that small microplastics are far more abundant than large microplastics due to degradation of particles over time. Particles resembling microplastics are often identified as plastic using selection criteria based on particle size, shape, colour and resistance of particles to pressure. However, this naturally lends itself to error; for example, particles identified as plastic films may in fact be skin or carapace, while those identified as microfibrils may in fact be plant material such as cellulose fibres or filamentous algae (fig. 11). Studies have observed that anywhere between 20–70% of particles identified as microplastics were later determined to be natural substances following spectroscopic identification. This error rate increases with decreasing particle size. It is therefore highly recommended that manual extraction is followed by the use of spectroscopy or other analytical methods to determine the chemical composition of the particles to confirm them as polymers (Löder and Gerdt, 2015). It must be noted that particles identified as ‘natural’ substances may still be of anthropogenic origin; for example, cellulose fibres can be dyed or chemically altered to produce viscose or rayon. Although not plastic, the additives may still lead these particles to pose a hazard to aquatic organisms (Remy et al., 2015). For this reason, researchers must be careful to define ‘natural’, ‘anthropogenic’ or ‘microplastic’ particles.



Figure 11. Image of a sediment sample following ZnCl_2 flotation, giving an indication of the difficulties encountered when manually identifying and extracting particles from environmental samples. Some particles can be easy to identify by colour or shape, while others are more difficult to see due to a small size, transparent/natural colour or being obscured by organic matter (*Image: Alice Horton*).

7.1.4. Identification

There are a few different methods used by researchers to determine chemical composition of particles following extraction from environmental matrices. The most commonly used in microplastics research are Fourier transform infra-red (FT-IR) spectroscopy and Raman spectroscopy. These are commonly carried out following quantification of particles as a validation of particle analysis, in addition

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to gathering data on polymer types and abundances. These spectroscopic methods both use similar physical concepts and work by directing a light source onto the surface of the sample. The amount of vibrational energy re-emitted by the chemical bonds within the compound is then translated into a spectrum (fig. 12). This can be analysed based on the position of peaks, which relate to different chemical bonds within the compound. However, these methods do have drawbacks as dirty or aged particles (as particles from environmental samples often are) can cause interference with the spectrum acquisition and impede analysis.

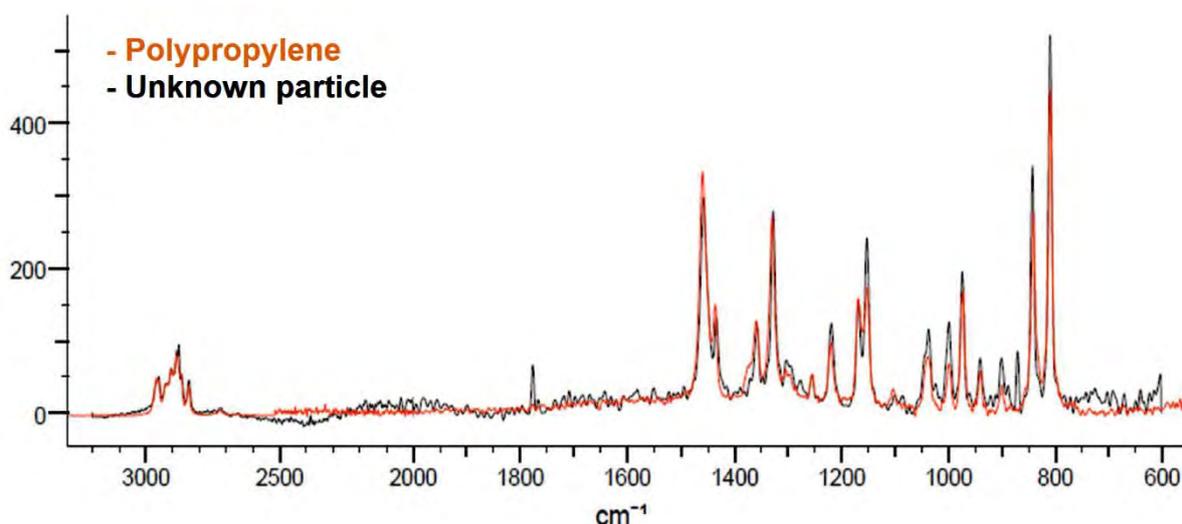


Figure 12. Example of Raman spectra comparing a particle of unknown composition to a known polymer. In this case the particle can confidently be determined to be polypropylene.

Recently, researchers have developed methods similar to those used for analytical chemistry for identifying types of polymers within samples. This can be applied either to particles that have been previously removed from samples, or to an unsorted environmental sample. These include pyrolysis gas-chromatography mass-spectrometry (Py-GCMS) (Fischer and Scholz-Bottcher, 2017) and thermogravimetric analysis (TGA) (Majewsky et al., 2016). Although it is not possible to determine number of particles, these methods can give an indication of the presence of specific polymers, and in some cases can provide data on mass and concentration.

7.1.5. Quality/contamination control

Some studies implement strict contamination avoidance measures (e.g. wearing of only cotton clothing while collecting and analysing samples, only working within fume hoods) while others do not. Some studies run parallel spiked samples or

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blanks to determine the extent of airborne contamination that could be introduced during the process of analysis, or to validate methods for removal of particles. The measures taken for contamination control/process validation are at the discretion of the researcher and are also not standardised across studies (Hanvey et al., 2017).

7.2. Laboratory toxicity testing

One of the critical aspects of microplastics research is to determine the ecological effects of microplastics. Environmental studies have shown that microplastics are ubiquitous within the aquatic environment (every sample taken across every survey worldwide has contained microplastics, including in Antarctica, the Arctic and the deep sea). However, insufficient research has been conducted to date to determine the potential short and long-term consequences of this contamination for organisms and ecosystems. Is it clear that organisms will ingest microplastics, as is evidenced by the number of wild-caught species that have been shown to contain microplastics within their guts, in addition to laboratory tests showing rapid ingestion of microplastics across a range of species and ecological guilds.

Currently, the main issue with laboratory testing currently is the (lack of) comparability of laboratory test conditions to those found within the natural environment. The freshwater environment is influenced by a very complex combination of physical, chemical and biological factors, all of which interact in very different ways depending on the conditions at any one time. This could be due to any number of elements including weather, seasonality, inputs to the system and human influences. Unfortunately, these factors are very difficult to replicate within the laboratory and with a number of variables and ever-changing conditions it would be impossible to determine which aspects were causing any changes seen within the system.

For this reason, laboratory tests are generally simple, with as few variables as is necessary to determine effects within the system. With microplastic studies it is generally the case that organisms are exposed to high concentrations of pristine microplastics over acute or chronic timescales. This approach is based on the principles of hazard assessment used in ecotoxicology, representing a 'worst case' scenario and exposing organisms to concentrations that are above those they will likely encounter under normal conditions. This gives a proof-of-concept to determine whether microplastics do, in fact, have detrimental impacts on organisms. The effects at the high concentrations can then be extrapolated to estimate likely effects of exposure within real systems. However, given the breadth of environmental data available for microplastic concentrations with water and sediments, it could be argued that it would be far more beneficial to use known

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environmental concentrations as a guidance to inform exposure concentrations for toxicity testing (Lenz et al., 2016). Barriers to introducing environmental realism are that the methods currently used for microplastics extraction may not accurately represent environmental concentrations (section 7.1.), in addition to the fact that not all microplastics within the environment will be encountered or be available to organisms (Huvet et al., 2016). Additionally, particles used in toxicity testing should attempt to more closely resemble those found within the environment (i.e. using fragments and fibres instead of pristine microbeads), as it has been shown that shape and size of particles can influence toxicity.

8 Nanoplastics

In addition to microplastics, it has recently been recognised that nanoplastics may also pose a threat within the environment. At 100 nanometres (nm) or less, these are plastics that are too small to be considered ‘micro’. It is not currently possible to detect nanoplastics within environmental samples with the methods presently available. As with microplastics, nanoplastics may form in one of two ways. Nanoplastics are manufactured for research and medical purposes, e.g. drug delivery to target sites within the (human) body. Alternatively, nanoplastics may also form within the environment from the breakdown of microplastics, in the same way as secondary microplastics can form from the degradation of larger plastic (or plastic-containing) items (Koelmans et al., 2015).

Due to their small size, it is likely that the behaviour of nanoplastics will be different to that of microplastics within the environment, both in terms of physical transport processes and also in terms of chemical and biological associations. The large surface area to volume ratio of nanoplastics gives them a greater potential for associating with chemicals and they may also accumulate a greater mass of biofilms than an equivalent mass of microplastics. Nanoparticles are understood to adsorb proteins and biomolecules secreted by organisms, forming a coating known as an ‘eco-corona’. This can change the way particles are perceived by organisms that may mistakenly identify them as organic matter, leading to interaction, ingestion and retention within the gut (Nasser and Lynch, 2016). Organic coatings can also lead nanoparticles to form aggregates which can change their behaviour within the environment, e.g. leading to sedimentation.

Toxicological effects of nanoplastics have been observed in freshwater species, including bacteria, algae, zooplankton and fish. These effects are similar to those observed with microplastic exposure and include effects on growth, metabolism, behaviour, reproduction and survival. The most concerning aspect of nanoplastics is that the small size of these particles allows them to pass through membranes, for

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such as example skin, which may allow them to be transported into body tissues or even into cells. This could lead to impairment of membrane stability and cell function (Rossi et al., 2014).

9 Microplastics and human health

So far, this report has focussed primarily on the ecological effects of microplastics within the environment. However, it is becoming recognised that microplastics (and nanoplastics) within the environment also have the potential to have health implications for humans. Studies have shown that microplastics are present within the air, implying that there is a high likelihood of inhalation (Wright and Kelly, 2017). It has been suggested that this could lead to respiratory inflammation and associated lung conditions. Additionally, microplastics have been found in a range of consumer products including honey, salt, sugar and beer. Recently, it has even been discovered that microplastics are widely present within tap water worldwide (Tyree and Morrison, 2017). This means that in addition to inhaling particles, we are highly likely to be consuming particles on a regular basis. In the case of both inhalation and ingestion, small particles have the potential to become lodged and accumulate within tissues. They may also lead to the transfer of associated organic chemicals or endocrine-disrupting plasticisers, such as Bisphenol A, to the body. The lack of clinical evidence for uptake and harm of microplastics means that we are currently unclear as to the long-term effects of human exposure to microplastics. This therefore remains a critical question to be addressed.

10 Management, policy and legislation

When it comes to the question of managing the release of microplastic waste to the environment, one of the biggest considerations is: who is responsible? Is it the plastic industry, product manufacturers, governments, the water industry, waste management companies or consumers? All play their part in determining what type, how and where plastic might enter the environment and all are being urged, in various ways, to consider how they might contribute to the reduction of plastic waste reaching the environment. This includes, for example, charity campaigns encouraging consumers to use less plastic or governments putting pressure on manufacturers and industry to consider their role and mitigate plastic inputs to the environment. However, this doesn't address the masses of plastic already present within the environment which, especially in the case of microplastics, will be impossible to remove. In reality there is not one simple solution to the problem. It is likely that, long term, the most effective method of control will be prevention, i.e. to only produce and use (micro)plastics when necessary, and to reuse or recycle any plastics that must be produced (CIWEM 2017a). As plastic has become a

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cheap and convenient material used in our everyday lives, changing methods of manufacture and public behaviours will be a challenge. With considerable time and money required to make changes to manufacture and management, where there is no clear view of who is responsible, there is little incentive for many to make a change.

As microplastics have only recently been recognised as an environmental contaminant with the potential to cause ecological harm, it is understandably taking time for governments to catch up with the possible need for policies and legislation regarding the manufacture, use and disposal of microplastics. The main complication currently is that the wide range of studies conducted to date give variable results in terms of demonstrating categorically damaging effects to organisms. This may be because some organisms tested are more tolerant than others; different sizes and types of microplastics will be differently available to organisms with different physiological effects; or effects may be seen but only over chronic timescales (whereas many experimental studies are acute). For this reason, justifying the development of any legislation has thus far been difficult. Despite this uncertainty, the UK government have noted that, given the potential for harm, precautionary measures should be taken and a ban will come into force in 2018 to cover the manufacture and use of microbeads added to ‘wash off’ personal care products such as exfoliating scrubs. Manufacture will be banned from 1st January 2018, while the sale of products containing microbeads will be banned from 31st June 2018 (Defra, 2017). Many other European and international countries are considering a similar ban (CIWEM, 2017b). While microbeads are only a small contributor to the overall problem of microplastic pollution (around 2%, fig. 13), this is a first step towards tackling the input of unnecessary microbeads into the environment; microbeads used for exfoliation purposes can be easily replaced with natural alternatives such as ground fruit stones and salt. This is also a way for the government to show their intentions towards confronting this issue going forwards (CIWEM, 2017b). Addressing the additional sources and inputs to the environment will be a much greater challenge and other pathways have not yet been addressed by legislation.

The issues of microplastics policy and management have recently been addressed in greater detail in a Policy Position Statement and separate report published by the Chartered Institution of Water and Environmental Management (CIWEM, 2017a & CIWEM, 2017b).

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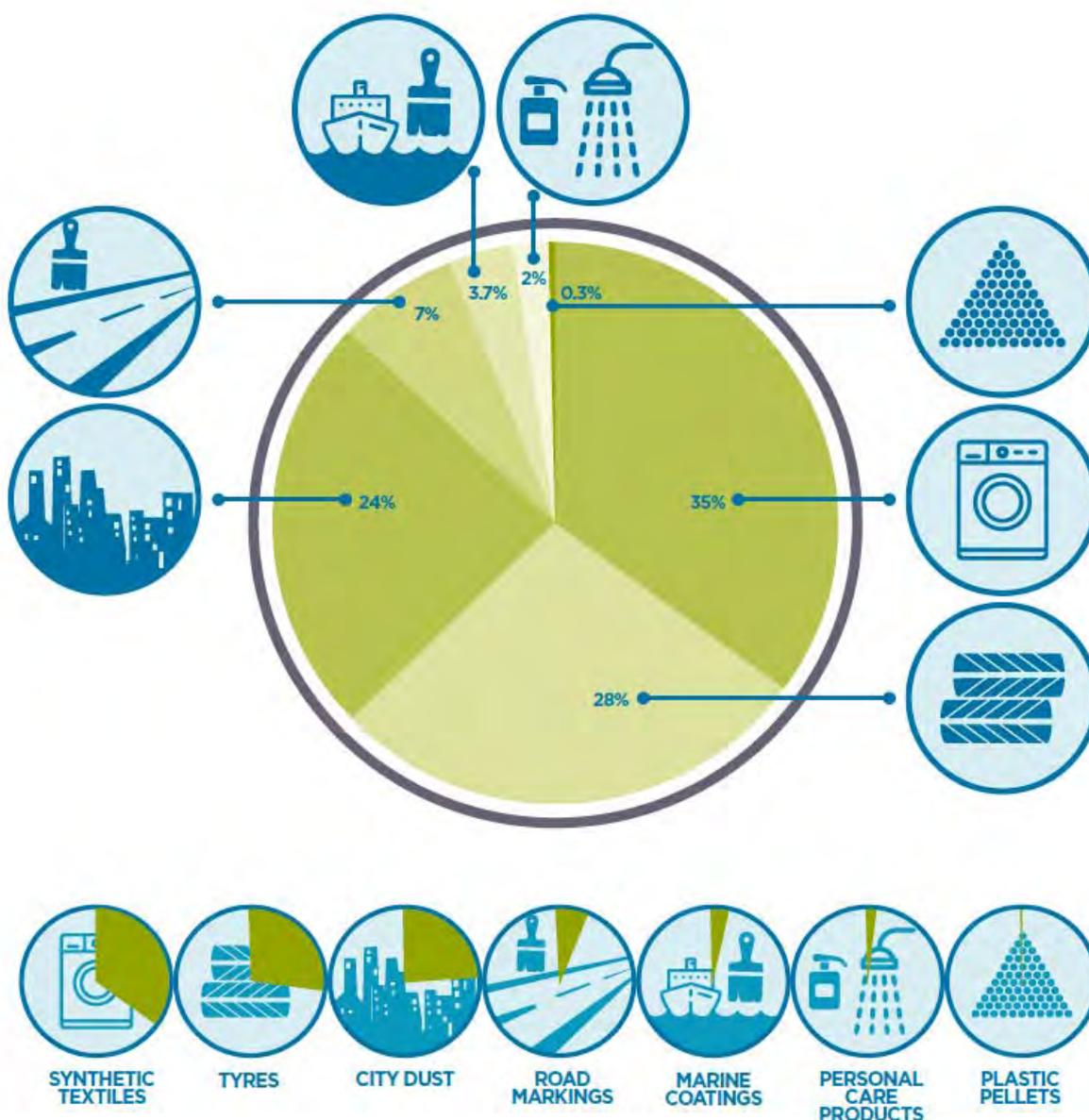


Figure 13. Sources of microplastics and proportional contributions to environmental microplastic pollution (Boucher and Friot, 2017)

11 The future (scenario and research)

Considering that plastic is such a persistent substance within the environment, in addition to the increasing rate of production, it is probable that the amount of plastic within the environment will continue to steadily accumulate. At all sizes, plastic pollution is ubiquitous throughout the environment worldwide. Although large ‘macroplastics’ can be manually removed from the environment to some degree, most of the plastic pollution is inaccessible or not possible to remove, and

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this applies especially to microplastics. It would be impossible to remove even a small proportion of microplastics from the environment due to technological limitations.

Where some of the information presented above is taken from the more extensive body of marine microplastic literature, this knowledge is widely applicable to freshwater systems, despite the comparative shortage of data. The uncertainty surrounding many aspects of microplastic pollution within freshwater systems is concerning as we rely heavily on freshwater resources for drinking water supplies, irrigation and healthy ecosystems. We do not currently understand the potential consequences of microplastic contamination within these systems, although there is plenty of evidence to show that microplastics are present and that there are a number of ecological interactions and chemical associations with plastic particles. All the freshwater studies to date have found microplastics in significant numbers within rivers and lakes, and the likelihood is that microplastic particles are as widespread throughout freshwater systems as they are in the oceans. The freshwater environment therefore needs to become a priority for research into the abundance, fate and effects of microplastics. It is also important to gain a better understanding of the factors that influence the concentrations and trends seen, including environmental and biological factors. This need for evidence also extends to terrestrial systems where many microplastics will originate, and where there is equal potential for ecological harm.

One of the most important actions for developing the research will be to determine the likely ecological effects of microplastics at the current and predicted future levels of contamination. Previous studies give us an idea of potential effects on organisms, although these are currently not widely applicable to real environmental scenarios, at the timescales that organisms will be exposed. Additionally, it is important to understand where microplastics fit under the vast umbrella of 'environmental pollutants' and how these physical and chemical pollutants might interact. This applies to both the physical and chemical properties of plastic, determining how likely and to what extent they will associate (and dissociate) with other contaminants.

There is a need for standardisation across microplastic studies to allow for comparison of data. For environmental studies this will involve developing standard protocols for sampling, extraction, quantification and identification of particles across a range of environmental media, whereas for laboratory toxicity tests this will require adhering to standard protocols for organism testing (e.g. OECD protocols) and characterisation of particles to allow effects to be attributed to specific aspects of exposure.

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In the meantime, assuming microplastics have the potential to cause long-term environmental damage, we need to focus on how, as a society, we can develop techniques and incentives for reducing and mitigating plastic inputs to the environment. This would need to work across a variety of stakeholders including regulators, manufacturers, industry, retailers and consumers.

12 Summary

Research into microplastics within the freshwater environment is rapidly advancing, but still lags behind the marine research. There are many questions which remain to be answered surrounding the sources, abundance, transport, behaviour, fate, associations with chemicals and ecological consequences of microplastics once they reach freshwater systems. This is despite the importance of freshwater resources for society, and is therefore a priority area for future research.

The evidence available indicates that microplastics are widespread throughout freshwater systems, as is the case with the marine environment. The factors influencing abundance and distribution are complex but, as would be expected, primarily correlate with urbanisation and drainage, both sewage and land-derived. As new information and evidence is constantly emerging, we have not yet managed to develop a comprehensive algorithm to predict where microplastics will travel and accumulate within freshwater environments.

It is clear from both environmental and laboratory studies that organisms will interact with, and ingest, microplastics. The effects of this will vary enormously dependent on the size, shape and type of polymer, in addition to the species exposed and the possible combined effects of additional stressors. Different species will interact and be affected by microplastics in different ways due to differing habitat preferences, feeding habits and behaviours. Studies to date suggest that the effects of microplastic ingestion will present as chronic sub-lethal effects with potentially long-term detrimental effects on organisms, leading to population-level effects. However, no studies have yet been carried out to determine the effects of realistic types and concentrations of microplastics on complex ecosystems over chronic, multigenerational timescales. These studies will be necessary to determine real, as opposed to perceived, risk.

Although a concerted effort is underway to develop our understanding of microplastics within freshwater systems, the research would benefit from a collaborative effort to ensure a harmonisation of methods. This would facilitate comparison between studies and extrapolation of data to a variety of organisms and environmental conditions. With ever-increasing quantities of plastic being

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manufactured and ending up in the environment, understanding the consequences of this will be critical to ensure the protection of ecosystems and freshwater resources.

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