Relevance of nano- and microplastics for freshwater ecosystems: a critical review

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Abstract

The current paper critically reviews the state-of-the-science on (1) microplastics (MP) types and particle concentrations in freshwater ecosystems, (2) MP and nanoplastics (NP) uptake and tissue translocation, (3) MP/NP-induced effects in freshwater organisms, and (4) capabilities of MP/NP to modulate the toxicity of environmental chemicals. The reviewed literature as well as new data on MP and NP concentrations in the river Elbe and on particle uptake into human cells indicate an environmental relevance of small particles in the low nano- and micrometer range higher than that of larger MP.

1. **Introduction**

Plastic pollution is an issue of environmental concern that has intensively been discussed in the scientific literature and public media in recent years. Against this background, problems related to the abundance of plastic waste in aquatic ecosystems have been communicated and transported into society by means of – quite often very emotional – reports supplemented by photographs displaying, e.g., birds and turtles either getting entangled in plastic waste or suffering from its ingestion.

Subsequently, microplastics (MP) emerged as a "novel" topic and attracted considerable attention in the scientific community during the last decade. Comprehensive research projects have been initiated, resulting in a massive increase in scientific output. Meanwhile, the topic of MP pollution has also reached the public as well as policy-makers *via* public and social media. However, in these media, the MP topic has often been reported in a less accurate way and has been intermingled with more general environmental problems associated with (macro)plastic waste.

Particularly with respect to consequences of MP for exposed organisms, a clear distinction between different types of plastics is essential. This differentiation is not only necessary for pointing out the differences between macro- and microplastics, but it is also key for the discrimination among effects caused by different types of MP depending on their size, shape and chemical composition.

Most commonly, MP are defined as synthetic polymer particles or fibers with a diameter of 1 to 5000 µm [1], even though the lower limit has been extended down to 100 nm by the European Food and Safety Authority (EFSA) [2]. This extension, however, interferes with the term 'nanoplastics' (NP) which is still under debate, because different studies have set the upper size limit at either 1000 nm or 100 nm. A recent opinion paper by Gigault et al. has defined NP as colloidal plastics within the size range from 1 to 1000 nm [3]. With respect to MP, primary and secondary MP have to be distinguished. Primary MP, e.g. pellets, granules or microbeads, are produced for distinct purposes,

while secondary MP arises from fragmentation of larger plastic items during use or in the environment [4].

MP can be found worldwide in the water and sediment phases of aquatic (marine and freshwater) ecosystems [5, 6] even in the most remote areas of the world, including the deep sea [7], the Arctic [8], mountain lakes [9] and in atmospheric fallout [10]. Usually, the major sources or pathways of MP to ecosystems are *via* rivers, drainage systems, abrasion from car tires [11], runoff from agricultural land [12], effluent of wastewater treatment plants [13] and erosion by wind and currents [14].

As of today, many studies on effects in biota were conducted with MP particle concentrations exceeding those measured in or (for particles < 10 µm) extrapolated for the environment by a factor of 10^2 - 10^7 [15]. Furthermore, there is still a striking discrepancy between high concentrations of smaller particles tested for toxicity and low concentrations of rather large particles analyzed in the environment. Only little information is available on the abundance and distribution as well as on biological effects of NP particles with a diameter < 1 µm, even though exactly this fraction is supposed to be of notable biological and environmental relevance [16, 17], particularly with respect to toxic and ecotoxic effects [18, 19].

In the present paper, the state-of-the science is critically reviewed with regard to toxicity studies with MP and NP focusing on (1) an evaluation of environmental relevance, (2) an assessment of their potential to transfer to tissues and (3) modulation capacities reported for MP and NP for the toxicity of other chemicals.

2. Environmental concentrations of MP and NP as prerequisites for the assessment of effect data

(References cited in table 1 are numbered as T1/1-16. Full citations are provided in the supplement).

In this chapter, data for MP/NP concentrations in freshwater ecosystems including effluent, surface water and sediment concentrations are reviewed as a basis for the assessment of the environmental relevance of the reviewed eco-toxicological studies. In addition, previously unpublished data for MP concentrations in the German River Elbe generated within the German research project MiWa are provided, which also serve as a basis for this assessment.

The concentrations of MP > 300 µm, e.g., have been reported to vary between 0.027 particles/m³ in Laurentian Great Lakes [T1/7] or 0.12 particles/m³ in Lake Hovsgol, Mongolia [T1/8], and up to 387 particles/m³ in the Rhine River [20]. Studies that investigated smaller particles, however, revealed very high particle concentrations especially for MP with a diameter < 20 µm [16, 17]. Around 10^5 particles/m³ were detected in urban Amsterdam canal water [T1/6]; 10^4 - 10^5 particles/m³ were

documented for the Three Gorges Reservoir, China [21]. Further studies on MP levels in freshwater systems are listed in Table 1.

Position Table 1

Within the scope of the project "MiWa – Microplastics in the Water Cycle", six sites along the German 538.5 km long stretch of the Elbe River were sampled with a micro-sieve filter cascade (5, 20, 100 µm mesh sizes) coupled to a submersible pump positioned in 0.5 m water depth [22]. Sample volumes between 250 L and 450 L were filtered for each site. The filter cakes were extracted in an ultrasonic bath, treated with ozone to reduce fluorescence, centrifuged and density-separated in a sodium polytungstate solution. MP particles down to diameters of 4 µm were analyzed on polytetrafluoroethylene (PTFE) filter membranes with micro-Raman spectroscopy (µ-RM). Additionally, total numbers of both inorganic and organic particles (1 - 200 µm) were quantified with a laser light extinction particle counter. The total particle concentration was in the magnitude of 10^{11} particles/m^3. The MP particle numbers for each sampling site varied between 10^5 particles/m^3 and 9 x 10^5 particles/m^3 (overall 96.5% polyethylene (PE), 1.8% polystyrene (PS), 0.7% polypropylene (PP), 0.5% polyamide (PA) and 0.5% polyoxymethylene). Thus, not more than 10 out of 10^6 particles consisted of plastic. The estimated MP loads were 4 - 30 kg/d (1.5 - 3.7 t/a).

Remarkably, 96% of the MP had diameters below 20 µm, and no MP particles larger than 100 µm were found. The largest particle diameter was 97 µm, and the smallest diameter was 4 µm. The high number of small particles (< 20 µm) accounts for more than 90% of the overall particle number, but results in a very small particle mass (ng mass range). This supports findings from previous studies (Table 1) and highlights the need to take the high number of small MP particles specifically into consideration for MP/NP research, in particular due to their probably higher relevance for biota and the environment.

Even though the knowledge on the impact of MP on aquatic ecosystems and human health is still limited, there is concern about the translocation of NP and MP to tissues (addressed in chapter 3), their toxicity in freshwater biota (chapter 4.1), and its possible interaction with environmental chemicals (4.2). In the following chapter, we therefore review the existing literature on this topic.

3. Tissue translocation of NP and MP in aquatic invertebrates and fish

(References cited in table 2 are numbered as T2/1-31. Full citations are provided in the supplement).

Besides the chemical toxicity of NP and MP as well as their potential to mechanically cause injuries (e.g., at the epithelia of gills or of the digestive tract), the translocation of plastic particles from the

external medium into tissues is a prerequisite for plastic-mediated adverse effects such as inflammation or necrosis. Already abundant membrane injuries can facilitate this passage. The terminology in the literature related to cellular uptake and tissue translocation of NP and MP, however, is often ambiguous or imprecise. From a biological point of view, the transfer of particles into the lumen of the gastrointestinal tract or an attachment to other external surfaces (e.g., gills) has to be regarded as external to the body. Nonetheless, some researchers use the term ˈuptakeˈ when describing microplastics adhering to gills or other soft tissues like the intestinal epithelium [T2/23]. To clarify, we use the terms ˈuptakeˈ and ˈtranslocationˈ only when particles have penetrated into either cells or tissues beyond the epithelial surface. This, for example, was shown in the project MiWa for polystyrene particles of 0,25 ± 0,06 µm, which were taken up by human keratinocytes (Figure 1).

Position Figure 1

3.1 Available data on tissue translocation

Overall, 31 studies were identified investigating the translocation of plastic particles (Figure 2 and Table 1), covering 18 different species from the taxa Mollusca (3 species), Crustacea (5), Osteichthyes (8), Echinodermata (1), and Nematoda (1). Among the five polymer types used in the majority were polystyrene (PS, 24 studies) and polyethylene (PE, 5 studies). Polyethylene terephthalate (PET), polymethylmethacrylate (PMMA) and polyester were used in one study each. Particle types (62 types tested) included mainly spheres (46 types, 74 %), shredded or commercially available irregular particles (13 types) and fibers (3 types). The majority of the particles were fluorescent, as fluorescence microscopy was the main detection method.

Most data are available for zebrafish (*Danio rerio*; 7 studies). Exposures to particle sizes between 20 and 20000 nm have led to partially conflicting results: Brun et al. [T2/21] found inflammatory responses associated with 25 nm PS particles, but no tissue translocation. In contrast, Van Pomeren et al. [T2/27] used the same particle size and material and reported an uptake into tissues (primarily in the eye), which is in line with various studies on nanoparticles [T2/22, T2/24-26]. There is also disagreement regarding the sizes of particles that can be taken up by cells and translocated among tissues. While in one study 0.25 and 0.7 µm particles did not translocate [T2/27], another study reported the uptake of 5 and 20 µm particles into liver tissue [T2/23]. Avio et al. [T2/29] described large particles up to the mm range into liver tissue of *Mugil cephalus*, and several studies in fish reported the accumulation of NP and MP in fatty tissues, such as the yolk of fish larvae [T2/24, T2/31] and brain [T2/19, T2/22].

Four studies on crab species exposed to PS cover *Carcinus maenas* and *Uca rapax* [T2/3-6]. Here, only Farrell and Nelson [T2/3] found the translocation of very large particles to the hemolymph and a

number of organs. All studies on *Daphnia* species and PS found translocation to either lipid droplets [T2/9, T2/11], or embryos [T2/7-8]. Translocation of PET fibers between 62 and 1400 µm length was not observed by Jemec et al. [T2/10]. Bhargava et al. exposed barnacles (*Amphibalanus amphitrite*) to PMMA particles < 200 nm and found distribution throughout the animals [T2/2].

Out of the six studies on mollusks, four focused on *Mytilus edulis* or *M. galloprovincialis* and one each on *Dreissena polymorpha* and *Scrobicularia plana*. All studies with PS found translocation to tissues or the hemolymph [T2/12-14]. While von Moos et al. [T2/15] reported a translocation for PE of up to 80 µm in *M. edulis*, Détrée and Gallardo-Escárate [T2/17] did not observe this for particles between 1 and 50 µm in *M. galloprovincialis*. In addition to fish, crustaceans and molluscs, Zhao et al. [T2/1] reported distribution of 100 nm PS particles throughout the nematode *Caenorhabditis elegans*, while sea urchin embryos did not show any tissue translocation of 40 and 50 nm PS particles [T2/18].

Position Figure 2

3.2 Approaches to investigate tissue translocation

Methodologically, most studies rely on fluorescence-labeled particles to investigate tissue translocation. These can be tracked in intact specimens or histological slides using advanced fluorescence microscopy, such as confocal laser scanning microscopy (CLSM) or hyperspectral microscopy [T2/19]. Alternatively, fluorescence was measured after digestion of tissues. Some studies used optical projections [T2/7] to locate particles. Electron microscopy (EM) was usually used as a supporting method, but not as the main means of investigation.

Apparently, fluorescence-based measures have certain limitations. Importantly, the fluorescent dye is often not covalently bound to the particle and subsequently may leach out. As the dyes are lipophilic, they may accumulate in lipid-rich tissues of animals under investigation. Sample treatment using detergents and solvents may further facilitate leaching. Although this issue is hardly addressed in the published literature, it is often unclear whether a fluorescence signal actually originates from particles or free dye [23, 24]. For instance, this might be the case in the study by Rosenkranz et al. [T2/11], where it could not be concluded that the observed fluorescence was particle-related. Particulate structures inside the lipid droplet visible under the electron microscope were considered translocated particles but were also present in control animals. Therefore, adequate controls are needed to ensure that the observed fluorescence actually represents translocated particles. Other possible sources of artefacts are discussed in [25].

Leaching of fluorescence may also occur during histological procedures, when solvents etc. are applied. In addition, histological approaches can be prone to contamination of slides by air-borne

particles or unintended relocation of particles through the cutting process, particularly when using high particle concentrations.

Along the same line, the tracking of NP within biota remains challenging. The resolution of fluorescence microscopes does usually not allow for the detection of individual nanosized particles and, therefore, may underestimate the frequency of translocation events. For example, for gold nanoparticles, Rothen-Rutishauser et al. [24] concluded that transmission electron microscopy (TEM) revealed 150-times more NP events per cell than CLSM. However, the low-density plastics are not readily detected using TEM. Alternative techniques, such as RM or coherent anti-Stokes Raman scattering, are limited in resolution by the optical diffraction to the micrometer range [16]. Accordingly, adequate methods to detect NP in tissues are missing.

3.3 Mechanisms of tissue translocation

Particle properties play a major role in mediating cellular uptake [23, 26]. Therefore, insights gained for one polymer type with specific properties are hardly transferable other ones [27]. Size and surface charge are important factors for uptake. Particles in the low nanometer range may passively cross membranes, whereas larger ones require active transport. Cationic particles more readily attach to cell surfaces than neutral or anionic particles and are, therefore, more likely to be taken up by active transport mechanisms [26]. In principle, higher particle concentrations should increase the chance of an interaction with epithelia, thus, enabling translocation in the first place. Additionally, weathering and the formation of an eco-corona (i.e., attachment of biomolecules) likely influence the interaction of particles with biota [26]. However, factors other than size have so far hardly been considered when studying tissue translocation.

Plastic particles can cross intact biological barriers by endocytotic processes such as phagocytosis and persorption [28, 29]. The former is an active vesicle-mediated process [30] involving a number of sub-mechanisms [23]. Persorption is a paracellular mechanical process driven by the kneading of particles through an epithelial layer into underlying tissues [29], potentially facilitated by the weakening of gap junctions [31]. Under normal conditions, persorption is restricted by junctional complexes [32]. They can be weakened by chemicals, disease, irradiation [31], tissue damage or the loss of cells [32]. This may promote translocation. The majority of these findings stem from cell culture experiments or mammals and cannot easily be extrapolated to invertebrates; thus, the relevance of persorption is currently not clear [32].

Size is a crucial factor for uptake, with very small particles being able to passively cross membranes while larger ones require active endocytosis [23]. Generally, processes facilitating active uptake into tissues appear to work on particles up to 1 µm [26]. Volkheimer [33] reported the persorption of silicate particles of up to 150 µm in dog tissues, however, as a rare occurrence.

In this context, it needs to be considered that even macrophages, i.e., cells with the highest capacity for phagocytosis, at best incorporate 1 - 3 µm particles [34, 35]. In rare cases, macrophages ingest latex beads > 20 µm [36], but such phagocytic potential is likely restricted to certain cell types. For mammals, Florence [37] discusses M-cells of the intestinal Peyer's patches as an additional route of entry into the lymphatic system or blood supply. This process was later deemphasized by Carr et al. [31] and is not applicable to invertebrates, which lack Peyer's patches. Obviously, our limited knowledge of these processes in mammalian cells and organisms leaves even bigger gaps regarding insight into invertebrates; therefore, we can only assume similarities.

Invertebrates have additional means to protect the integrity of their tissues. Most arthropods, for example, produce a peritrophic membrane, a chitinous layer enveloping the gut contents. This physical barrier prevents direct interaction of epithelial cells and particles [38]. In that case, penetration or destruction of the peritrophic membrane is another obligate requirement for particles to get in contact with the gut epithelium. In *Daphnia magna*, the peritrophic membrane is permeable for 130 nm, but not for 327 nm latex beads [39]. In contrast, Lehane [38] suggests pore sizes between 2 and 650 nm diameter in a number of different arthropod species. Shedding of the peritrophic membrane during ecdysis has been shown to be a significant mechanism for the depuration of CeO_2 nanoparticles in *Daphnia* [40].

Overall, 62 particle sizes or size fractions were investigated in the 31 studies reviewed. Here, tissue translocation was observed in 49 cases. While this implies that a tissue translocation of NP and MP is common, methodological limitations may cause false-negative and false-positive results that need to be addressed using more stringent quality controls, particularly in studies using rather large MP that cannot be taken up by phagocytosis. As many of these issues have already been faced by researchers investigating engineered nanoparticles, we can learn from other fields, accordingly [23, 41]. In addition, a broader range of taxa needs to be considered to widen the scope and to potentially gain insight into prerequisites, mechanisms, and conditions of tissue translocation. Importantly, translocation events need to be linked with adverse impacts to evaluate their biological relevance.

In summary, the potential translocation of NP and MP to tissues is of major toxicological concern. The biological mechanisms by which small plastic particles may enter cells and tissues include passive diffusion, endocytosis and persorption. At the same time, animals have evolved mechanisms preventing particles from entering tissues, such as peritrophic membranes in a range of invertebrate taxa. Out of the 31 available experimental studies, 22 report an uptake of NP or MP into the tissues of several species. While this is biologically plausible for very small plastic particles, some studies claim a tissue transfer of very large MP (Table 2). Since mechanisms facilitating the passage of such large particles are unknown, their tissue translocation is questionable. There is a number of factors

that may result in false-positive results, including leaching of the fluorescent dye, dislocation of particles during histological processing and general sample contamination. This highlights the need to apply rigorous quality assurance and controls in tissue translocation experiments.

Position Table 2

4. Effects of MP and NP in freshwater invertebrates and fish

4.1. Effects of particles alone

(References cited in table 3 and 4 are numbered as T3/1-34 and T4/1-33. Full citations are provided in the supplement).

In parallel to the development of sampling and detection methods for MP and NP, in recent years numerous studies have been published aiming at shedding light on potential effects of MP and NP in freshwater invertebrates and fish. In fact, two recent reviews on environmental issues of MP [42, 43] summarize the state-of-the-science for the toxicity of MP in freshwater biota, however, without critical assessment of their environmental relevance and validity. It is, therefore, one major aim of this communication to evaluate existing toxicity data in such respect and to identify methodological limitations.

When reviewing the toxicity data published until August 2018 (Tables 3 and 4), a multifaceted image becomes obvious with a wide range of particle types, particle sizes and plastic amounts tested in a variety of different species. Most frequently, PE and PS particles in a size range from 0.05 to > 700 µm were tested. PA, PP, PMMA, PVC, PET and other polymers were only used in a few studies. Only few papers also addressed effects of mixtures of different plastic types [T4/21, T4/33]. With respect to test organisms, more than half of the studies use a variety of fish species (including some brackish water species). NP or MP effects in invertebrates were mainly studied in crustaceans (mainly water fleas and gammarids) and, to a minor extent, in mollusks (snails and bivalves), sediment-dwelling organisms such as chironomid larvae, nematodes and annelids. With respect to the investigated effects, a wide range of different toxicological endpoints was addressed. These include studies on mortality, morphological alterations, growth, reproduction, behavior, different cellular, immunological, and metabolic parameters as well as a series of stress indicators including e.g., microbial dysbiosis in the gut.

Likewise, information on exposure conditions in general remains highly inconsistent. Only 27 studies could be identified which either directly provided information on the particle concentrations used or allowed calculation of this parameter. Out of these, 17 studies exclusively used concentrations ≥ 10^4 particles/L, eight studies [T3/6, T3/11, T3/13, T3/20, T3/30, T4/22, T4/24, T4/28] included < 10^4

particles/L, mainly in addition to higher concentrations. Two studies tested MP in sediments [T4/1, T4/29].

In general, there was a trend to test larger particles in lower concentrations and smaller particles (NP) in higher concentrations. The lowest water concentration tested was 50 particles with a size of 420 - 500 µm/L by De Sá and colleagues [T3/30], and the maximum concentration was 1.1 x 10^{13} particles per liter with a size of 70 nm in the study by Lu et al. [T3/8]. Another study of Sjollema et al. [T4/6] who also tested concentrations in the 10^{12} particles/L range used 50 nm NP particles. Despite their small size, however, such extraordinarily high concentrations result in high ratios of plastics in the medium (calculated for the Lu et al. study about 0.15 % of the medium with a rather large surface of about 0.15 m^2, assuming spherical particles). The study with the lowest proportion of plastics in the medium was conducted by Guven et al. [T3/20], who tested 97 µm MP at a concentration of 100 particles per liter.

The studies providing information on particle concentrations were assessed with respect to the environmental relevance of at least one of the conditions tested. For that purpose, exposure concentrations were compared to environmental concentrations of MP of the respective size shown either in Table 1 or in chapter 2 (data for the river Elbe, Germany). However, since no exposure data for particles < 5 µm were available, seven studies which used such small particles could not be assessed for their environmental relevance [T2/13, T3/24, T4/15, T4/18, T4/22, T4/26, T4/31]. Two further papers [T3/6, T3/11] could also not be assessed, since no exact particle size was given. Out of 18 papers assessed, only three studies tested environmentally relevant concentrations [T3/20, T4/28, T4/29]. Only in one [T4/29] of these three studies, in which PE particles (1 - 126 µm) were added to sediment, an MP-induced impact on mortality, growth and emergence of chironomids was found. The two other studies [T3/20, T4/28] did not report any adverse effect of PS or PET particles in fish or in gammarids after exposure to environmentally relevant concentrations of these polymers. In addition, it has to be considered that, most probably, negative results are not fully represented in the published literature.

In 11 out of the remaining 15 studies, which often used very high particle concentrations, MP negatively affected at least a single one – though not always the same – of the endpoints investigated [T2/10, T2/23, T3/10, T3/13, T3/16, T3/30, T4/1, T4/21, T4/24, T4/25, T4/27]. For fish, histopathological effects in the intestine and the gills, inflammation in the liver, negative impact on the immune system and on blood parameters, alterations of metabolic profiles indicating disturbed lipid and energy metabolism, increased activity of biotransformation enzymes, decreased activity of acetylcholinesterase, significantly increased oxidative stress parameters, alterations in the composition of the gut microbiome, and changes in feeding / predatory behavior were reported. In

invertebrates, mainly influences on mortality, growth, reproduction and stress responses were described. One paper has reported an impact of MP on bacterial richness in sediments [T4/1].

For 36 studies, we could not evaluate their environmental relevance due to either lacking information on particle concentrations or since solely mass concentrations were provided. Among these, 21 studies addressed effects of MP, ten studies those of NP [T2/7, T2/19, T3/3, T3/4, T3/22, T3/27, T3/28, T4/3, T4/5, T4/7] and four studies covered sizes in both the micro and the nano ranges [T4/2, T4/11, T4/12, T4/20]. In a single study, no information on particle size was given [T3/7]. In 16 of the MP studies, adverse effects were reported for at least one of the endpoints investigated in either fish, invertebrates or algae. All ten studies investigating NP found effects of NP particles, as did the four studies on both NP and MP.

In summary, although there is a considerable number of toxicity studies, a conclusive assessment of the risk posed by NP and MP for aquatic organisms is hardly possible up to now. This is due to the fact that, for larger particles, most MP concentrations tested were orders of magnitude higher than environmental concentrations, thus only allowing conclusions on potential hazard. In addition, for smaller MP and NP, environmental exposure data are still scarce due to still existing restrictions of analytical methods. Lacking information on the exact particle qualities and quantities in some of the studies poses a further drawback in this context. Despite these shortcomings and based on (limited) tissue translocation, toxicity and exposure data, the risk for freshwater organisms exposed to MP or NP seems to increase with decreasing particle size. However, to distinguish plastic effects from general particle effects possibly exerted by, e.g., diatom shells or zeolites, non-plastic micro- or nanoparticles of natural origin are recommended as additional 'control treatments' within the experimental designs of future studies.

Position Table 3

Position Table 4

4.2 Modulation of bioavailability and toxicity of chemicals by NP and MP

Recently, a review paper by Wang et al. [56] on the interaction of toxic chemicals with MP summarized existing knowledge on environmental partitioning of compounds, focusing on the occurrence of chemicals in and absorbed to MP, their distribution patterns and the mechanisms of interference between chemicals and plastics, as modulated by extrinsic factors such as pH, salinity and temperature. However, this work largely focuses on the marine environment and summarizes effects on organisms only briefly. Nevertheless, it seems common sense to assume processes to be

similar in freshwater and marine systems and that MP can sorb and concentrate a vast number of environmental toxicants and may thus transfer these into organisms [57, 58]. Upon desorption of such compounds, or release of plasticizers and additives from the plastic itself, these chemicals may exert toxic effects to exposed organisms [T2/4, 59, 60]. Despite existing reports on biological effects of chemicals that have desorbed from MP, it is still under debate whether exposure to dietary MP-sorbed compounds really plays a biologically relevant role, if compared to the high number of other routes of exposure [61, 62]. In a study on the sorption of high molecular weight polycyclic aromatic hydrocarbons (PAHs) to plastic debris, thermodynamics revealed the role of plastics as a vector to transfer PAHs to living organisms to be minimal [63]. Similar conclusions were drawn by Rehse et al. [T4/19] based on a study with bisphenol A in daphnids. Nonetheless, MP undoubtedly represent a novel matrix that provides an alternative surface not only for pollutants, but also for bacteria. MP, at least potentially, act as a vector for sorbed organic matter and, therefore, pose a challenge to our society to react before they cause irreversible harm [64]. Such contradictory views call for a closer look into toxicity-modulating properties of MP, at least based on what we know today.

About ten years ago, Teuten et al. [65, 66] described the sorption and subsequent release of phenanthrene by three types of MP (PE > PP > PVC), and, in 2014, Bakir et al. [67] confirmed the conclusion that transfer of sorbed contaminants to organisms is possible under physiological conditions such as in the presence of gut surfactants and at varying pH and temperature. In a thermodynamic approach, Gouin et al. [61] used a food-web model and calculated that chemicals with log K_{OW} > 5 had the potential to partition at > 1% to PE, and that reductions in body burden concentrations for nonpolar organic chemicals were likely to occur for chemicals with a log K_{OW} between 5.5 and 6.5. Thus, the relative importance of MP as a vector of persistent, bioaccumulative, and toxic substances (PBT) to biological organisms would be of limited importance, relative to other exposure pathways. However, these predictions are based on purely theoretical approaches. In contrast, experimental data from studies on biota are rather scarce, which caused Wagner et al. (2014) in their review [4] to deplore a serious lack of knowledge on biological effects exerted by contaminants associated with MP. Whereas the overall number of data on the impact of MP on vertebrates in field studies has increased in the last years [28, 68], the number of controlled laboratory studies that address the transfer of contaminants to biota *via* MP particles have remained insufficient [5, 69]. The absence of standardized laboratory bioassays is one of the major reasons for the ever-increasing controversy over the fate and biological impact of MP.

In addition to effects in fish by virgin MP [T2/23, T3/6, T3/10, T3/13, T3/15, T3/16, T3/18, 68, 70 - 72] there is increasing concern about its potential carrier function for inorganic [73 - 75] and organic chemicals [T3/9, T3/10, T3/12, T3/14, T3/25,T3/29, T3/33, 76, 77]. Khan et al. [73] investigated the uptake and localization of PE beads ranging from 10 µm to 100 µm and radiolabeled silver in

zebrafish gills, intestine and body tissue over 24 h and did not observe an impact of these MP beads. In contrast, Ag-coated MP beads lead to a significantly reduced uptake rate. However, after 24 h, silver levels were similar in all treatment groups, leading to the conclusion that MP may alter bioavailability and uptake routes of contaminants. However, due to treatment-unrelated mortalities the sample size had to be reduced to facilitate a valid statistical analysis. Nevertheless, this observation supports the idea of equilibrium partitioning as suggested by Koelmans et al. [78].

In a recent study, Lu et al. [75] exposed adult zebrafish to virgin 5 µm PS particles and to a mixture of particles and cadmium (10 µg/L) over 3 weeks to study effects of chronic cadmium toxicity *via* histopathology as well as oxidative stress and functional gene analyses. According to Lu et al. [75], the presence of MP increased the accumulation and toxicity of Cd in zebrafish liver, gut and gills. However, the histological analysis may be questioned for technical reasons, and it is hard to conceive why inorganic materials such as cadmium should adsorb so strongly to MP. In another study, mercury was shown to sorb to MP, and MP altered the bioaccumulation of Hg in sea bass (*Dicentrarchus labrax*) [T3/19]. Another study [T3/31] reported 1 - 5 µm MP to enhance the neurotoxicity (AChE inhibition) of chromium (VI) in common goby (*Pomatoschistus microps*), which made the authors claim 'toxicological interactions' between MP and Cr, even though they do not provide a mechanistic explanation for such an interaction. Particular care seems appropriate in the interpretation of some recent studies on the effects of MP, since part of the studies applied extraordinarily high concentrations without any relevance for the environment (see chapter 3.1) and/or used technically questionable data for their conclusions [79].

In a preliminary descriptive study on the impact of virgin and polluted MP, Pedà et al. [T3/18] documented histopathological lesions in the distal part of the intestine of sea bass (*Dicentrarchus labrax*) not only for virgin particles, but even more conspicuously for polluted MP. In a study with common goby (*Pomatoschistus microps*), Oliveira et al. [T3/29] observed that MP particles modulated either the bioavailability or biotransformation of pyrene. Here, simultaneous exposure to MP and pyrene decreased the energy available through the aerobic pathway of energy production, and MP inhibited AChE activity. A similar study with MP and pyrene, however, failed to show modulations of acute pyrene toxicity to the predatory performance of barramundi (*Lates calcarifer*) and revealed, at best, a slight decrease in swimming speed when applied together [T3/20].

Effects of endocrine disrupting chemicals on fish can be diverse after sorption to MP, including changes in sexual maturation, in gonadal development or in behavioral patterns. Chen et al. [T3/10] described effects of 17α-ethinylestradiol (EE_2) associated to 45 µm PS spheres on zebrafish larval locomotion and gene expression patterns as well as oxidative stress. However, the authors could not distinguish between the portion of 17α-ethinylestradiol taken up directly from the water and the

portion that had been taken up *via* adsorption to MP. Most likely, in this study, alleviation of effects by EE2 was simply due to the availability of freely dissolved 17α-ethinylestradiol.

Likewise, reduced availability of EE2 and phenanthrene due to binding to PVC particles (200 – 250 µm) was investigated by Sleight et al. [76]. The authors observed that gene expression patterns of cytochrome P4501A and vitellogenin were significantly reduced upon the presence of MP. However, it should be noted that the exposure scenario actually excluded the transfer of contaminants into zebrafish *via* MP, since 200 - 250 µm particles are definitely too large to be taken up by larval zebrafish. However, the authors observed that phenanthrene was 48 % more bioavailable than predicted by a linear sorption model, thus indicating potential interference of MP with the transfer of the compound into biota.

Rainieri and colleagues [T3/14] investigated the effect of low-density PE MP of 125 - 250 µm after sorption of a mixture of perfluorinated flame retardants, polychlorinated biphenyls and methylmercury in adult zebrafish over 3 weeks. Particularly in the liver, the diet supplemented with 2 % MP and the contaminant mixture produced strong effects, whereas MP alone did not produce any relevant effects. However, again, important information on the amounts of MP applied, density of the polymer type and concentration of single substances of the contaminant mixture are missing, which makes it difficult to agree with the authors´ conclusions that contaminants have been transferred from the plastic particles to zebrafish tissues. In another study, however, Nematdoost Haghi and Banaee [T3/7] argued in similar directions. According to the authors, their results indicate that increasing concentrations of MP increased toxic effects of paraquat on biochemical blood parameters in common carp (*Cyprinus carpio*). Likewise, Guilhermino and colleagues [T4/31] found MP to increase florfenicol-elicited oxidative stress and anti-oxidant defenses in the freshwater clam *Corbicula fluminea*.

Similar results were reported for the marine microalga *Tetraselmis chuii* which had been exposed to MP of small size (1 - 5 µm in diameter), pharmaceuticals (procainamide or doxycycline), or mixtures of MP with one of the pharmaceuticals [80]. Whereas MP alone had no significant effect on the growth rate of the algae, the toxicity of both pharmaceuticals was enhanced in combination with MP. However, concentrations of both MP particles and pharmaceuticals were probably orders of magnitude higher than what may be expected for field situations. In another study, PE beads increased triclosan toxicity in *Acartia tonsa*, a marine copepod 81], which speaks in favor of the role of MP to potentially act as a vector for toxicants.

In a mechanistic study on the potential transfer of benzo[a]pyrene *via* PE along a laboratory model aquatic food chain comprising *Artemia* and zebrafish, Batel et al. [T3/9] were able to track the uptake of benzo[a]pyrene into *Artemia* and the subsequent transmission into zebrafish. In contrast, virgin

particles that were not loaded with polar organic pollutants (POPs) did not cause any observable physical harm in the intestinal tracts, although parts of the particles were retained within the mucus of intestinal villi and might even have been taken up by epithelial cells (at low rates, however). The transfer of associated benzo[a]pyrene could be documented by both an ethoxyresorufin-*O*-deethylase (EROD) assay for CYP1A induction in zebrafish gastrointestinal tract and *via* fluorescence analyses (autofluorescence of benzo[a]pyrene). Thus, Batel et al. [T3/9] were the first to document in an experimental approach that contaminants adsorbed to MP may desorb upon contact with the intestinal milieu and can be taken up across the intestinal epithelium. Yet, although MP could thus be shown to function as vectors, benzo[a]pyrene accumulation was not high enough to induce adverse effects in zebrafish.

In a follow-up study on MP accumulation patterns and transfer of benzo[a]pyrene to adult zebrafish as well as to zebrafish embryos, Batel et al. [T3/12] demonstrated that these do not permanently accumulate at high amounts on adult zebrafish gills: Most particles only superficially adhered to the mucus layer on the filaments, which is constantly renewed. In contrast, especially small MP (10 µm) accumulated at significant rates on the surface of zebrafish egg chorions. Most importantly, in either exposure scenario, a transfer of benzo[a]pyrene from adhering MP to biota could clearly be visualized by means of fluorescence tracking and EROD induction. Yet, again, benzo[a]pyrene from spiked MP did not reach concentrations sufficient to induce morphological effects in both adult and embryonic fish.

The key to understand the potential of MP to act as vectors of toxicants likely lies in experiments that consider different particle sizes of MP. In a comprehensive study on both MP and NP, which were tested for their joint toxicity with phenanthrene to *Daphnia magna*, Ma et al. [T4/20] showed 50 nm NP to significantly enhance bioaccumulation of phenanthrene-derived residues in the daphnids and to inhibit dissipation and transformation of phenanthrene in the medium, while MP of 10 µm size did not exert any effects. Likewise, NP have been reported to enhance uptake and neurotoxicity of bisphenol A in adult zebrafish [T2/22]. In contrast, larger PA particles failed to increase and even lowered the toxicity of bisphenol A in *Daphnia magna* [T4/19]. Thus, it seems reasonable to assume that NP are generally more relevant as vectors for toxicants, not only because of their relatively higher surface ratios [82], but – more importantly – because their low size allows them to be subject to cellular phagocytosis (see chapter 3). In addition, the role of surface-sorbed proteins or mucopolysaccharides, which are released by the intestinal epithelium and bound to nanoplastic particles (eco-corona) needs to be considered. Such an eco-corona has been shown to be responsible for an increased uptake of NP and a less efficient removal of these particles in the gut of *Daphnia magna,* which affects the rate of subsequent feeding [83]. Indeed, the accumulation of particles in the intestinal lumen and insufficient clearing seem to affect feeding and absorption of substances

mechanically. MP were shown to reduce predatory performance in the common goby [T3/34], and PA fibers reduced the assimilation efficiency in *Gammarus fossarum* [T4/26]. In consequence, the toxicity of toxicants, even pesticides, can be reduced in the presence of high particle concentrations, although sorption processes were shown to be excluded [84].

For the potential transfer of contaminants *via* MP particles, several conclusions can be drawn: (1) Sorption of substances to the surfaces of MP and NP seems to be a prerequisite for the particles´ role as vectors, but the crucial parameter for toxicity is the desorption in the vicinity of cells and tissues, particularly in complex media secreted by, e.g., intestinal epithelia. Without desorption of contaminants from MP, even bacterial communities remain unaffected in the presence of high concentrations of PAHs loaded to MP [T4/1]. (2) Probably simply due to their smaller size, NP seem to be more relevant in modulating the toxicity of chemicals than MP. Several mechanistic explanations appear likely for this, such as a possible interaction with secreted biomolecules/mucus, mechanical constraints to chemical diffusion or a more efficient cellular uptake and tissue translocation (chapter 2). However, none of these has been proven so far. (3) There is increasing evidence for the potential of a trophic transfer of MP-associated contaminants [85], but this also holds true for chemicals adsorbed to natural micro-sized particles. In fact, under field conditions, plastic particles compete with both natural particles (e.g., suspended matters) and a vast variety of dissolved organic matters for binding of contaminants. As of today, however, controls with both suspended and dissolved natural materials have never been considered in laboratory experiments. Whether and to what extent the observations made under controlled laboratory conditions so far are relevant for the situation in the field therefore still remains to be clarified. Given the low abundance of plastic particles relative to natural particles present in freshwater and marine systems, exposure to contaminants *via* plastics is most probably of far less importance than exposure *via* ˈnaturalˈ particles taken up by filter or sediment feeding or predation [59, 78, 82]. Finally, effects of polymer composition and morphology, particle fouling and degradation as well as more realistic exposure scenarios and particle concentrations need to be considered in future laboratory studies [4, 69].

5. Conclusions

The present review provides a multifaceted, but fragmentary image of MP- and NP-induced effects in freshwater fish and invertebrates. It documents the use of numerous different particle qualities and quantities under numerous different exposure conditions (MP applied as particles/L; mg/L; mg/kg; % of diet etc.) in a wide range of species. Studies using high particle concentrations – even though these presumably occur in the environment only for small MP or NP – are overrepresented in number. In addition, shortcomings exist in sampling and detection of NP/MP [86], which is especially

true for small MP or NP, both preventing a solid risk evaluation to date. This is even more important, when considering that, in addition to plastic particles, water bodies contain large quantities of natural particles in comparable size ranges competing with plastics as possible stressors for aquatic organisms. In this context, the concentration data collected for the river Elbe by the MiWa consortium are important, because these demonstrate that less than 10 out of 10^6 particles in a surface water sample consist of plastics.

On the basis of existing data for MP concentrations in freshwater ecosystems and the results obtained by MiWa, part of the reviewed effect-related publications was evaluated with respect to their environmental relevance. It becomes obvious that only in very few cases environmentally realistic MP concentrations were tested, out of which two observed adverse effects.

Capacities of MP/NP for modulation of adverse effects exerted by other chemicals were reported by a series of papers, and most of them showed the toxicity of chemicals to be reduced by MP. It has to be considered, however, that, also here, relatively high particle concentrations have been tested.

Furthermore, it should be noted that MP uptake by organisms needs a differentiated view considering particle sizes and surface charges. It is necessary to distinguish between particle passage through the intestinal tract and a translocation into cells and tissues. Whereas only very small NP particles are able to passively pass membranes, processes like phagocytosis or persorption also allow uptake of larger particles into cells and tissues. Importantly, quality assurance needs to be improved to avoid false-positive results for tissue translocation.

Based on available environmental concentration and existing toxicity data the risk posed by larger MP to cause harm to biota may be rather low. Much higher putative risk has to be allocated to small MP with a diameter of just few micrometers and NP, because (1) their environmental concentrations are expected to be much higher than currently known and (2) smaller particles run a higher risk of uptake into cells and tissues. In addition, NP have a much higher surface area ready to sorb and desorb a range of chemicals, and thus, as they can more easily enter cells, adverse effects in organisms by NP are more likely. For these small particles, however, scientific knowledge is fragmentary at best. In this context, comparing the toxicity of plastic and naturally occurring particles is recommended as a key to better understand the hazard posed by MP and NP to freshwater organisms.

Author contributions:

RT initiated this paper and wrote the abstract, introduction and conclusions. MP and YM wrote chapter 2 and NZ, SH, TPK, ASR and MJ contributed to it. ChS and MW wrote chapter 3 and TG and CS provided Figure 1. RT, SK and HS wrote chapter 4.1. TB, H-RK and LH wrote chapter 4.2.. RT wrote chapter. ASR, H-RK, MW, RT, TB and TK revised the whole manuscript.

Abbreviations

AChE: acetylcholinesterase

BkF: benzo(k)fluoranthene

Chg H: choriogenin

COX: cytochrome oxidase

CS: citrate synthase

CYP1A: cytochrome P450 1A

Erα: estrogen receptor α

EROD: ethoxyresorufin-O-deethylase

ETS: electron transport system

GPx: glutathione peroxidases

GR: glutathione reductase

GSH: glutathione

GST: glutathione-S-transferase

LDH: lactate dehydrogenase

LPO: lactoperoxidase

MiWa: Microplastics in the Water Cycle

MP: microplastics

n.d.: not determined

NP: nanoplastics

PA: polyamide

PE: polyethylene

PMMA: polymethacrylate

PP: polypropylene

PS: polystyrene

PTFE: polytetrafluoroethylene

PVC: polyvinyl chloride

ROS: reactive oxygen species

SOD: superoxide dismutase

Vtg: vitellogenin

µ-RM: micro-Raman spectroscopy

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