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# It's a matter of microbes: a perspective on the microbiological aspects of microand nanoplastics in human health

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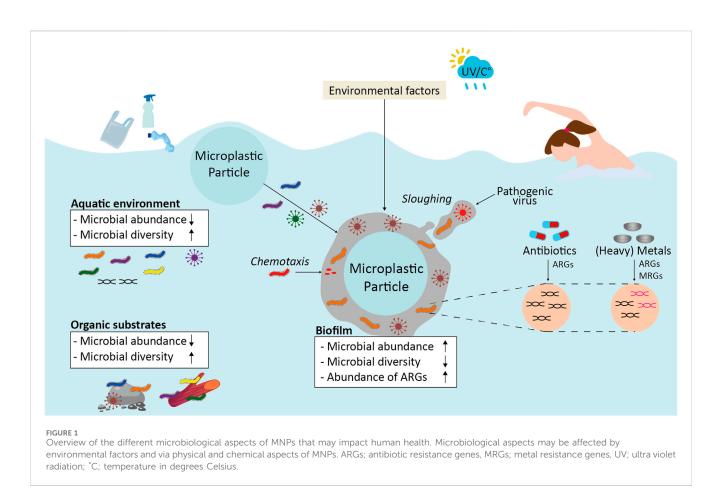
Micro- and nanoplastics (MNPs) are everywhere: in the air we breathe, in our food and in virtually every type of water. Currently, it is unknown whether, and to what extent, these MNPs are hazardous to human health. Identifying risks of physical and chemical aspects of MNPs has gained a lot of attention over the last few years, and efforts have been made to quantify these risks. In our opinion, the momentum delivered by these efforts should be used to highlight the relevance of including another important aspect of MNPs: their associated microbes. Although more and more studies describe MNP-associated microbes, the interplay between physical, chemical and microbiological aspects are lacking. Hazard identification parameters describing this interplay are crucial to risk assessment strategies, yet the majority of effort has been directed towards optimizing human exposure parameters. Here, we address the importance of including microbiological aspects of MNPs in the risk assessment of MNPs. Physical and chemical aspects of MNPs impact the diversity and abundance of microbes and antibiotic resistance genes (ARGs). In turn, environmental factors (e.g., UV radiation, antibiotics) may impact the microbial composition directly, or indirectly by impacting physical or chemical MNP aspects. Future efforts should be directed towards investigating this interplay in order to determine the effects of these dynamic outcomes on human health. We believe that elucidating these pieces of the puzzle is needed to ultimately perform a more holistic risk assessment of MNPs on human health.

#### KEYWORDS

micro-and nanoplastics, microbes, risk assessment, biofilms, pathogens, human health, antimicrobial resistance

#### **1** Introduction

Plastic debris including microplastics (<5 mm, MPs) and nanoplastics (<1 µm, NPs) are ubiquitous in environmental compartments such as air, soil, water and certain foods (Vethaak and Legler, 2021). Humans are exposed to MNPs mainly via inhalation, ingestion or skin contact (WHO, 2019a). Although the European Union has adopted several proposals aimed at limiting intentional plastic pollution, 200–600 olympic size swimming pools of MPs are unintentionally released in the environment every year (European Comission, 2024). Due to their ubiquity, toxicity and ability to cover large distances in aquatic environments and in the atmosphere, MNPs are a human health concern (Vethaak and Legler, 2021). However, the (quantitative) risks of physical and chemical properties of MNPs on human health are unclear. Literature addresses



the current limitations in MNP reference material, available analytical methodology and study comparability (Brachner et al., 2020) and highlights the effects of MNPs on environmental ecosystems (Sana et al., 2020) and human health (Gonzalez-Acedo et al., 2021; Yee et al., 2021; Gruber et al., 2023). Additionally, MNPs may be colonized by microbes, hereby potentially posing microbiological threats to human health (Loiseau and Sorci, 2022).

The human body is continuously exposed and home to microbes. Some microbes are beneficial to human health (e.g., Lactobacilli in the gut), while others are pathogenic and may cause respiratory infections (e.g., Legionella pneunomphila), gastrointestinal infections (e.g., noroviruses) or skin infections (e.g., Pseudomonas aeruginosa). Whether microbes are pathogenic depends on the levels and nature of their virulence factors and thus their ability to evade or inhibit host defense mechanisms. Infectious diseases caused by water- and airborne pathogenic microbes and antimicrobial resistance (AMR) are considered some of the most important threats to human health (WHO, 2019b), of which the latter one contributed to 4.95 million global deaths in 2019 (Antimicrobial Resistance Collaborators, 2022). Understanding the role that plastic debris plays in the spread of pathogenic microbes and AMR in environmental compartments is critical for quantification of the overall impact of aspects (physical, chemical and microbiological) of MNPs on human health.

Although an increasing number of studies assess the microbiological aspects of MNPs, information on the physical or

chemical properties of MNPs are often lacking since no standardized material is used or since laboratory equipment, funding or expertise only allows for the detection of one of those MNP aspects. This results in only static microbiological parameters, while understanding the dependability between MNP aspects helps to understand and predict these microbiological parameters. Here, we provide perspective on how this dynamic interplay can be addressed. The parameters contributing to this interplay are, despite the increasing number of publications on the role of MNPs in the spreading of microbes via air, soil and foods, best characterized in aquatic environments. Notably, aquatic environments combine the presence of (pathogenic) microbes while facilitating long-distance transport of plastic debris and MNPs, hereby enabling particle dissemination and human exposure. First, we provide perspective on which different microbiological aspects of MNPs may impact human health (Figure 1). Next, we describe how physical (particle size) and chemical (polymer type) MNP properties affect MNPassociated microbes. Lastly, we describe how environmental factors (e.g., antibiotics and UV radiation) may affect these microbes directly or indirectly, via MNPs.

## 2 MNP-associated biofilms are dynamic

MNPs were collected in various aquatic environments ranging from marine environments to freshwater. Planktonic microbes that stick to the surface of MNPs may form a microbial biofilm in which

different types of microbes form a complex network of cells that stick to each other and to the MNP surface. The ecosystem that surrounds the MNP surface is referred to as the 'plastisphere' (Zettler et al., 2013). Potentially pathogenic bacteria (e.g., Vibrio spp., Arcobacter spp. and members of the Campylobacteraceae) (Zettler et al., 2013; Amaral-Zettler et al., 2020; Mughini-Gras et al., 2021; Metcalf et al., 2022), diatoms and algae (Audrezet et al., 2021), fungi (e.g., Aspergillus) and viruses (e.g., Rotavirus, Poliovirus, Norovirus, SARS-CoV-2) (Moresco et al., 2021) may inhabit the plastisphere of various MNP polymer types. Thus, MNPs that accumulate in these aquatic environments may serve as a habitat for a wide range of pathogenic microbes. The composition of biofilms is dynamic, since it is affected by physical and chemical properties of the MNP, as well as by environmental factors such as temperature, pH, salinity, UV radiation, nutrient limitations and natural organic matter (NOM). These combined factors might lead to competition between microbes for nutrients or binding sites on the particles, constantly allowing the biofilm to change in composition. Continuous subjection to changes in environmental factors allows for growth, attachment and detachment of (pathogenic) microbes which affect the environmental fate of MNPs. Detachment of a larger portion of the biofilm is called 'sloughing'. Sloughs may allow for the sudden release of (multiple) pathogenic microbes from the biofilm at once (Skraber et al., 2005).

#### 3 Humans may be exposed to MNPassociated biofilms

MNPs with biofilms have been found in virtually every type of water, including drinking-water (WHO, 2019a). The human health risk of these MNP-associated biofilms in drinking-water has been described as low considering that 1) drinking-water sources can contain pollution leading to a higher concentration and diversity of pathogens and 2) the concentrations of MNPs in fresh water are relatively low compared to other possible attachment sites for microbes or biofilms (e.g., drinking-water pipes) (WHO, 2019a). In contrast, a recent study by Yang et al. has detected higher levels of MPs in drinking-water (Yang et al., 2024). They showed that changes in water quality (chlorination, heating) and in environmental factors (freeze-thawing) expedited the release of MPs from polypropylene pipes, suggesting that this phenomenon occurs in a wide range of drinking-water pipes. Additionally, MNPs (that are for instance released from drinking-water pipes) are mostly light weight and persistent and may therefore, together with their associated biofilms, be transported over a long distance. Through sloughing, pathogenic microbes could be released miles from where microbes first attached (Skraber et al., 2005), in environments where these microbes are otherwise not present. Therefore, even whilst overall concentrations found in drinking-water may be relatively low, MNPs and their biofilms are still relevant for human health.

Once MNPs enter the human body they may impact human health via gastric, intestinal, pulmonary and dermal exposure. Local inflammation, apoptosis or oxidative stress may be induced, depending on the cellular uptake mechanism (Yee et al., 2021). It has been described that MNPs can lead to dysbiosis in the human intestinal microbiome (Fackelmann and Sommer, 2019). It is currently unclear whether pathogenic microbes in MNPassociated biofilms affect cellular uptake mechanisms and aggravate the effects elicited by MNPs, for instance via enhancing intestinal dysbiosis.

# 4 MNP size and chemical composition define their microbial composition

The dominant narrative is that the microbial content in biofilms of different types of MNPs found in aquatic environments is less diverse than the microbial content in biofilms of natural substrates (such as stone, wood and plant materials), or in the surrounding aquatic environment (Miao et al., 2019; Mughini-Gras et al., 2021). In contrast, microbial abundance was found to be higher in biofilms of MNPs compared to biofilms of organic material (McCormick et al., 2016; Miao et al., 2019). Furthermore, metabolic pathways in microbes in MNPassociated biofilms are different compared to microbes in biofilms on other substrates (Miao et al., 2019), possibly due to toxic properties of the plastics. Investigating the effect of diversity, abundance as well as the (metabolic) function of MNPassociated microbes is important to get a complete picture of the microbiological risks of MNPs.

The composition of MNP-associated biofilms appears to be determined by physical properties of MNPs such as particle size. In general, the microbial composition of biofilms on MPs is less diverse than those of NPs (Mughini-Gras et al., 2021). NPs are small in size but more abundant than MPs, therefore, the total surface to which microbes can attach is larger for NPs compared to MPs. The larger the total surface, the more space will be available for different microbes to attach. An increased surface-to-volume ratio also means an increase in chemical reactivity and hydrophobicity of the particle, which may contribute to the difference in microbial composition. For instance, adsorption rates for viruses are higher on smaller particles (2  $\mu$ m) than on larger particles (100  $\mu$ m), possibly due to a higher electrical (zeta) potential in smaller particles, allowing for enhanced adsorption of viral particles to the plastic surface (Lu et al., 2022). Whether this is similar for virus adsorption to NPs, needs to be investigated. After virus absorption, the zeta potential of the plastic particle surface changes (Lu et al., 2022), which may affect the binding of other viruses, or other types of microbes. Studies with pharmaceuticals have shown that in addition to hydrophobicity of the MNPs, ionic strength also affects the sorption potential of MPs (Puckowski et al., 2021; YangLi et al., 2023). When measuring biofilm compositions, these physical properties of MNPs should be measured and reported as well, to correlate the biofilm composition to physical properties and link this to human exposure and uptake mechanisms.

Notably, the composition of MNP-associated biofilms is also affected by the chemical composition of MNPs, including the release (leaching) of chemicals (Li et al., 2022; Nelis et al., 2023). This (chemical) composition varies per plastic polymer type and may therefore differentially affect the microbial composition of MNPs. For instance, MNP polymer types polyethylene and polypropylene can release dissolved organic matter (Amaral-Zettler et al., 2020). Microbes may migrate towards these hydrocarbons to take up dissolved organic matter via chemotaxis (Amaral-Zettler et al., 2020). In contrast, leachates may exert cytotoxic effects on microbes (Sarker et al., 2020; Liu et al., 2022), potentially leading to microbial degradation or inactivation and altered environmental fate of the plastic particles. Notably, the presence of different microplastic-specific functional groups (COOH, NH<sub>2</sub>) affect the microbial composition. COOH groups enhance, while NH<sub>2</sub> groups decrease, virus adsorption rate to plastics compared to plastic particles without functional groups (Lu et al., 2022), hereby selecting for the type of pathogen present on the particle.

The detection and composition of MNP-associated pathogenic microbes (e.g., E. coli and Vibrio spp.) is subject of study and generally performed with sequencing techniques or quantitative molecular detection methods. However, the virulence (pathology) of these microbes is severely understudied, yet provide important pieces to the risk assessment puzzle. A clear example was found in the virulence genes of Rhodococcus equi, a bacterium that can infect immunocompromised humans causing pulmonary infections. These virulence genes were upregulated when in contact with disposable polypropylene, but not when in contact with glass (Hansen et al., 2022), suggesting that MNPs have the potency to affect virulence factors of microbes. However, which properties (physical, chemical or both) attributed to this observation, remains unclear. Studies should therefore focus on the effect of (standardized) MNPs on virulence factors of pathogenic microbes in different exposure-relevant environments. These studies would aid in understanding the plastic-mediated infection risks upon inhalation, ingestion or direct contact of MNPs.

#### 5 MNPs may facilitate the emergence of antimicrobial resistance

Aquatic environments are often polluted with antibiotics, responsible for the rapid emergence of AMR (WHO, 2019b). AMR is achieved via various antibiotic resistance mechanisms including the reduction in bacterial cell permeability, intra- and extracellular sequestration of antibiotics, efflux pumps, mutations at target sites and the formation of biofilms (Imran et al., 2019). Bacteria in the plastisphere harbor different antibiotic resistance genes (ARGs) and pathogenic bacteria profiles than biofilms on natural substrates or bacteria in the surrounding aquatic environment (Wu et al., 2019). Furthermore, the abundance of ARGs (e.g., sul1, ermB) in MNP-associated biofilms was higher compared to bacteria in the surrounding environment (Wu et al., 2019; Shi et al., 2020). ARGs can be transferred to other (pathogenic) bacteria via horizontal gene transfer of mobile genetic elements, leading to antibiotic resistance in the receiving bacterial cell. Horizontal gene transfer was found to occur faster between microbes on MPs than between planktonic microbes (Arias-Andres et al., 2018). This was corroborated by the increase in abundance of ARGs and mobile genetic elements on MNPs over time (Imran et al., 2019; Shi et al., 2020). Considering that MNPs might serve as a vector for AMR and are ideal substrates for the long-distance transport of biofilms, MNPs

might expose people to resistant microbes, for example, while swimming, hereby posing a human health threat.

Since the size of MNPs affects the microbial composition of the MNP-associated biofilm, it may also affect the diversity or abundance of ARGs (Yang et al., 2019). NPs contain more reactive oxygen species (ROS) and microbes with a lower membrane permeability than MPs, which may lead to more bacterial cell damage (Shi et al., 2020). This lower membrane permeability found in NP-associated microbes may enhance AMR. Overall, the effects of the physical and chemical properties of MNPs on the diversity, abundance and function of ARGs is understudied, yet would help in assessing the integrated risks of MNPs and would predict which measures could be taken to minimize the change of MNP-facilitated AMR emergence.

AMR is, in addition to antibiotics, driven by other environmental factors, such as the presence of metals in aquatic environments. Some metals are beneficial or even required for physiological functions of microbes (e.g., MNO<sub>2</sub>, Fe, Co) while other metals are toxic for microbes even when present at a low dosage (e.g., Pb, Cd). The presence of (heavy) metals in aquatic environments drives the selection for metal resistance genes (MRGs) (Bartkova et al., 2021). When MRGs and ARGs are present on the same mobile genetic element, co-resistance can occur (Imran et al., 2019; Li et al., 2021). This co-resistance leads to metal-driven antibiotic resistance in nearby bacterial cells for instance when present in an MNP-associated biofilm. Both MRGs and ARGs have been found in potential clinically relevant bacteria including Salmonella enterica, E. coli, S. aureus, Pseudomonas spp., Acinetobacter spp., Listeria spp., Aeromonas spp. and Proteus spp. (Imran et al., 2019), some of which have the ability to form biofilms. Metals may exert a long-lasting selection pressure on ARGs because they cannot easily be degraded. Thus, the environmental effect of (heavy) metals may also exert a long-lasting effect on AMR via MNPs.

# 6 Environmental factors affect the physical, chemical and microbiological properties of MNPs

In addition to the presence of antibiotics or (heavy) metals in aquatic environments, other environmental factors, such as UV radiation, temperature and pH also affect the composition and function of microbes in MNP-associated biofilms. The effect of these environmental factors on the microbes are either exerted directly, or indirectly via affecting the MNP.

Different environmental factors may affect the MNPs (Al Harraq et al., 2022). They may lead to leaching of MNP-associated chemicals (Imran et al., 2019), that could in turn affect microbial survival, composition and function. For example, changes in water quality (heating) and freeze-thawing of plastic pipes accelerated chemical leaching with a distinct chemical profile. Incubation of these leachates with indigenous bacteria in drinking-water led to enhanced cytotoxicity as measured by bacterial ROS production and cell wall permeability and led to a higher abundance of pathogenic bacteria and a change in virulence gene and ARG profiles (Yang et al., 2024).

In addition to leaching, environmental factors might lead to the degradation of particles for instance via UV radiationmediated ageing of the particles. Such a process may result in changes in size, available chemical groups and (bio)availability of surface area of particles (Al Harraq et al., 2022). These smaller particles exhibit more surface area and may therefore lead to more microbial adsorption. The ageing of particles by UV radiation was found to affect virus adsorption rates, with more virus adsorption on UV-aged particles and on particles with no functional groups, or with NH<sub>2</sub> groups (Lu et al., 2022). Furthermore, a higher surface area can lead to enhanced adsorption of chemical pollutants (Al Harraq et al., 2022) that may result in altered attraction and selection of, or toxicity to, microbes. Adsorption of antibiotics and metals by MNPs (Imran et al., 2019), could for instance drive the selection mechanisms for AMR. Notably, adsorption rates of chemical and microbiological components are also (positively or negatively) affected by the presence of NOM in aquatic environments, depending on the type of water, source of NOM and other environmental factors (Junaid and Wang, 2021; Ali et al., 2022).

Environmental factors are dynamic and can, due to the high number of relevant factors and rapid changes within these factors, never fully be mimicked *in vitro*. However, the interplay between environmental factors, microbes and MNPs is unclear yet critical for the risk assessment of MNPs on human health. Studies should therefore focus on elucidating this interplay first, for instance by assessing which factors are dominant when it comes to microbial formation and microbial compositions.

## 7 Discussion

MNPs may play a facilitating role in two major threats to global health: firstly, the potential spread and, secondly, the emergence of infectious diseases and AMR. Currently, the physical and chemical impacts of MNPs on human health are unclear, and the microbiological impacts of MNPs are even more indeterminate. The impact of microbiological aspects of MNPs should be included when assessing the overall risk of MNPs on human health (Figure 1). For this, quantitative risk assessment frameworks and input data are needed. We believe that steps should be taken towards further characterization of microbial biofilms on MNPs found in different environments. This should include the elucidation of biofilm properties such as the characterization of different biofilm-associated microbes and their persistence and virulence. Since MNPs are continuously exposed to environmental factors, their physical, chemical and microbiological properties are dynamic. The interplay between these different properties of MNPs should be studied, rather than snap shots of their compositions, in order to understand these dynamics better. Investigating AMR traits generated under different environmental conditions and in the presence of antibiotics and metals is of particular importance. Of note, NPs, rather than MPs, and their associated biofilms may enter the human body and translocate across human tissues. The routes of exposure and translocation of NPs impact their location in the human body and thus the concentration of both NPs and microbes in different tissue compartments. Understanding the various ways in which microbiological aspects of MNPs may impact human health will provide insight in the occurrence and emergence of infectious diseases and AMR and thus in selecting the best preventative strategies possible to avert integrated MNP health impacts.

### Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

### Author contributions

MS: Conceptualization, Visualization, Writing-original draft, Writing-review and editing. AR: Funding acquisition, Writing-original draft, Writing-review and editing.

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# **Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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