A critical review of microbiological colonisation of nano- and microplastics (NMPs) and their significance to the food chain

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The risks posed to human health by NMPs associated microorganisms are hard to quantify at this time. There is clear scientific evidence that NMPs have found their way to every part of the biosphere from urban centres to the poles, remote oceans and the deep sea. However, research has particularly focused on the marine environment, leaving gaps of knowledge on environments which are actually closer to most humans such as fresh waters and soils. The research team that we have assembled for this project (Cefas, FERA, and University of Exeter) represents a unique consortium of UK governmental and regulatory laboratories, and academic partners to achieve this desk-based critical review.

INTRODUCTION

- This project provides an authoritative and critical review regarding microbial interactions with microplastics and the potential risk that they pose to human health through the food-chain.
- This review summarises the available scientific evidence (including peer review and grey literature sources) concerning the diversity of microorganism that colonises microplastics, outline the key pathways that these microbiologically contaminated microplastics are able to enter the food chain from environmental sources (e.g. from water, soil and air) and the associated risks these may pose to human health.
- However, the actual risk of nano and microplastics (NMPs) as a potential vector of pathogens into the human food chain is currently unclear. To address this, this report reviews the latest literature available on four topics:

  - **NMPs in the environment**
  - **Pathways of colonised NMPs into food chains**
  - **Interactions between NMPs and microorganisms**
  - **NMP-specific microbial risks to consumers**

- For each topic, peer reviewed and grey literature was reviewed with a structured approach as described in the 'review methodology' section .
- After an overview of the four topics (WPs 1 to 4), findings are discussed, and gaps identified which need to be researched to better determine the potential risks of interactions between environmental NMPs and human pathogens.
- The knowledge acquired from this project will be disseminated through peer-reviewed publications, conference presentations and lay-audience events.
- The key output from this review will be the production of an **authoritative full synthesis report and special topic report document that will provide a collated and impartial summary of the scientific evidence on the impacts of microplastics and human health, utilising the most relevant and contemporary scientific data available**.

Review methodology

- The literature searches for this review were carried out using a combination of database searches, the application of expert knowledge of existing literature and the use of team-member networks.
- All search results were saved in a bibliographic database created with Microsoft Excel. This database was used during the sifting process to record those references that were kept or rejected. Following sifting, the references kept were stored in a Mendeley Group to allow for easy sharing between the project team and for easy citations in the report.
- Before literature searches were carried out, a list of search terms was agreed by consensus between the review delivery team and FSA.
- To achieve this, search terms were initially developed separately for each of the four review sections by the teams working on those sections. The search terms were then be reviewed by the rest of the project team and FSA before being finalised.
- Each section of the review had its own set of search terms to allow the search results to be categorised between the review sections. This allowed the results to be easily separated later on in the sifting process and to be reviewed by topic experts.
- The search terms were developed to be inclusive to ensure that the as many relevant articles were found as possible. However, due to the broad scope of this review, it was also necessary to include exclusion terms within the searches to limit the results that were not relevant.
- To search for the peer-reviewed literature used in this review, the Web of Science database (http://www.webofknowledge.com) was used. Web of Science was selected for this purpose by consensus with the project team and FSA due to its broad coverage of scientific literature and the universal access available to members of the project team. Web of Science searches were carried out on 03/02/2020.
- For each search that was conducted, the results were exported to Excel for inclusion in the bibliographic database. After all Web of Science searches were completed, duplicate entries in the bibliographic database were removed.
- The total number of unique search results from the Web of Science searches was **8,703**.
- Addition of some grey literature and other relevant literature brought the total number of articles considered for review to **10,016.**

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WP1: Overview of NMPs in the environment

- WP1 provides an evaluation of where NMPs are found; their sources; the type of plastics from which NMP are made; the residence time of NMP in the environment; and their accumulation zones.
- Several authoritative reviews and scientific studies have attempted to provide a detailed analysis of where NMPs are found in the marine environment. [1-7]
- It should be noted that sampling protocols, analysing methodologies, and the units of MPs abundance are yet to be standardized, making direct comparisons between datasets difficult. [4]
- Furthermore, because sufficient measurements of plastic pollution are not available for all the world's oceans, mapping the microplastic abundance across the entire global ocean system has proven technically challenging. [3].
- Currently, the most widely used synthetic plastics are low- and high-density polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS) and polyethylene terephthalate (PET). Altogether, these plastics represent ~90% of the total world production [8, 9].
- Several studies have attempted to provide a global mass of plastics in the ocean system, using collected using surface-trawling plankton net-based approaches. Cózar et al 2014 estimated that there were 7–35 thousand tons of plastic waste in the ocean system [11]
- A substantially higher global burden of plastic contamination was estimated by van Sebille et al. (2015) which indicated that there were 93 to 236 thousand metric tons of waste globally [5].
- Limited field evidence from higher trophic level organisms in a variety of habitats suggests that trophic transfer of microplastics may be a common phenomenon and occurs concurrently with direct ingestion [12] .
- A recent estimation that 99% of plastic entering the oceans will eventually reach the ocean floor, including buoyant polymers. In the marine environment plastics degrade through physical, chemical and biological processes [8,13] .

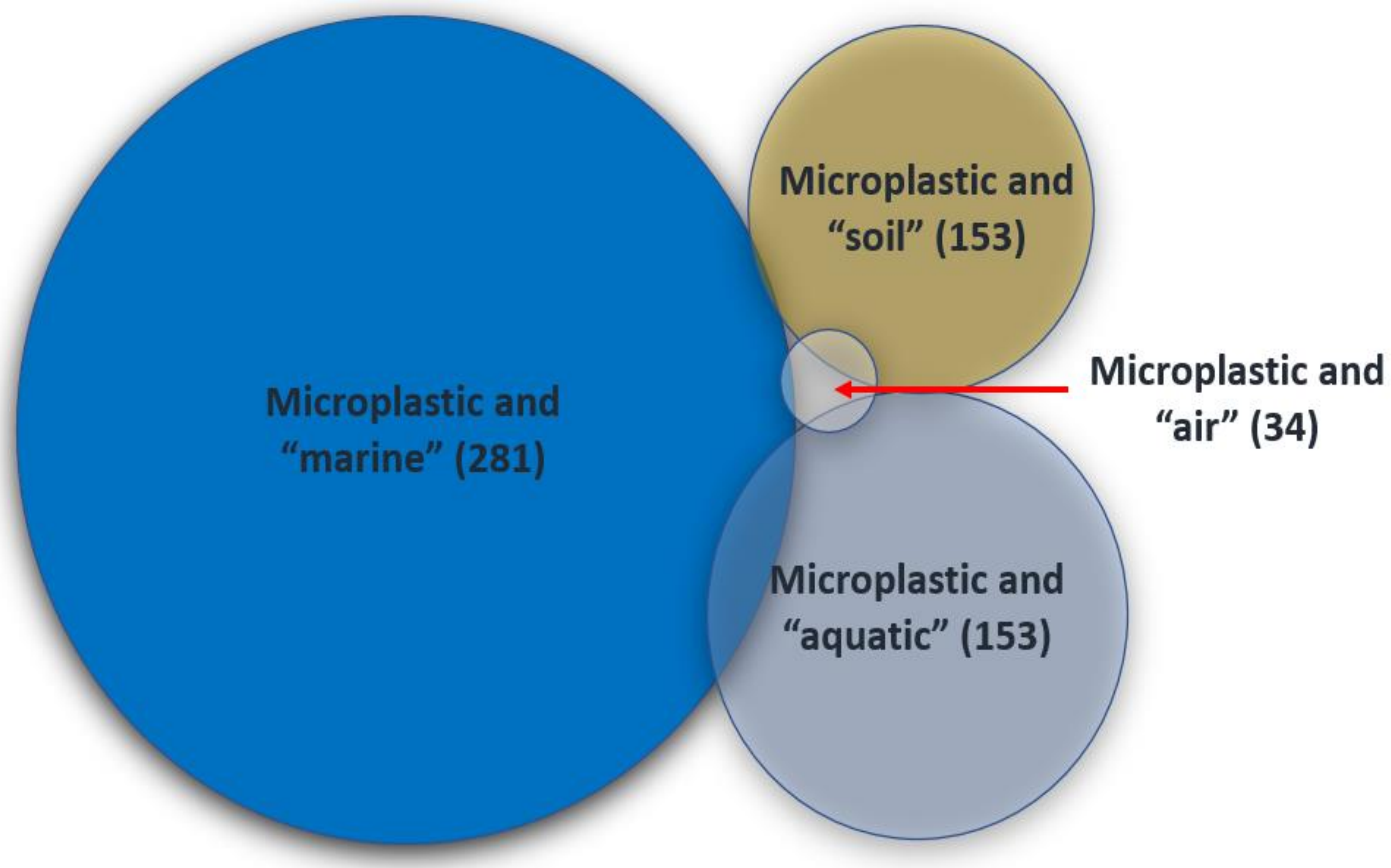

Figure 1. Published scientific studies with the term "microplastic" in the title alongside other environmental search terms (until Feb 2021) available on pubmed.gov. Figure generated 26th February 2021.

WP1: Evidence gaps and recommendations for further work

1. There are currently a lack of standardised and universally-applied methods to collect data
2. The need for internationally accepted definition regarding NMPs.
3. A huge number of studies have looked at the fate of NMPs in the environment, in particular marine settings, however less emphasis on other environments.
4. Little is known about the transformations of plastics in seawater, including the time scales of degradation and its ultimate sinks.
5. A lack of standardised, laboratory-based data on long-term fate of NMPs.
6. Although the presence of NMPs have been widely reported in certain food commodities, there are very little detailed studies on these at ecosystem-levels.
7. There is a lack of data on microplastic contamination and the cycling of these contaminants between different environmental compartments.

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WP2: Interaction of NMPs and microorganisms

- In the environment, plastics are exposed to a wide variety of conditions. Once exposed to a non-sterile environment, plastics will immediately start to interact with the microorganisms that are present in the environment (**Figure 1**).
- The degree to which microorganisms interact with surfaces can vary widely from transient contact, to the development of complex structures of microorganisms and extracellular matrices known as biofilms. Where microorganisms are not physically attached to surfaces, they may be relatively easily removed by water or air currents. If on the other hand, micro-organisms develop complex biofilm structures, they will be more firmly bound to those surfaces [1].
- There is a large diversity of plastics in the environment in terms of their chemical composition, their particle size, their hardness and their roughness. Due to their ubiquity in the environment, they are also exposed to all of the extremes of environmental conditions on Earth and so it would be expected that the interactions between plastics from macro to nano in size will be equally diverse.
- Most studies on biofilm formation re on macroplastics. There is a lack of evidence on the differences in microbial communities between macro- and microplastics.
- There are some reports of enrichment of pathogenic and AMR bacteria on microplastics relative to the surrounding water column. But there is a lack of comparisons with non-plastic substrates, so it is unclear whether this enrichment is specific to plastics or if it is a non-specific substrate effect.
- Most pathogenic bacteria associated with microplastics appear to be transiently bound, and not permanently attached. Leading to the suggestion that the pathogens are "hitchhiking" on the plastic surfaces. A primary microbial colony is needed for permanent attachment of pathogens.
- Horizontal gene transfer is often enhanced in biofilms. There is some evidence of unique AMR gene profiles in microplastic associated bacterial communities.
- Formation of biofilms on microplastics may affect buoyancy, thereby altering their transport in the water column.
- The presence of biofilms on plastics can increase the attractiveness of plastics to other organisms. This may lead to increased shredding (generating smaller plastic particles) and increased consumption and uptake into food webs.

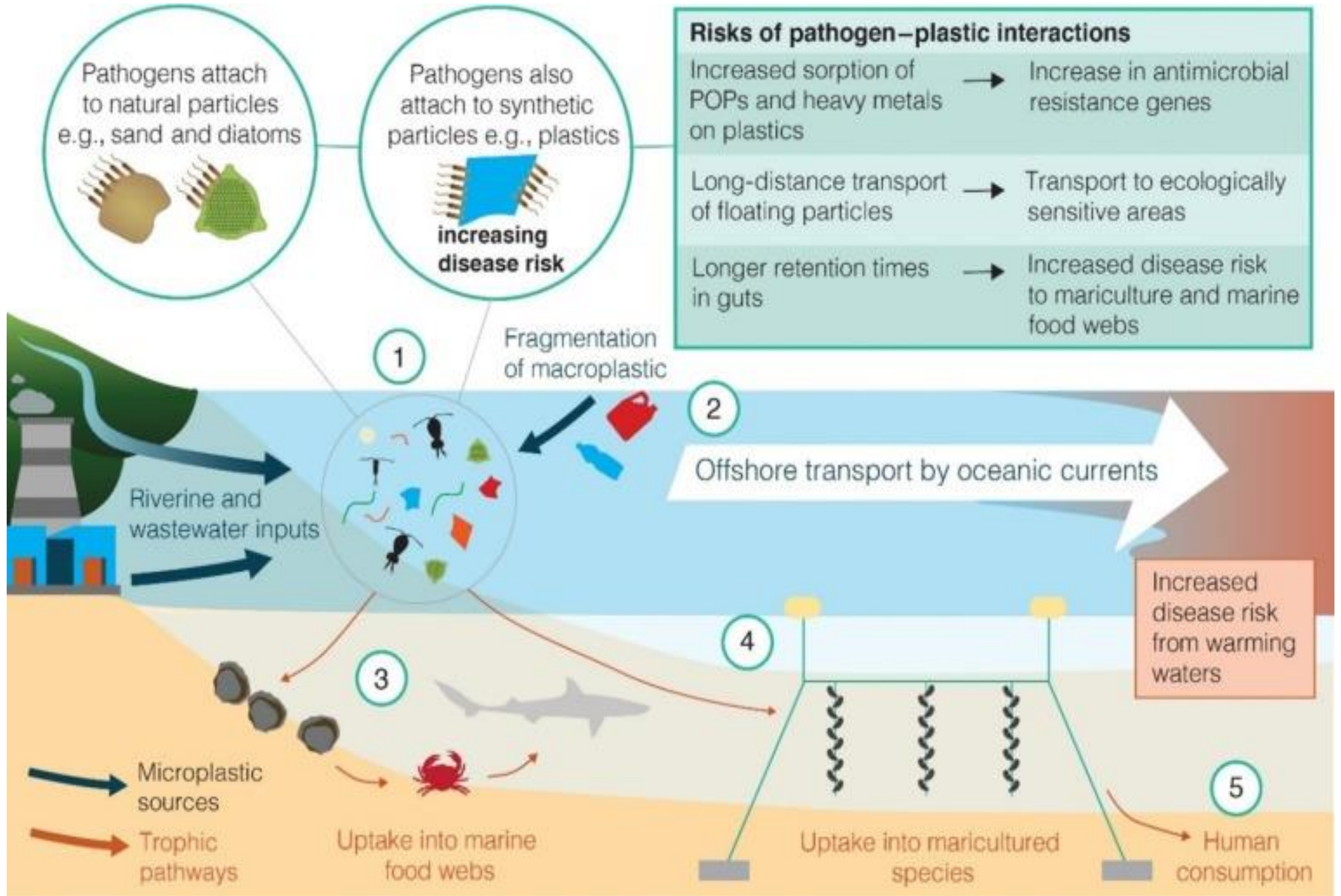

Figure 1. Summary of the Potential Interactions of Vibrios with Microplastic Particles in Marine Ecosystems and How These Might Differ from Interactions with Natural Particles in Terms of the Following Factors. Source: Bowley et al., 2021.

WP2: Evidence gaps and recommendations for further work

1. There is a lack of standardised approaches to investigate the interactions between plastics and microorganisms. Isolating DNA resulting in different result depending on libraries compared to. Lack of characterisation of surface properties makes it difficult to identify key properties for colonisation.
2. There is a lack of inclusion of non-plastic substrates in controlled exposure experiments to better understand the factors influencing colonisation and to determine whether plastic substrate-driven selection is occurring.
3. Further work is needed to determine the role NMP-associated biofilms play in selecting for and/or transporting pathogenic bacteria. , Standardisation
4. Research needs to be expanded in the relatively new field of determining whether NMPs themselves, or sorbed antimicrobials exert a selective effect for antimicrobial resistance through species sorting or de novo acquisition of AMR through mutations or horizontal gene transfers.

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WP3: Pathways of colonised NMPs into food chains

- The articles from the reviewing process were assessed and allocated to categories according to the type of pathway that they represented:
  1. **Airborne**
  2. **Terrestrial**
  3. **Drinking water**
  4. **Shellfish**
  5. **Salt**
  6. **Fish**
  7. **Other foods**

- To date , a limited amount of studies have demonstrated the direct potential for uptake of plastic particles from **airborne vectors** [1,2] . Results indicated that the quantity of particles ingested via inhalation was as important as the quantity consumed via diet. Estimates of quantities of microplastics ingested from airborne sources varied greatly between studies [3] .

- Contamination of **terrestrial systems** by microplastics have been well documented recently. There is however a limited amount of information whether microplastics can be transferred to edible plants. Li et al. (2019a and 2019b) demonstrated the potential for PS beads (0.2 μm) to be transported to roots, shoots, and leaves of wheat indicating potential for transfer to humans following consumption [4,5].

- Lwanga et al. (2017) reported an increase in microplastics from **soils**, to earthworms' casts to chicken faeces indicating potential for transfer of microplastics in terrestrial food webs. While no evidence of microplastic translocation is available, it is unclear whether micro and nano-plastics can be transferred to tissues [6].

- Freshwater sources of **drinking water** have been shown to contain abundant levels of microplastics contamination. Microplastics have been found in many brands of bottled water [7-9] .

- NMPs have been reported for **shellfish** globally. Most studies have been primarily focusing on the detection and quantification of microplastics in mussels and oysters. Annual intakes of NMPs from **shellfish** was estimated in a few studies and ranged from 22.73 to 4919.83 items/person/year depending on the mollusc species being consumed (**Figure 1**) [10,11] .

- The estimations for annual intake of MPs from **salt** consumption are variable, and some numbers low compared to other matrices such as mussels. However, as salt is used and consumed every day, it is important to consider this when looking at long term exposure of microplastics. It is estimated that humans could be ingesting hundreds or thousands of microplastic particles every year, just from **salt** [12] .

- Overall, the data for microplastics in the parts of **fish** generally considered edible is mixed. A wide range of percentage occurrence from 0 to 100%, with an estimate of 0 to 0.7 plastic items per gramme or 0 to 21.8 plastic items per fish.

- Contamination of **food and drink** with microplastics facilitates their entry into the human food chain. Toussaint (2019) reported a microplastic fibre burden in air of 0.3 to 1.5 fibres/m3 and 1.0 to 65 per m3. This equated to 190 to 670 fibres per milligram of settled dust. This dust when landing on plated food or drink could be ingested directly [13] . A similar study was reviewed by Ribeiro (2019) who also found that entry into humans via inhalation of airborne particles was as common as ingestion of deposited microfibres on food from household dust [14].

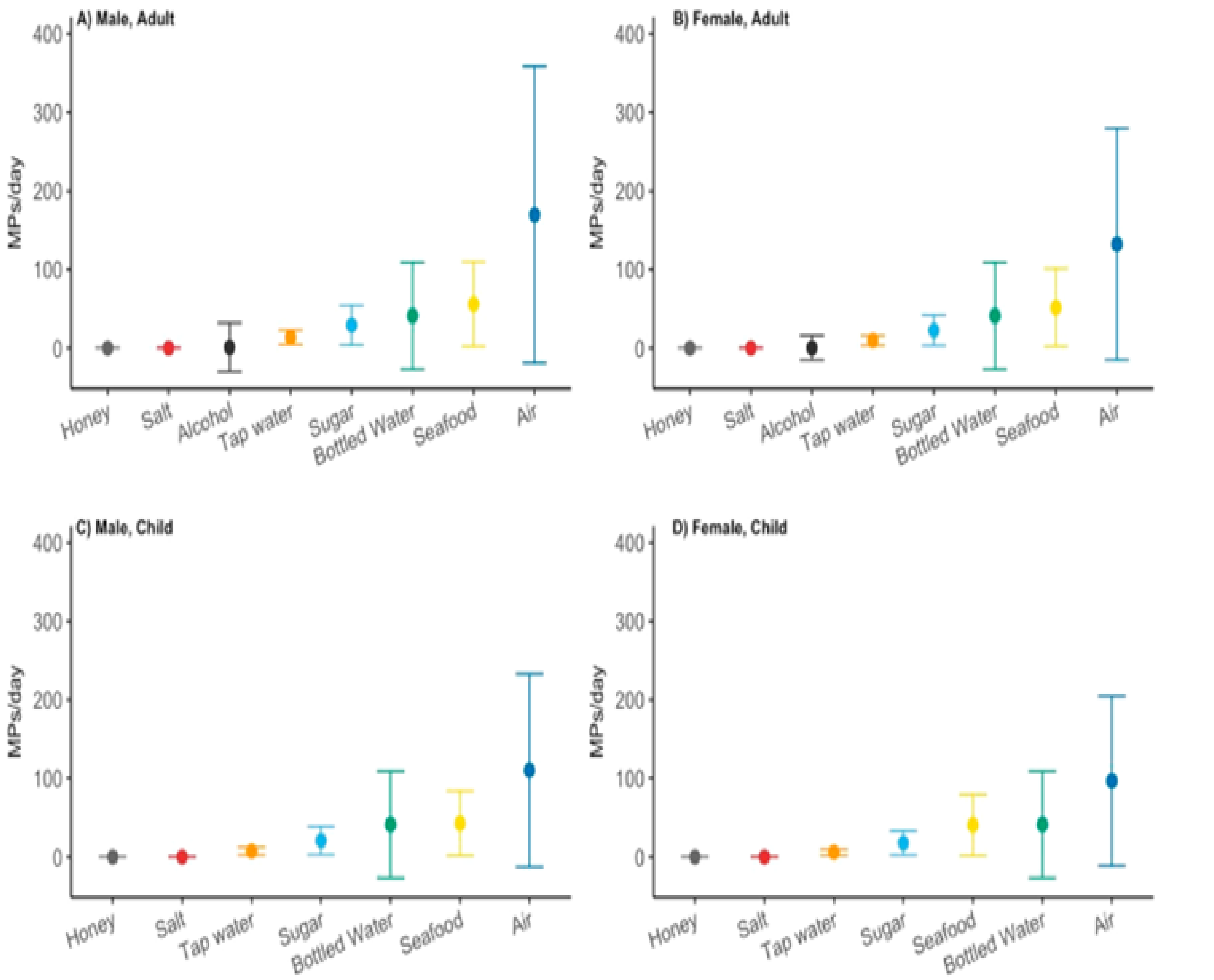

Figure 1. Mean and standard deviation of MPs concentration within each source of ingested MPs including salt, alcohol (beer), seafood (fish, shellfish and crustaceans), added sugars (sugar and honey), water (bottled and tap), and air in (A) male adults, (B) female adults, (C) male children, and (D) female children. (from Cox et al., 2019).

WP3: Evidence gaps and recommendations for further work

1. There has been a strong focus on microplastics in the environment. Far less has been done on the route between that environment and the consumer.
2. Large amounts of data are available on seafood but not for the product that has passed through processing and packaging to points of sale and people's home.
3. For most other types of food, there is no data for any part of the process. This may lead to an exaggeration of the importance of inhaled NMPs as many major parts of our diet (meats, vegetables) lack sufficient data.
4. There is also data missing for very small NMPs in all parts of the process from environment to plate while studies have found smaller particles to be far more abundant.
5. Throughout the research, there is a lack of standardisation which inhibits comparisons between studies. Analysis methods and size classes investigated differ strongly, leading to widely different NMP concentrations in comparable food types.
6. While there is little data on many typical types of food, information on toxic effects of NMPs and subsequent risks to human health are even rarer, making any risk assessment very tentative.

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WP4: NMPs specific microbial risks to consumers

- In WP4, we reviewed the existing literature to determine whether there is any evidence that links the consumption of foods contaminated with NMP with human disease risk.

- Several research areas were considered as part of this WP:
  1. **Dysbiosis**
  2. **Evidence of AMR bacteria and AMR exchange on NMPs**
  3. **The presence of key human pathogens on NMPs and evidence of novel and non-monitored pathogens on NMPs**
  4. **NMPs and human disease risk/evidence of disease**

- Pathogens of human health relevance have been found on MPs and NMPs evidenced from a variety of published scientific studies (**Figure 1**).

- Most of the published research in this area assessed through WP4 were focussed on marine settings, however there are also studies providing insights into this phenomenon in aquatic and soil-focussed studies.

- Importantly, a variety of human pathogens have been found on microplastics in environmental settings, but we do not know their pathogenicity and virulence potential or what, if any, human pathogen transmission occurs via this potential route of exposure [1]

- With regards to microbial resistances, many of these studies suggest that MPs can be colonised by antibiotic resistant bacteria (ARB), and that densities are frequently higher than in the surrounding natural environment (again, frequently marine and aquatic settings).

- There is however, a lack of robust experimental data to demonstrate this phenomenon in laboratory settings.

- There also is little evidence and few published studies to suggest that MPs are any more likely to be colonised by ARB than other particle substrates.

- Although many published studies show entry points of NMPs as well as specific impacts of NMPs in humans, including respiratory systems, digestive and excretory system, the central nervous system etc, conversely, little data on NMPs and microbes associated with specific human disease risks was identified in this WP.

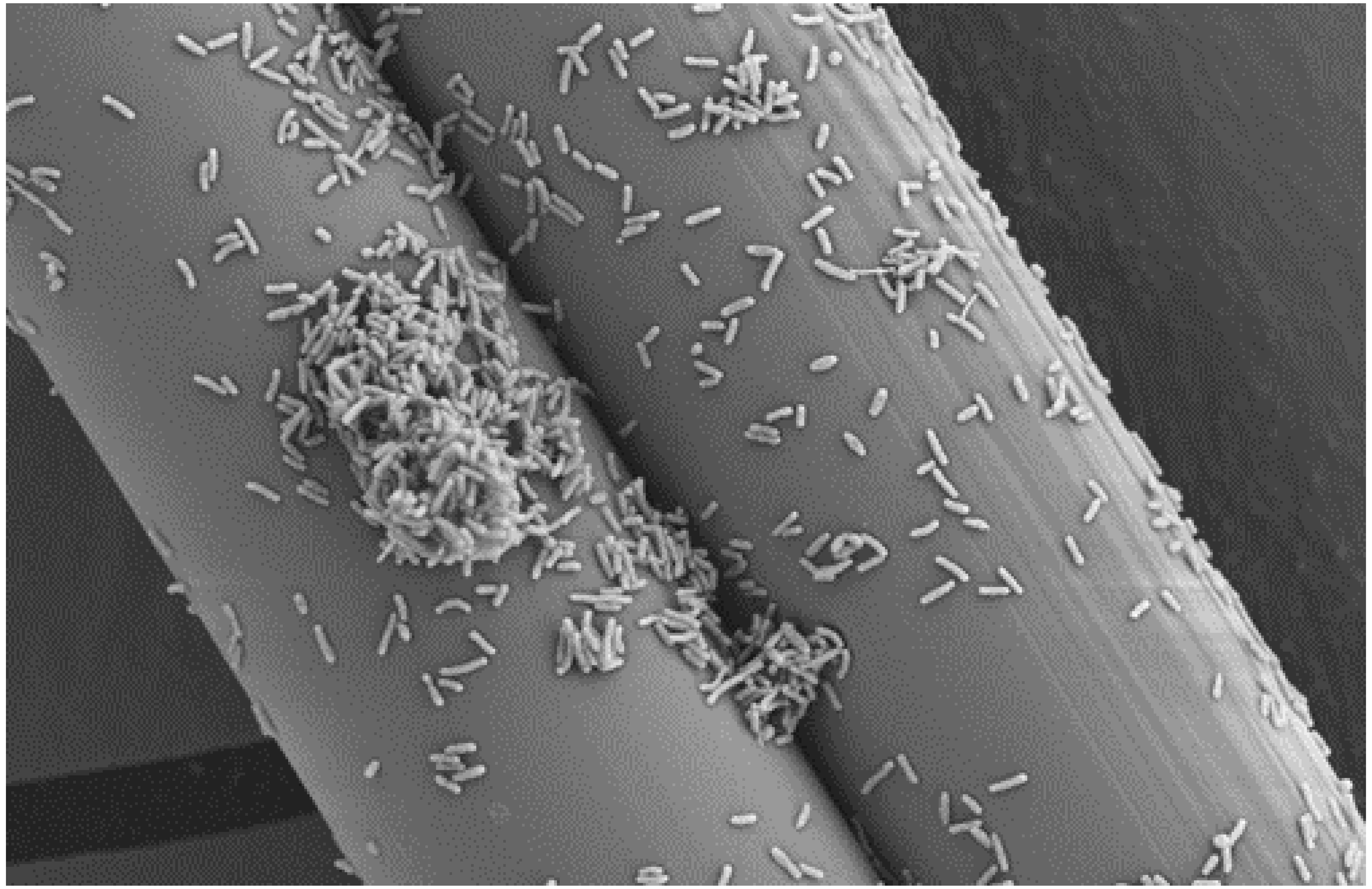

Figure 1. Scanning electron micrograph image of the attachment of the foodborne pathogen Vibrio parahaemolyticus to microplastic fragments. Picture courtesy Jake Bowley (University of Exeter)

WP4: Evidence gaps and recommendations for further work

1. Data on the presence of viruses on NMPs is currently lacking.

2. Research into human disease risks and NMPs focuses on toxicological effects, but very little attention is focused on the potential role of associated microorganisms.

3. Many studies regarding the presence of pathogens on NMPs in environmental settings are purely anecdotal and lack robust controls such as comparisons to other substrates.

4. Studies on AMR tend to lack appropriate controls, making direct comparisons as well as ascertaining overall relevance to risk difficult to gauge.

5. There is a lack of published evidence regarding dysbiosis in humans, although there are studies in other model organisms (e.g. zebrafish, mice).

6. There is little current data regarding impacts of NMPs on pathogens and human health outcomes including a clear lack of epidemiological data.

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