Microplastics: A Novel Suite of Environmental Contaminants but Present for Decades

Christian Laforsch, Anja F. R. M. Ramsperger, Simona Mondellini, and Tamara S. Galloway

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Abstract

The ubiquitous contamination of the environment with plastic debris and the possible associated risks to ecosystems and, ultimately, human health has recently attracted a great deal of public and scientific attention. Among the plastic materials found in aquatic environments, microplastic particles have attracted particular attention since harmful effects on various organisms have been discussed, especially related to their ingestion. However, possible risks associated with microplastics cannot be generalized, as microplastics comprise a very heterogeneous group of particles that differ in their physicochemical properties. At present, there is a considerable lack of knowledge on the effects of microplastics at the molecular, cellular, tissue-specific, and organismic levels and the

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C. Laforsch (✉) · A. F. R. M. Ramsperger · S. Mondellini
Animal Ecology I and BayCEER, University of Bayreuth, Bayreuth, Germany
e-mail: christian.laforsch@uni-bayreuth.de; anja.ramsperger@uni-bayreuth.de; simona.mondellini@uni-bayreuth.de

T. S. Galloway
College of Life and Environmental Sciences: Biosciences, University of Exeter, Exeter, UK
e-mail: T.S.Galloway@exeter.ac.uk

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resulting consequences on environmental and human health. This chapter addresses the benefits of plastic products but also why plastic has turned into an environmental problem. It briefly explains how environmental contamination is assessed and shows on which biological levels potential harmful effects are expected.

Keywords

Plastic debris · Microplastics · Nanoplastics · Environmental risks · Tissue translocation

Introduction

Modern Plastics: A Success Story Turned into an Environmental Problem

Plastics have become essential components of our everyday life and have made a wealth of technical and medical innovations possible (Andrady and Neal 2009). Plastic products are light yet stable and corrosion-resistant and have excellent insulating properties, to name only a few of their advantages. Due to the versatile material properties and the low production costs compared to other materials, plastics can be found in a variety of products. For instance, plastic packaging reduces food waste by extending shelf life. Plastics play a central role in the lightweight construction of vehicles, ensuring that they consume less fuel and reduce greenhouse gas emissions such as carbon dioxide (CO_2). In freight transportation over long distances, fewer greenhouse gases are emitted with the use of plastic products compared to alternative heavier materials like glass. Plastics are also used in the insulation of houses to reduce energy consumption, and they play a crucial role in the construction sector as pipes and cables, cladding, seals, adhesives, and gaskets. Plastic is essential in medicine, for instance, to ensure the sterility of medical products, and without plastic, neither computers nor smartphones would make our lives easier. Although there are a huge variety of plastics, the majority of plastics processed are limited to only a few types: polyethylene, polypropylene, polyvinyl chloride, polyurethane, polyethylene terephthalate, and polystyrene.

Since the 1950s, plastics' global production has risen from 1.5 million tons to 359 million tons in 2018, with production rates forecast to continue to rise sharply, doubling in 20 years (Plastics – the Facts 2019). China has the largest share of world production with 25%, followed by the European Union with 20% and North America with 19%. Among the main applications for plastics (~40%) are short-lived disposable products in the packaging industry. Correspondingly, the amount of plastic waste produced has also risen rapidly over the years. The proportion of plastics going into the recycling process was estimated to be only 31% in Europe in 2016. The remainder of plastic waste continues to be dumped in landfills across Europe or sent for other forms of exploitation, such as incineration (Plastics – the

Facts 2017). However, it can be assumed that in developing countries in particular, which often lack a proper collection system, the proportion of recycled plastic is far lower than in Europe.

Unfortunately, a significant proportion of plastic waste is released into the environment through careless and improper disposal (Browne et al. 2011; Dubaish and Liebezeit 2013). Especially this improper disposal of plastic waste inevitably leads to a long-term environmental problem. That is why plastic has changed from being a cheap problem-solver to an environmental problem itself.

The World Economic Forum has calculated, for example, that every year, approximately 32% of plastic packaging material alone is improperly disposed of in the environment (World Economic Forum 2016). The main problem of plastics in the environment arises from the high resistance and durability of the material. Due to the slow degradation dynamics, it is assumed that many plastics are persistent in the environment for hundreds of years, depending on the polymer type (Barnes et al. 2009). Therefore, it can be assumed that most of the plastic that has been released into the environment since the beginning of mass production is still to be found there and represents far more than just an aesthetic problem.

Once released into the environment, plastic disintegrates over time into ever-smaller particles due to weathering processes. Fragmentation occurs due to various environmental influences such as solar radiation and chemical and biological degradation. As a result, the material becomes cracked and brittle and continues to break up due to mechanical effects such as wave movements. The resulting particles are referred to as microplastics (Fig. 1).

Fig. 1 Microplastics: Different kinds of microplastics found on the shoreline of Lanzarote. (© Christian Laforsch, University of Bayreuth)

Microplastics: A Young Field of Research

The term “microplastics” refers to fragments, fibers, films, foams, and spherical particles of plastics smaller than 5 mm. Accordingly, this definition covers a wide range of materials that, due to their specific chemical and physical properties, are likely to exhibit different behaviors and effects in the environment. Till now, there is no consensus on the definition of the actual size range of microplastic particles. The upper size limit of 5 mm is widely accepted, whereas the lower size limit is still under debate. The suggestions of the lower size limit range from 100 μm down to 100 nm, depending on the scientific field. For instance, for analytical studies of microplastics in the environment, the definition of the lower size limit often refers to the technological detection limitations, whereas for toxicological studies, lower size limits are proposed, as these may promote its bioavailability under laboratory conditions (Wright et al. 2013b; Frias and Nash 2019). Furthermore, there is no consensus on the definition of nanoplastics because it depends on the lower size limit of microplastics. However, an overall accepted distinction is made between “primary microplastics” and “secondary microplastics.” “Primary microplastic” is manufactured industrially as a component of, for example, cosmetics, cleaning products, or abrasives and is discharged into the environment mainly via the wastewater (sewage treatment plants, rainwater, and combined sewerage systems). “Secondary microplastic” results from mechanical, chemical, and/or biological degradation from large waste fragments (macroplastics) or an abrasion from various plastic products (e.g., agriculture, construction industry, traffic, clothing) and can enter the environment in large quantities via various input paths. One example is tire abrasion from motor vehicles, consisting mainly of polyisoprene in the form of tiny particles. It is now considered certain that microplastics occur worldwide in all habitats, in some cases in considerable quantities.

Although the occurrence of microplastic in marine systems was reported as early as 1972 (Carpenter and Smith 1972), it took over 30 years until it became a hot topic with the publication of Thompson et al. (Thompson 2004) “Lost at Sea: Where Is All the Plastic?”. Since then, microplastic has been detected in marine ecosystems worldwide and classified as a potential threat to biota, economy, and society (Fig. 2). Although 50–80% of the waste found in the sea is produced and disposed of on land until recently, research has focused mainly on the supposed main sink of plastic waste, the ocean, where significant amounts of plastic waste are floating on the surface (Eriksen et al. 2014). Extrapolations indicate that between 1.1 and 12.7 million tons of plastic waste are discharged into the oceans via rivers worldwide each year (Jambeck et al. 2015; Lebreton et al. 2017). Despite a large number of publications on the marine system’s microplastic contamination, there is still a lack of decisive information, for example, on the spatial distribution of microplastic in the oceans. Nevertheless, microplastics are reported to occur from tropical to pristine polar areas and from beaches to deep-sea sediments.

Freshwater ecosystems such as rivers and lakes have more recently received attention, and plastic particles have been found in areas used by tourists and even in remote mountain lakes (Imhof et al. 2013; Dris et al. 2015). Considering that the

Fig. 2 Beach on Lanzarote. Visible contamination with large microplastic fragments. (© Christian Laforsch, University of Bayreuth)

majority of plastic waste is generated and emitted on land, it is not astonishing that plastic particles have only recently been found in the atmosphere and terrestrial ecosystems, especially in urban and agricultural soils (Fig. 3) (Dris et al. 2016; Piehl et al. 2018; Weithmann et al. 2018).

Studies show that limnetic and terrestrial systems can serve, as well as marine systems, as sinks of plastic waste. No clear correlation could be established between the occurrence of microplastic in the environment and population density and

Fig. 3 Plastic on agricultural soils. All larger fragments can disintegrate into microplastics over time. (With kind permission from © Stefan Leible (University of Bayreuth))

proximity of industrial plants, which underlines both the complexity of the issue and the need to understand the mechanisms of the environmental behavior of microplastic. An estimate of the abundance of microplastic on agricultural land suggests that the pollution in Europe and North America alone is higher than the total amount of microplastics in the oceans (Nizzetto et al. 2016). Recent estimates suggest that there is almost 40 times more macro- and microplastics on land than in the ocean (Kawecki and Nowack 2019). The latter underlines the fact that plastic contamination of the environment not only affects the world's oceans but is of global relevance as a terrestrial pollutant.

It is predicted that, as a result of global population growth, increasing urbanization, and rising consumption in developing and emerging countries, the production and consumption of plastics will continue to grow strongly on a global scale and that the problem of the entry of microplastic into the environment will, therefore, become increasingly important. The resulting publicly discussed need for action is enormous, from which an urgent need for research can be derived to close the considerable gaps in knowledge that are becoming increasingly apparent despite or because of the topicality of the issue.

According to current estimates, "secondary microplastic" represents the main component of environmental contamination by microplastics. However, the extent of the contamination of the environment cannot yet be fully determined. Microplastics in the environment, unlike soluble pollutants, are neither temporally nor spatially homogeneously distributed, and therefore most studies on microplastic contamination are snapshots in time. In addition, microplastic analysis methods are only just being developed, as it is far from trivial to isolate and analyze microplastic particles smaller than the diameter of a human hair from complex environmental samples. Particles in the nanometer size range have not yet been detected in the environment. Further, data on contamination of the environment with microplastics are often not comparable with each other, as no uniform methodology for the detection of microplastics has yet been established, and different methods are used for sample processing and analysis.

Microplastics: Analysis of Environmental Concentrations

Representative sampling is among the most critical step in the analysis of microplastics. Non-representative sampling leads to unreliable data, regardless of how reliable the subsequent sample processing and analysis is. Each sampling design must be adapted to the specific research question.

To chemically identify and quantify each plastic particle occurring in environmental samples, potential microplastic must be extracted from the sample volume. Usually, an environmental sample contains more natural particles in the form of plant, animal, and mineral constituents than microplastic. Water samples usually contain only a few mineral particles and a high proportion of organic material. For non-homogeneous solid samples such as soils, microplastic isolation is even more challenging. The difficulty in the purification of the samples is to avoid method-related damage or fragmentation of the microplastic particles as far as possible. The simplest method for microplastic isolation is sieving and manual sorting using a stereomicroscope. This method is not only limited to sizes >500 μm but is also very susceptible to misidentifications and observer bias, so a subsequent reliable polymer identification is essential.

Suggested methods for removing the mineral fraction include electrostatic separation, oil extraction, froth flotation, magnetic extraction, vertical density gradient separation, and density separation. Usually, saturated salt solutions of sodium chloride, zinc(II) chloride, or sodium polytungstate are used. A density of 1.6–1.8 g/mL is suitable for all environmentally relevant plastics. Various methods have been developed based on the principle of density separation for sample preparation, such as the Munich Plastic Sediment Separator (MPSS) (Imhof et al. 2012).

For the removal of the organic fraction, the use of strong acids and bases has proven to be unsuitable, as some polymer types can be strongly damaged or even completely dissolved. Small microplastics and fibers are particularly affected. The treatment with various technical enzymes combined with mild oxidizing agents such as hydrogen peroxide has proved to be a gentle method of extracting microplastic from environmental samples (Löder et al. 2017).

The pretreated samples are then applied to filters for the chemical analysis. Raman spectroscopy and Fourier transform infrared (FTIR) spectroscopy are the most commonly used state-of-the-art analytical methods in microplastic research. Both vibrational spectroscopy techniques enable the precise identification of polymer types and their abundance, shape, and size. Software-based automatic detection of microplastics has been developed (Fig. 4) (Hufnagl et al. 2019). However, next to

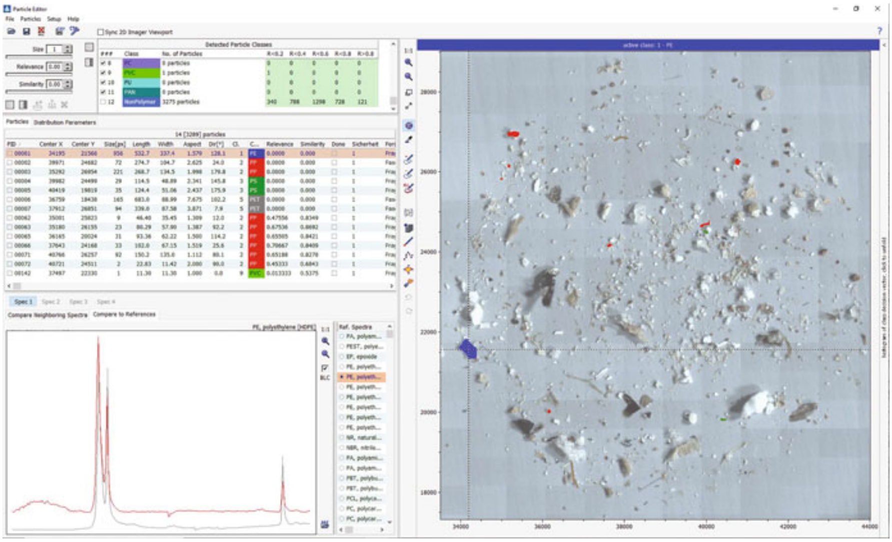

Fig. 4 Software-based automatic detection of microplastics using focal plane array (FPA)-FTIR (Hufnagl et al. 2019). Right-hand side: optical image of a filter after sample processing. The blue-labeled particle is identified as polyethylene (PE). Left-hand side: respective fingerprint spectra of the polyethylene particle. (© Martin G. J. Löder and Christian Laforsch, University of Bayreuth)

the restricted size limit of the respective methods (10 μm for FTIR, 300 nm for Raman), the spectra of environmentally aged plastics cannot always be clearly identified because biofilms on the particle surfaces can interfere with the spectroscopic methods.

If the shape and size of microplastic, which is indispensable if the toxicity on organisms is considered, are not in the focus of the study, microplastic can further be analyzed using pyrolysis gas chromatography mass spectrometry (Pyr GC-MS) or thermal extraction desorption gas chromatography mass spectrometry (TED GC-MS). With these methods, the polymer components, as well as the contained additives, can be examined under defined thermal conditions. However, these methods are restricted in the sample volume, which can be used (Fries et al. 2013; Dümichen et al. 2017).

In general, during sampling, sample processing, and analysis of microplastics, it should be noted that the risk of contamination of environmental samples is very high, as synthetic polymers are ubiquitous. Therefore, precautions must be taken at each processing step: blank samples should be used at each step, and plastic material should be avoided and replaced by alternative materials such as metal or glass wherever possible.

Woodal et al. (2015) comprehensively describe the application of a forensic-scientific approach to minimize sample contamination. A comprehensive discussion on the advantages and disadvantages of further and all described methods are listed in Möller et al. (2020).

Microplastics: Environmental Risks

The ubiquitous contamination of the environment with microplastics and the possible associated risks to ecosystems and ultimately to human health has recently attracted a great deal of public and scientific attention. Potential biological risks of plastic particles in the environment arise from the small size of the particles through which it can easily enter the food chain, mistaken as food or by inhalation. Microplastic particles can further degrade into even smaller particles, which have been termed "nanoplastics." Nanoplastics, with their smaller sizes, may have a higher bioavailability than microplastics and may even pose a higher environmental risk. Contamination of the environment with plastic particles, therefore, represents a global challenge and is (G7 Summit2015) classified as a "top emerging global issue" due to the as yet unassessed hazard potential and ubiquitous occurrence (GESAMP 2015).

The ingestion of plastic particles, together with natural food, has already been investigated in various organisms from aquatic and terrestrial habitats. The resulting effects on organisms and human health are still under discussion. It has to be noted that possible risks associated with plastic particles cannot be generalized since micro−/nanoplastics comprise a very heterogeneous group of particles that vary in polymer composition, additive content, size, shape, aging state, and, consequently, their physicochemical properties.

Hence, microplastic is only a collective term for small particles (< 5 mm) of various types of plastics with a wide range of chemical and physical properties as well as different surface conditions (e.g., functional groups, zeta potential). Non-polymerized monomers, as well as adsorbed organic material and coating with biomolecules and inorganic substances, contribute to the further complexity of the particles. Overly broad generalization over the potential biological effects predicted for microplastic is hence of limited value.

A central bottleneck in the assessment of the environmental relevance of microplastic is the lack of comprehensive data on biological mechanisms of action of microplastics as a function of the chemical and physical properties of the various plastics degraded in the environment. A comparison of microplastic with naturally occurring particulate materials, which can also be ingested with food, is indispensable. Considerable knowledge deficits currently exist at the molecular, cellular, tissue-specific, and organismic levels and the resulting ecological consequences.

Next to direct effects on organisms, which are discussed in the following paragraphs, plastic debris could also exert indirect effects in the environment leading to ecological consequences. Plastic debris could, for instance, act as a substrate and transport vector for alien species (Rech et al. 2016). Further, Trotter et al. (2019) found the interspecific communication between predator and its prey being interfered by the sole occurrence of plastic in the aquatic environment. The authors assume that the allelochemicals used for communication may adsorb to the surface of the plastic particles. The resulting misperception of the chemical cues may lead to a false adjustment of the prey's defensive strategies and may, therefore, affect population dynamics in higher orders of the food web.

To date, most studies about the effects of microplastic pollution mainly focused on the direct effects resulting from exposure. In particular, physical effects like injuries due to entanglement can cause severe inflammation of the affected tissue. Other possible consequences are drowning due to reduced mobility.

Moreover, if plastic particles are mistaken with natural food, the stomach capacity can be reduced, or the stomach passage blocked, which can lead to a false sense of fullness and, in turn, to a slow death from starvation.

Ingestion: The Main Entrance Route of Microplastics into Organisms

The degradation of plastic particles into smaller and smaller fragments increasingly affects organisms at lower trophic levels. Microplastics can float on the surface of water bodies, disperse in the water column, or accumulate in the sediment, making them accessible to a wide array of organisms in different habitats. Hence, the ingestion of plastic particles together with natural food has already been investigated in a variety of organisms, ranging from low trophic levels like zooplankton and mussels right up to higher trophic levels like vertebrates, from aquatic and terrestrial habitats. Several laboratory experiments, mainly conducted with aquatic organisms (including ciliates, cnidarians, rotifers, annelids, copepods, cladocerans, amphipods, mysids, euphausiids, barnacles, mussels, tunicates, and fishes), confirmed microplastic ingestion and uptake across the gill (e.g., Duis and Coors 2016). Some studies have already been carried out with terrestrial organisms, and ingestion was confirmed, for example, in detritivore soil invertebrates (Zhu et al. 2019). The ingestion of microplastic particles is additionally enhanced for microplastic being environmentally aged (Hodgson et al. 2018; Vroom et al. 2017). Moreover, some studies suggest that the presence of a microbial biofilm makes the microplastics more palatable for those organisms (Helmberger et al. 2019).

Upon ingestions, laboratory experiments suggest the excretion of microplastic particles within hours or days (Duis and Coors 2016), although the knowledge about microplastics retention time and excretion is still scarce. Some studies carried out with the Mediterranean mussels (*Mytilus galloprovincialis*) had conflicting results. Kinjo et al. (2019) reported that 99% of the ingested microplastics were excreted within 2 days after exposure. This study pointed out that very small microplastics were excreted faster than the larger ones. Those were detected in the feces up to 40 days after the exposure. Opposite results were obtained by Fernández and Albentosa (2019), as their work suggested that larger particles are excreted faster from the intestinal tract than the smaller ones. These contrary results are likely due to the different size range used in the two independent studies.

An investigation carried out on fathead minnows (*Pimephales promelas*) described excretion occurring within hours of exposure and dependent on the microplastics' size, with again the larger particles being excreted faster (Hoang and Felix-Kim 2020). The excreted microplastics were left available for reconsumption and were detected within the gut at all monitoring points. The excreted microplastics were coated with intestinal liquids, resulting in their aggregation and precipitation to the bottom of the exposure beakers. This suggests that the excretion process may contribute to the vertical movement of microplastics from the

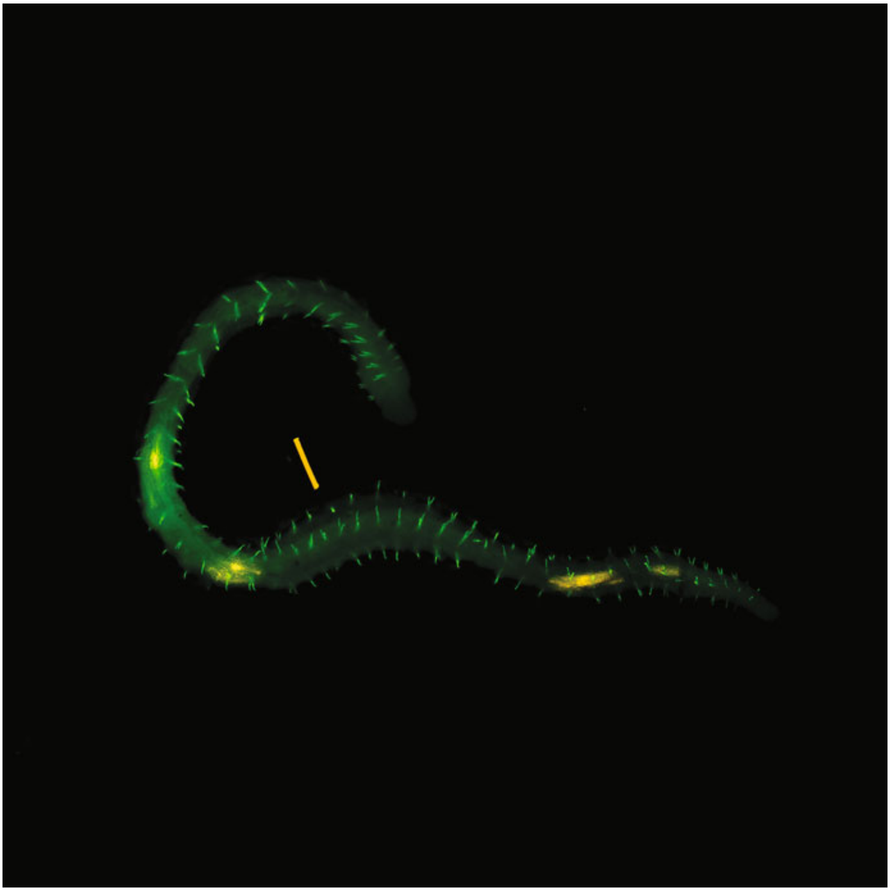

Fig. 5 Ingestion of fluorescent microplastic fibers (orange color) by an aquatic worm. The distinctly visible fiber is outside the worm (Lumbriculus variegatus). All other fluorescent particles are ingested and inside the gut. (© Christian Laforsch, University of Bayreuth)

water column to the waterbed, making them potentially more bioavailable for benthic organisms (Fig. 5).

Nevertheless, it has been shown that microplastics often remain in the digestive tract longer than natural particulate material, such as clay minerals (Wright et al. 2013a, b). Thus, organisms are confronted with this foreign substance for a longer time. With the enhanced retention period of microplastic particles within the gastrointestinal tract, the probability of bioaccumulation and biomagnification within the food web is much higher.

Trophic Transfer

Active selection of microplastic particles might occur when animals mistake plastic for food (Nelms et al. 2018). Further, microplastics are often in the size range of particles that are eaten by indiscriminate filter feeders. Especially those microplastic particles with a neutral or a positive surface charge can further adhere to phytoplankton and can, therefore, be found on the surface of suspended seaweed, rendering also herbivorous organisms prone to microplastic ingestion. As microplastics are persistent contaminants and often retain longer in the digestive tract than naturally occurring particles, all these mechanisms can lead to bioaccumulation in organisms from different functional feeding groups. To date, more than 690 species were found to be contaminated with microplastics (Toussaint et al. 2019; Wang et al. 2019). When such contaminated prey is consumed by a predator, the latter also ingest microplastics unintentionally. Thus, even organisms that do not have a food preference in the size range of microplastics can still ingest these particles indirectly via their food. This process leads to trophic transfer of microplastics along the food web, which may result in biomagnification. As a result, even in areas contaminated with relatively low concentrations of microplastics, the continuous ingestion of microplastic-containing organisms by predators may result in high concentrations in keystone predators (Au et al. 2017).

Trophic transfer has already been demonstrated on a laboratory scale, for instance, using low-density polyethylene microspheres on a model food chain relevant to North American estuaries (Athey et al. 2020). Further, Farrell and Nelson (2013) studied the transfer from the blue mussel (*Mytilus edulis*) to the shore crab (*Carcinus maenas*), since both are prevalent species in marine environments. Their analyses confirmed that the ingestion of contaminated blue mussels determined the concentration of microplastics in the crab, which was persistent even after 21 days post-exposure. Although different laboratory studies have assessed trophic transfer in low trophic level organisms, data on trophic transfer in the wild are still scarce. However, microplastics have been found in many wild fishes' gastrointestinal tracts, making the transfer to predators likely. Nelms et al. (2018) studied a correlation between the presence of microplastic particles in the gastrointestinal tract of Atlantic mackerels (*Scomber scombrus*) and the guts of their predators, the gray seals (*Halichoerus grypus*), suggesting that trophic transfer occurs in natural environments as well.

Since it has been shown that seafood is often contaminated with microplastics, the consumption of the latter may lead to a trophic transfer up to humans, which may also have implications on human health (Smith et al. 2018).

Ingested Microplastic Particles: Possible Effects in the Gastrointestinal Tract

The effects of microplastics at an organismal level can be separated in physical and chemical effects (Campanale et al. 2020). According to the authors, physical effects are related, for instance, to the particle size, shape, and concentration of microplastics, and chemical effects are related to chemicals that are associated with microplastics.

Effects Caused by Additives

Microplastics should not be considered as chemically inert particles. Due to the plethora of additives used during their production or intended to exert specific characteristics, the physicochemical properties of the microplastic particles may lead to chemical effects at an organismal level. Plastic additives, which enable the various material properties of plastic or incompletely converted starting materials (monomers, oligomers), can leach out in the environment or upon ingestion in the gastrointestinal tract of organisms. Among chemical additives added to the polymers in the versatile production process are plasticizers, colorants, fillers, or flame retardants, just to name a few. The plasticizer to polymer ratio strongly depends on the material's desired property but can amount up to 50%, for example, in PVC.

Carcinogenic and hormonal effects on organisms have already been proven for some of these additives. Well-known examples are bisphenol A and phthalates (Prata et al. 2020). However, the release of additives in the digestive tract of organisms is controversially discussed in the scientific community and is considered low, since a biodynamic model (Koelmans et al. 2014) has shown a negligible release of additives in the digestive tract. Nevertheless, it has been shown that rigid PVC particles (PVC without phthalate) did not affect the growth rate and the number of offspring in *Daphnia magna*, whereas flexible PVC (the phthalate DiNP was added) did show adverse effects (Schrank et al. 2019). Other types of additives are trace metals. Trace metals are used as flame retardants, stabilizers, or biocides, which have been shown to induce effects on human health. For instance, trace metals potentially induce allergic reactions; have endocrine reactivity, which enhances the probability of hormone-induced cancer; and show genotoxic effects, the formation of reactive oxygen species, and other cytotoxic effects (Campanale et al. 2020).

Effects Caused by Adsorbed Pollutants

Besides being used as additives, trace metals and other environmental pollutants like pesticides are widely discussed to adsorb from the surrounding environment to the surface of microplastic particles. In this context, once again, the physicochemical properties of the specific microplastic particle, such as hydrophobicity or surface roughness, play a critical role in the adsorption of pollutants. Once organisms ingest microplastic particles, either with additives within their polymer matrices or adsorbed pollutants from the environment, they can suffer from adverse effects. Nevertheless, the ecological relevance of this vector effect is still under discussion. The transfer of adsorbed pollutants to organisms and the resulting possible effects of these substances are considered negligible, since there is currently a consensus, resulting from laboratory and modeling studies, that the quantities of substances absorbed by this route are small compared to those absorbed directly from the water (Bakir et al. 2014; Koelmans et al. 2016).

Effects on the Gut Microbiome

Although the mere passage of plastic particles through the digestive tract may have no direct effect on organisms, it may alter the gut microbiome instead. A recent study in mice shows that the intestinal microbiome is altered by the intake of polystyrene microplastic, resulting in a disturbance of fat metabolism (Lu et al. 2018). Similar results on the murine model system have also been found by Li et al. (2020). Microplastic exposure resulted in a significant increase in bacterial abundance and diversity in mice fed with high microplastic concentrations. Further, microplastic exposure induced intestinal dysbacteriosis and inflammation. Even in fishes, microplastic exposure leads to alterations in the gut microbiome's composition, though the mechanism is still unknown (Triebskorn et al. 2019). Furthermore, a dysbiosis (disturbance of the intestinal microbiome) after the intake of polystyrene microplastic particles in zebrafish was found (Jin et al. 2018). Whether other organisms were also affected at this level and what role the type of plastic with the corresponding physical and chemical properties plays in this is entirely unexplored. Since gut microbiomes affected by microplastics may have different effects on the immune function, further studies are required to better understand this topic and its potential threat to animal and human health (Li et al. 2020).

Microplastics: Tissue Translocation

One potential risk that has been intensively discussed but not yet sufficiently investigated and understood is the translocation of microplastic particles from the digestive tract and respiratory system into cells and tissue. It has been shown that microplastics not only pass through the intestinal tract but are also absorbed on and encapsulated within the tissue, which can lead to inflammatory responses, as shown in mussels (von Moos et al. 2012). Further microplastics were found to be translocated from the digestive tract into the mussels' circulatory system (Browne et al. 2008). In the shore crab (*C. maenas*), microplastics were detected in the hemolymph, hepatopancreas, ovaries, and gills, indicating that some particles can cross the gut epithelium (Duis and Coors 2016). The translocation of microplastics in different body compartments is not just described in invertebrates but recently also in vertebrates including zebrafish, where hyperspectral imaging was used to identify nanopolymer particles translocated from the intestine into the liver (Galloway et al. 2017a, b). Additionally, the translocation of microplastic is not exclusively found under laboratory conditions but also occurs in natural environments in fish (Barboza et al. 2020).

As mammalian model systems to study tissue translocation, mainly mice and rats or murine cell lines are used. Feeding experiments showed the translocation of micro- and nanoplastics from the gut to excretory organs like the liver and kidney (Yong et al. 2020). Particulate substances in the size range of microplastic particles, as found in environmental samples, can, therefore, potentially also be translocated into the tissue of humans, which underlines the environmental relevance of this issue.

To date, neither the corresponding interactions between the cells of the intestinal tract and the microplastic particles have been understood, nor has it been investigated whether microplastics are internalized by cells directly or which internalization mechanisms are potentially involved.

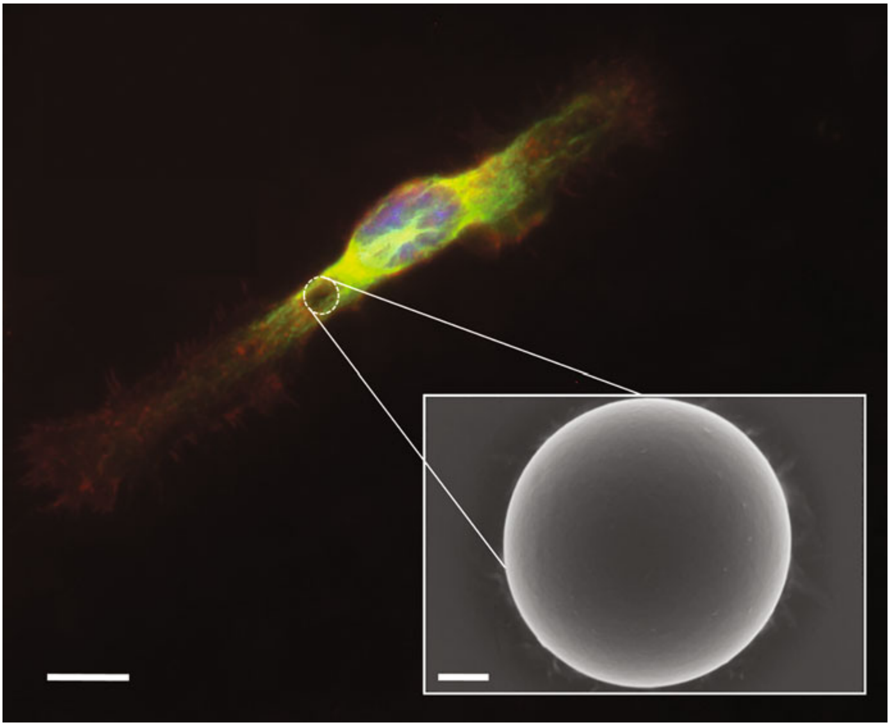

Fig. 6 Cellular internalization of a spherical microplastic particle. Fluorescently labelled J774A.1 murine macrophage cells exposed to 3 µm polystyrene microplastic particles. The cytoskeleton of the cell surrounds the microplastic particle. False-color image; blue, DAPI stain of the nucleus; red, Alexa Fluor™ phalloidin stain for filamentous actin; and green, immunolabelling of the microtubules, scale bar: 10 µm. Scanning electron microscopy image of the 3 µm spherical polystyrene microplastic particle, scale bar: 500 nm. (© Anja F. R. M. Ramsperger, University of Bayreuth)

It is known that particles in the micrometer size range can, in principle, be internalized by epithelial cells and identified as potential pathogens by cells of the immune system, e.g., macrophages (Fig. 6). Particles are further internalized by cells via, e.g., phagocytosis when coated with antibodies (Desjardins and Griffiths 2003).

There are mainly two possible pathways of how micro- and nanoplastic particles may translocate into tissues. Either the particles are transported paracellularly, which means in between cells through, e.g., tight junctions, or transcellularly, which means the endocytosis of particulate matter into cells directly (Wright and Kelly 2017). Endocytosis mechanisms depend on several factors. Besides receptor-mediated internalization mechanisms, which require ligand-proteins and suitable receptors, the size of the particles is an essential factor (Doherty and McMahon 2009). Smaller particles are suggested to become internalized passively, whereas larger particles in the lower micrometer size range are discussed to become internalized by cells in an energy-dependent active manner (Shang et al. 2014; Wright and Kelly 2017). However, even the particle's surface properties are responsible for tissue translocation, and therefore, the environmental coating of the particle may play a crucial role in cellular internalization (Galloway et al. 2017a, b).

The Coating of Microplastic Particles: Biofilm, Coronas, and Pathogens

Once micro- or nanoparticles enter aquatic environments, a biofilm can develop on the particles' surface. In the marine environment, the development of a biofilm has been shown on various polymer types (Oberbeckmann et al. 2015). The development of a biofilm can generally be described to occur in five successive steps. In the first step, microorganisms reversibly attach to the surface. Within the second step, microorganisms excrete extracellular polymeric substances (EPS), enabling them to stick to the surface better and, in the third step, start to proliferate within the EPS matrix. By forming 2D and 3D colonies, the biofilm grows within the fourth step. A biofilm is a highly dynamic system, as, within the fifth step, microorganisms are also able to detach from the surfaces (Renner and Weibel 2011). Next to the adhesion of microorganisms within the first step, another important factor is the adhesion of biomolecules on the particle surfaces. It has been suggested that the adhesion of biomolecules on surfaces appears within seconds (Loeb and Neihof 1975). The initial biomolecule coating is not a stable system, as biomolecules with higher binding affinities may substitute biomolecules with lower binding affinities. Over time a so-called (more or less) stable hard corona develops on the surface of the particle. On top of this hard corona, an additional corona develops, which is highly dynamic with its surrounding environment. This dynamic corona is called the soft corona and is in high exchange with its surrounding environment (Monopoli et al. 2012). The process of the development of a protein corona, especially on the surface of nanoparticles, has intensively been studied in medical research, e.g., for drug delivery using target nanoparticles.

A rather new research field is the coating with biomolecules from complex environments like marine or limnetic ecosystems. The coating with environmental biomolecules is said to be an ecocorona, referred to the similar term "protein corona." An ecocorona can consist of different kinds of biomolecules, like proteins, lipids, or carbohydrates, just to name a few (Galloway et al. 2017a). On a cellular level, it has been shown that particles coated with protein coronas interact differently with cells (Monopoli et al. 2012; Francia et al. 2019). On an organismal level, it has already been shown that the coating with an ecocorona mediates the impact of polystyrene nanoparticles to *D. magna*. Additionally, *D. magna* was less sufficient in removing particles with an ecocorona from their digestive tract compared to particles without an ecocorona. This highlights the importance of including micro- and nanoplastic particles coated with an ecocorona in future experimental attempts, as these may show severe effects that may not occur by using pristine microplastic particles (Nasser et al. 2019).

Another critical aspect that has been widely discussed in the context of environmental impact is the adherence of pathogens on the surface of plastic particles or within the biofilm matrix (Zettler et al. 2013). However, it is not clear whether or not there is a difference in pathogen load on microplastics compared to occurring natural particles coated with a biofilm (Rummel et al. 2017). This again highlights that besides the use of pristine microplastics and plastic particles coated with an ecocorona, natural particles must also be included as a reference for the risk assessment of microplastics.

Effects on Cellular, Tissue, and Physiological Level

Once ingested, it has been shown in laboratory studies that microplastics can exert adverse histopathological effects in fish, i.e., causing damage to villi structures in the gastrointestinal tract and on the gill membranes. Further, the longer the residence time of microplastic within the organisms is, the more damage occurs to the immune system and blood parameters. In addition, alterations of metabolic profiles indicating disturbed lipid and energy metabolism were reported (Triebskorn et al. 2019). Furthermore, it has been reported that maternal exposure to polystyrene in mice led to metabolic disorders in the offspring (Luo et al. 2019). Beyond laboratory feeding experiments, Barboza et al. (2020) analyzed the microplastic ingestion and accumulation in wild fish. For fish containing microplastics in their brains, gills, and dorsal muscles, they found a significantly higher lipid peroxidation level and increased brain acetylcholinesterase enzyme activity. Some studies have also shown effects at the molecular level using different terrestrial and aquatic organisms by applying an "omics" approach (e.g., Limonta et al. 2019). Here, the authors report on alterations in the expression of immune system genes, indicating that microplastics are identified as stressors.

First attempts have been made to investigate possible negative effects on human cell lines. Similarly, when investigating effects at an organismal level, contradictory results were found. In general, the most often reported effects in human cell lines are the generation of reactive oxygen species and the increase in inflammatory responses (Yong et al. 2020), whereas, in some studies, no effects were found. This inconsistency may originate from various factors. Different cell lines may interact differently with the used particles. The sizes of the particles, as well as the concentrations used, were profoundly different and could, therefore, lead to different results. Another critical aspect in cytotoxicity studies is the choice of particles. The use of surfactants for colloidal stable particle solutions may alter the surface of the used particles. Additionally, surface-functionalized particles may behave entirely different compared to non-functionalized particles.

Microplastics: Effects on Morphology, Behavior, Population, and Life History

The ingestion and possible accumulation of micro- and nanoplastics within body compartments has been suggested to pose a risk to organisms. Depending on the sampling site, environmental concentrations can vary accordingly from few to several thousand particles/L; hence, experimental designs have to consider this. Micro- and nanoplastic particles can alter the behavior, morphology, or life history of an organism at concentrations relevant to environmental exposures. For instance, the exposure of zebrafish (*Danio rerio*) to plastic particles (100 and 1000 μg/L, 50% polystyrene +50% high-density polyethylene) resulted in alterations of their circadian timekeeping mechanism, resulting in an increased activity during the dark and the loss of the regular diurnal pattern of activity (Limonta et al. 2019). Alterations in the phototactic behavior were shown for *D. magna*, along with increased swimming activity and reproduction after the exposure to three different polystyrene microplastic concentrations (0.125, 1.25, and 12.5 μg/mL) (De Felice et al. 2019). The authors suggested that increased swimming activity might be explained as an avoidance behavior or an attempt to eliminate the microplastic particles.

Energy depletion caused by a large number of microplastics in the digestive tract results in reduced food intake, and it can additionally lead to a significantly reduced survival rate, increased development time, and reduced fecundity, as it has been shown for copepods (*Tigriopus japonicus*) and lugworms (*Arenicola marina*) (Duis and Coors 2016). In *Daphnia magna,* microplastic exposure resulted in a reduction in the population growth rate and so in an impairment in the cladoceran's fitness, probably due to a decrease in food intake in the presence of microplastic particles (Martins and Guilhermino 2018).

Impairment in fertility and larval growth was also examined in sea urchins (*Paracentrotus lividus*). In detail, a lower fertilization rate was observed in eggs exposed to plastic particles along with larvae abnormalities and a decreased developmental time (Martínez-Gómez et al. 2017). Other studies reported similar results on plutei larvae (Messinetti et al. 2017). Adverse effects on fertility and larval development were assessed in oysters (*Crassostrea gigas*) as well. In particular, both the sperms and oocytes' numbers decreased and were deteriorated in quality compared to the control organisms. Further, the larval developmental was significantly slower (Sussarellu et al. 2016). The mentioned effects of micro- and nanoplastic exposure to organisms are not only shown for aquatic organisms. Effects from microplastic exposure have already been shown for terrestrial invertebrates, like nematodes, oligochaeta, collembola, or isopods. Studies on nematodes (*Caenorhabditis elegans*) have revealed that smaller microplastic particles impaired the survival rate, the average lifespan, and body growth. Collembolans were found to be more sensitive to microplastic exposure, showing a significant inhibition in growth and reproduction (Zhu et al. 2019).

Considering morphological alterations due to microplastic exposure, the current results are less consistent. Some suggestions have been made that the exposure to microplastic particles may also alter the morphological parameters of daphnids. For instance, it has been shown that *D. magna* shows a larger body size than the control treatment when exposed to microplastics (De Felice et al. 2019). For similar concentrations used, Eltemsah and Bøhn (2019), on the other hand, show now alterations in the body length of daphnids.

This highlights that the causes of the effects on the organisms investigated are not yet understood, primarily since exposure to the same type of plastic has led to adverse effects in some studies, but in others, no effects on the organisms investigated could be observed. Some authors suggest that the alterations on a cellular level may subsequently lead to morphological, behavioral, or life history changes. For instance, Limonta et al. (2019) discussed that the behavioral alterations observed in zebrafish might originate from very small microplastic particles crossing the blood-brain barrier.

Microplastics in Plants: A Fairly New Research Field

A fairly new topic on micro- and nanoplastic research is the contamination of plants (Fig. 7). In general, due to the application of sewage sludge from wastewater treatment plants, which has been shown to contain a massive amount of microplastic particles and fibers (Corradini et al. 2019), or due to the application of organic fertilizers (Weithmann et al. 2018), agricultural fields can be contaminated with plastics (Piehl et al. 2018). The possible effects of micro- and nanoplastic pollution on plants can be divided into indirect and direct effects. For terrestrial plants, indirect effects may come from altered soil structure. Depending on the physicochemical parameters as well as the size and shape of the micro- and nanoplastic particles, the soil bulk density, structure, and water holding capacity may be altered (Rillig et al. 2019). This may have effects on the root growth or microbial community composition, which in turn may affect nutrient acquisition. The aquatic plant *Lemna minor* was found not to be altered in its leaf growth rate and amount of photosynthetic pigments but negatively affected in its root growth. The authors suggest the microplastic particles being adsorbed onto the surface of the roots and mechanically blocking the root growth (Kalčíková et al. 2017). A direct effect of microplastic and nanoplastic contamination is the uptake of the particles into plant tissues. This has been suggested to be unlikely for microplastics but generally accepted for

Fig. 7 Plastic and plant growth. A plastic bottle degrades on an agricultural field. (© Simona Mondellini, University of Bayreuth)

particles in the nano-size range. Recently, it has been shown that functionalized nanoplastic particles adhere to the root surface of *Arabidopsis thaliana* and reduce the root growth correlating with particle concentrations. Furthermore, the nanoparticles may even translocate into the root epidermis cells and the catheter of the xylem (Sun et al. 2020). Although the authors used functionalized particles which are unlikely to occur in nature, these findings indicate that the translocation and further accumulation of nanoplastics in plants are generally possible. Due to methodical and technical limitations in identifying nanoplastics in environmental matrices, the environmental pollution of nanoplastics is currently unknown. Nevertheless, the fact that plants used for food production may accumulate plastics within their tissues may elicit environmental and human health risks.

Microplastics Risk to Humans?

The exposure of humans to plastic particles has extensively been discussed and investigated in the last years. The ubiquitous occurrence of plastics in the environment and consumer products makes human exposure to microplastics inevitable. The most reasonable pathway is discussed to be via ingestion. Microplastic contamination of food and beverages has already been shown. For instance, microplastic particles were found in salt, sugar, processed food, and beverages like beer and drinking water and seafood (Wright and Kelly 2017). Once in the digestive system, there are several adverse effects discussed. Microplastics can potentially be adsorbed by M-cells in the intestine, penetrating the intestinal mucus (Prata et al. 2020). Moreover, the ingestion of microplastics and transfer of endocrine-disrupting chemical additives potentially could be associated with a range of chronic metabolic effects, including infertility, obesity, and cancer (Sharma and Chatterjee 2017).

A further as yet unexamined risk is the intake of microplastic particles via the air we breathe, as it has been shown that up to 16 microplastic particles can be present per m^3 of air (Vianello et al. 2019). Microplastic fibers are shown to be possibly inhaled, likely most of them undergo mucociliary clearance, but in some cases, they can persist in the lung causing obstructions and inflammation, especially in individuals with compromised clearance mechanisms. Persistence in the lungs seems to be connected to the particles' dimension. The longer fibers are the more persistent and the more likely to create obstructions or to penetrate deep in the lung. Furthermore, microplastics can determine granulomatous lesions in the lung tissues and respiratory irritation, a phenomenon mainly observed in the textile industry workers after chronic exposure (Gasperi et al. 2018).

Although the addition of plastics to cosmetics was banned in several countries (Conkle et al. 2017), the uptake via derma is generally discussed to be a possible pathway to enter human bodies, especially for nanoplastics. Nevertheless, the exposure to the associated additives such as bisphenol A or phthalates is discussed to be more alarming (Prata et al. 2020). The extent to which harmful additives such as phthalates are transferred into the human body via microplastics or directly through water, food, or contact with consumer items remains unknown, but it is known that virtually everyone has plastic-associated chemicals in their bodies (CDC 2020).

Synopsis

Overall, the mechanisms underlying the direct and indirect effects of plastics on organisms, as shown so far, are not yet understood. In general, macroplastics cause more obvious ecological effects, whereas the effects of microplastics are not so easy to elucidate. The nature of the effect probably depends mainly on the particles' physicochemical properties, the particle shape, the corresponding degradation stages, and environmental coating and concentration. Yet, the majority of the studies on biological effects were carried out with unrealistically high concentrations of microplastics, whereby the sheer quantity of foreign substances could have caused the observed effects and not the plastic particle per se.

The discrepancy that in some studies, although the same type of plastic was used, effects were found at different biological levels, but not in other studies, is due to the complexity of the issue.

As a result, there are still considerable gaps in knowledge regarding the biological effects of microplastics under realistic conditions. There is a dearth of studies that have been carried out concerning the physicochemical properties of microplastic particles and in comparison to naturally occurring particulate material since ecotoxicological studies have mostly used virgin ground plastics whose physical and chemical properties have not been characterized. Hence, more environmentally relevant studies are needed to assess the risk of microplastics for environmental and human health.

Cross-References

- ▶ Dose-Response Analysis, Identification of Threshold Levels for Chemicals
- ▶ Exposure Scenarios in Toxicology
- ▶ Green and Sustainable Chemistry as Regulatory Levers
- ▶ Importance of Physicochemical and Physical Properties for Toxicological Risk Assessment
- ▶ Nanoparticles and Their Regulation
- ▶ National and International Collaboration in Regulatory Toxicology

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