



Review

Quantifying the importance of plastic pollution for the dissemination of human pathogens: The challenges of choosing an appropriate ‘control’ material

Rebecca Metcalf^{*}, David M. Oliver, Vanessa Moresco, Richard S. Quilliam

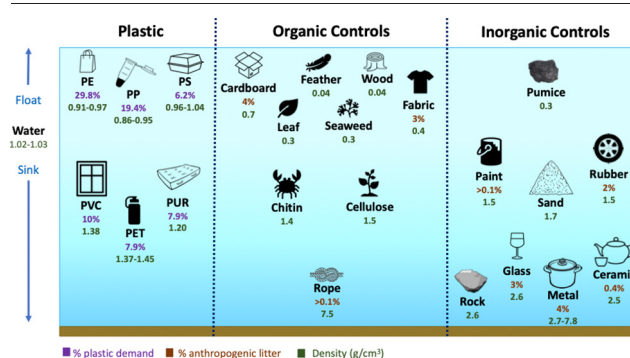
Biological and Environmental Sciences, Faculty of Natural Sciences, University of Stirling, Stirling, FK9 4LA, UK



HIGHLIGHTS

- Human pathogens can colonise environmental plastic pollution.
- Persistence and transport of pathogens can be facilitated on plastics.
- Experiments need an appropriate ‘control’ to understand potential risk.
- No single control substrate can control for all relevant variables.

GRAPHICAL ABSTRACT



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ABSTRACT

Discarded plastic wastes in the environment are serious challenges for sustainable waste management and for the delivery of environmental and public health. Plastics in the environment become rapidly colonised by microbial biofilm, and importantly this so-called ‘plastisphere’ can also support, or even enrich human pathogens. The plastisphere provides a protective environment and could facilitate the increased survival, transport and dissemination of human pathogens and thus increase the likelihood of pathogens coming into contact with humans, e.g., through direct exposure at beaches or bathing waters. However, much of our understanding about the relative risks associated with human pathogens colonising environmental plastic pollution has been inferred from taxonomic identification of pathogens in the plastisphere, or laboratory experiments on the relative behaviour of plastics colonised by human pathogens. There is, therefore, a pressing need to understand whether plastics play a greater role in promoting the survival and dispersal of human pathogens within the environment compared to other substrates (either natural materials or other pollutants). In this paper, we consider all published studies that have detected human pathogenic bacteria on the surfaces of environmental plastic pollution and critically discuss the challenges of selecting an appropriate control material for plastisphere experiments. Whilst it is clear there is no ‘perfect’ control material for all plastisphere studies, understanding the context-specific role plastics play compared to other substrates for transferring human pathogens through the environment is important for quantifying the potential risk that colonised plastic pollution may have for environmental and public health.

^{*} Corresponding author.

E-mail address: rebecca.metcalf@stir.ac.uk (R. Metcalf).

Contents

1. Introduction	2
2. Biofilms on environmental plastics	2
3. Human pathogens in the plastisphere	2
4. What is an appropriate 'control' substrate for plastisphere studies?	4
5. Material properties affecting microbial colonisation	5
6. Conclusion.	9
CRedit authorship contribution statement	9
Declaration of competing interest	9
Acknowledgements	9
References	9

1. Introduction

Plastics are inexpensive, lightweight, strong and durable, making them the ideal material for a diverse array of products and applications with widespread societal benefits (Thompson, 2006; Andrady and Neal, 2009). This has resulted in an increase in the global production of plastics, from 35 million tonnes in the 1950s to 335 million tonnes in 2019 (PlasticsEurope, 2020). However, less than a fifth of plastic is recycled globally and large amounts of plastic are continuously released into the environment either directly or indirectly via multiple pathways, e.g., from wastewater treatment plants (WWTPs), agriculture (e.g. mulching film or seed coating) and littering (Ivleva et al., 2017; Ren et al., 2021; Woodward et al., 2021), and due to their longevity, can persist and accumulate in terrestrial, freshwater and marine ecosystems (Thompson, 2006; Ivleva et al., 2017; Karbalaee et al., 2018). Plastics in the environment can have multiple negative impacts, including blocking drains, wildlife entanglement, the accumulation of toxins (e.g. PCBs, DDTs and HCHs) and the transport of non-native species (e.g. mussels, barnacles and diatoms) (Wang et al., 2018; Napper and Thompson, 2020; Welden, 2020).

2. Biofilms on environmental plastics

Intrinsic properties of plastics and microplastics (defined as plastic particles <5 mm), such as their hydrophobicity, density, and high surface area to volume ratio, can promote microbial colonisation and biofilm formation (Harrison et al., 2014; Frere et al., 2018; Cai et al., 2019). Organic matter, nutrients and biomolecules can also rapidly adsorb to plastic surfaces in the environment, forming a unique ecocorona that further attracts microbial colonisers (Bhosle et al., 2005; Galloway et al., 2017; De Carvalho, 2018). Many microorganisms prefer being attached to a surface rather than remaining planktonic; and biofilms can provide several benefits, including the capture of nutrients, protection from environmental stressors and predation, and enhanced dispersal (Lee et al., 2008; De Carvalho, 2018; Santos et al., 2018). Consequently, biofilms are composed of a dynamic array of successional microbial communities, including the bacterial groups Gammaproteobacteria (during the initial 24 h), Alphaproteobacteria (from 24 h) and Bacteroidetes (Dang and Lovell, 2000; Oberbeckmann et al., 2015; Wright et al., 2021), together with a diverse range of fungi, diatoms, algae, and viruses (Audrézet et al., 2020; Gkoutselis et al., 2021; Moresco et al., 2021).

Biofilms can form on any surface, including living tissues, indwelling medical devices, water system piping, wood and plastics (Donlan, 2002; Lobelle and Cunliffe, 2011; De Carvalho, 2018). However, Zettler et al. (2013) coined the term 'plastisphere' to describe the distinct microbial communities that colonise environmental plastic debris. Plastisphere communities are highly variable, diverse and genetically different from the free-living communities that surround them, implying that plastic provides a novel ecological habitat (Kirstein et al., 2019; Wu et al., 2020; Li et al., 2021). Importantly, biofilms on plastics can also support, or even enrich, microbial communities, including human pathogens (Oberbeckmann and Labrenz, 2020; Sun et al., 2020; Wu et al., 2020).

Although an increasing number of studies have found human pathogens within the plastisphere, the relative risk to human health has not yet been

quantified (Noventa et al., 2021); thus, there is a pressing need to increase our understanding of the dynamics of human pathogens colonising environmental plastic pollution. However, we argue that in order to determine whether plastics play a greater role in enhancing the survival and dispersal of human pathogens within the environment compared to other substrates (either natural materials or other pollutants), future experiments need to incorporate a 'control material' as a direct comparison. Very few published studies have attempted to compare the behaviour of human pathogens in the plastisphere with human pathogens in the biofilm on a control substrate. Therefore, in this paper, we summarise all of the published studies that have detected human pathogenic bacteria on the surfaces of environmental plastic pollution and critically discuss the challenges of selecting an appropriate control material for plastisphere experiments.

3. Human pathogens in the plastisphere

Human pathogens (and potential pathogens) have been identified within the plastisphere of several different plastic polymers, including polyethylene, polypropylene, and polystyrene (Table 1). Pathogens that are frequently detected within plastisphere communities include *Vibrio* spp.; although not all vibrios are pathogenic, some human pathogen species have been found colonising marine plastics, particularly in summer months, e.g., *Vibrio parahaemolyticus*, *V. cholerae* and *V. vulnificus* (Kirstein et al., 2016; Silva et al., 2019; Laverty et al., 2020; Rasool et al., 2021). Such pathogens can cause diarrhoea, cellulitis and septicemia in humans and are responsible for significant levels of mortality, particularly in developing sub-Saharan African and Southeast Asian countries where medicines and resources are less readily available (Ali et al., 2015; Heng et al., 2017). Due to the benefits of living within the biofilm, the infectiousness of pathogens such as *V. cholerae*, can be increased (Lyons et al., 2010; Bowley et al., 2020; Wu et al., 2020). Other human pathogens identified within the plastisphere, include *Escherichia coli*, *Providencia rettgeri* and *Salmonella* spp., which can cause diarrhoea, gastrointestinal, urinary tract and eye infections (El-Liethy et al., 2020; Moore et al., 2020; Shi et al., 2021). Human pathogens are often identified within the plastisphere by sequencing or culture-based approaches, therefore, testing for virulence genes is required to determine their actual pathogenesis (Wright et al., 2020).

Plastics in the environment have the potential to facilitate the dispersal of human pathogens and transport them large distances through terrestrial, freshwater, and marine environments (Debroas et al., 2017), particularly as certain species of human pathogen have been detected colonising plastics in more than one environmental matrix (Table 1). The potential for human pathogens in the plastisphere to survive, persist and be transported between different environments, could increase exposure routes and opportunities for coming into contact with humans, e.g., through direct exposure at beaches or bathing waters (Keswani et al., 2016; Rodrigues et al., 2019), or via the consumption of shellfish or water (Cox et al., 2019; Bowley et al., 2020; Fabra et al., 2021). The survival dynamics of pathogens colonising environmental plastic pollution is yet to be fully understood, although it has been suggested that *Staphylococcus aureus* is able to survive on dry plastic for up to three years (Chaibenjawong and Foster, 2011); which highlights the high potential for pathogens to be transported large distances during this time.

Table 1

Potential human pathogenic bacteria detected in the plastisphere of environmental plastic pollution and on the surfaces of control materials.

Plastic type ^a	Control material ^b	Pathogenic bacteria	Environment	Method	Reference
PVC, PA, PE, PS	–	<i>Burkholderia</i> sp. <i>Enterococcus faecium</i> <i>Klebsiella pneumoniae</i> <i>Listeria monocytogenes</i> <i>Mycobacterium</i>	Terrestrial	16S rRNA	(Zhu et al., 2021)
PP, PS, PET, PE, PVC	–	<i>Klebsiella pneumoniae</i> <i>Vibrio cholerae</i>	Terrestrial	Selective media, 16S rRNA	(Rasool et al., 2021)
PS	–	<i>Pseudomonas aeruginosa</i>	Terrestrial	16S rRNA	(Shi et al., 2021)
PE, PP, PS, PET, PAN	–	<i>Escherichia coli</i> <i>Coxiella</i> sp. <i>Legionella</i>	Freshwater	16S rRNA	(Galafassi et al., 2021)
PE, PP, PS	–	<i>Streptococcus</i> sp. <i>Arcobacter</i> sp. <i>Campylobacteraceae</i> <i>Enterobacteriaceae</i> <i>Klebsiella pneumoniae</i> <i>Moraxellaceae</i> <i>Pseudomonas</i> spp.	Freshwater (WWTP)	16S rRNA	(Kelly et al., 2021)
LDPE, HDPE, PP, PC, PS	Glass	<i>Vibrio cholerae</i> <i>Vibrio parahaemolyticus</i> <i>Vibrio vulnificus</i>	Freshwater	16S rRNA	(Lavery et al., 2020)
Undetermined	–	<i>Arcobacter</i> sp. <i>Pseudomonas</i> spp. <i>Campylobacteraceae</i>	Freshwater	16S rRNA	(McCormick et al., 2014)
PE, PP, PS	–	<i>Arcobacter</i> sp. <i>Campylobacteraceae</i>	Freshwater	16S rRNA	(McCormick et al., 2016)
PE	–	<i>Enterobacter</i> spp. <i>Helicobacter</i> spp. <i>Arcobacter</i> sp. <i>Clostridium perfringens</i> <i>Escherichia coli</i>	Freshwater	16S rRNA	(Murphy et al., 2019)
PE, PS	Sand	<i>Raoultella ornithinolytica</i> <i>Stenotrophomonas maltophilia</i>	Freshwater	16S rRNA	(Pham et al., 2021)
PVC, PE	–	<i>Legionella</i> <i>Mycobacterium</i> <i>Neisseria</i> <i>Arcobacter</i>	Freshwater (Sewage)	16S rRNA	(Wang et al., 2021)
PBT, PE, PP, PS	–	<i>Pseudomonas</i> spp.	Freshwater	16S rRNA	(Xue et al., 2020)
PE, PP, PET, PS, PU	–	<i>Vibrio</i> spp. <i>Pseudomonas</i> spp. <i>Bacillus</i> spp. <i>Rhodococcus</i> spp.	Freshwater	16S rRNA	(Zhang et al., 2021c)
PVC	–	<i>Mycobacterium</i> <i>Legionella</i> spp. <i>Rhodococcus</i> spp.	Freshwater	16S rRNA	(Y. Zhao et al., 2021)
PS, PP, PE, PET, PVC	–	<i>Bacillus</i> sp. <i>Mycobacterium</i> sp.	Estuary	16S rRNA	(Guo et al., 2018)
LDPE	–	<i>Arcobacter</i> sp.	Estuary	16S rRNA	(Harrison et al., 2014)
PS, PP, PE	–	<i>Pseudomonas</i> spp. <i>Vibrio</i> spp.	Estuary	16S rRNA	(Jiang et al., 2018)
Undetermined	–	<i>Vibrio</i> spp. <i>Shewanella</i> sp.	Estuary	Selective media	(Li et al., 2019)
Undetermined	–	<i>Escherichia coli</i> <i>Enterococci</i>	Estuary	Selective media	(Pazos et al., 2020)
PE, PP, PS, PET, PU	–	<i>Pseudomonas</i> spp.	Estuary	16S rRNA	(Wu et al., 2020)
HDPE, LDPE, PP	–	<i>Francisella</i> <i>Rickettsia</i>	Marine	16S rRNA	(Barral et al., 2018)
PE, PP, PS	–	<i>Mycobacterium</i> <i>Staphylococcus</i> spp.	Marine	Microscopy, 16S rRNA	(Basili et al., 2020)
Undetermined	–	<i>Pseudomonas alcaligenes</i> <i>Vibrio</i> spp.	Marine	16S rRNA	(Curren and Leong, 2019)
PE	–	<i>Vibrio</i> spp.	Marine	16S rRNA	(De Tender et al., 2015)
LDPE, OXO, PHBV	–	<i>Staphylococcus aureus</i> <i>Vibrio</i> spp.	Marine	16S rRNA	(Dussud et al., 2018)
PET, PP, PE	–	<i>Acinetobacter oleivorans</i> <i>Escherichia coli</i> <i>Vibrio fischeri</i> <i>Vibrio splendidus</i>	Marine	16S rRNA	(Hou et al., 2021)
PS	Glass, chitin	<i>Arcobacter</i> sp.	Marine	16S rRNA	(Kesey et al., 2016, 2017)
PE, PS	Wood	<i>Vibrio</i> spp.	Marine	16S rRNA	(Kesey et al., 2019, 2021)
PE, PP	–	<i>Vibrio parahaemolyticus</i>	Marine	Selective media	(Kirstein et al., 2016)
PS	–	<i>Shewanella</i> sp.	Marine	16S rRNA	(Lagana et al., 2019)
PE, PET	–	<i>Vibrio</i> spp. <i>Pseudomonas</i> spp. <i>Streptococcus</i> spp.	Marine	16S rRNA	(Li et al., 2020)

(continued on next page)

Table 1 (continued)

Plastic type ^a	Control material ^b	Pathogenic bacteria	Environment	Method	Reference
Undetermined	–	<i>Providencia rettgeri</i>	Marine	Selective media	(Moore et al., 2020)
PET	–	<i>Vibrio</i> spp.	Marine	16S rRNA, 18S rRNA	(Oberbeckmann et al., 2016)
HDPE, PS	Wood	<i>Vibrio</i> spp.	Marine, freshwater	16S rRNA	(Oberbeckmann et al., 2018)
Undetermined	Sand, seaweed	<i>Vibrio</i> spp.	Marine	Selective media	(Quilliam et al., 2014)
PET	–	<i>Pseudomonas</i> spp. <i>Morganella morganii</i> <i>Vibrio parahaemolyticus</i>	Marine	Whole genome sequencing	(Radisic et al., 2020)
PVC	Metal		Marine	Selective media, 16S rRNA	(Rajeev et al., 2019)
Undetermined	–	<i>Escherichia coli</i> <i>Vibrio</i> spp.	Marine	Selective media	(Rodrigues et al., 2019)
Undetermined	–	<i>Vibrio</i> spp.	Marine	16S rRNA	(Schmidt et al., 2014)
Undetermined	–	<i>Escherichia coli</i> <i>Vibrio cholerae</i>	Marine	16S rRNA	(Silva et al., 2019)
Undetermined	Feather	<i>Vibrio</i> spp.	Marine	Microscopy	(Sun et al., 2020)
Undetermined	–	<i>Escherichia coli</i> <i>Bacillus cereus</i>	Marine	NGS	(Van der Meulen et al., 2015)
PP, PVC	–	<i>Vibrio</i> spp.	Marine	16S rRNA	(Xu et al., 2019)
PP, PE	–	<i>Vibrio</i> spp.	Marine	16S rRNA	(Zettler et al., 2013)
Undetermined	–	<i>Vibrio</i> spp.	Marine	16S rRNA	(Zhang et al., 2020)

^a PP-polypropylene, PE-polyethylene, HDPE-high density polyethylene, LDPE-low density polyethylene, PET-polyethylene terephthalate, PS-polystyrene, PU-polyurethane, PVC-polyvinyl chloride, PC-Polycarbonate, PBT-Polybutylene terephthalate, OXO-Additivated PE with pro-oxidant, PHBV-poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PAN-polyacrylonitrile.

^b Only 8 of 45 studies included a control material and also detected human bacterial pathogens within their biofilms.

Colonisation and persistence of human pathogenic bacteria in the plastisphere are probably influenced by the plastic polymer type, with recent studies suggesting that the highest diversity of human bacterial pathogenic species were found colonising polyethylene (Table S1); although some species, e.g., *Vibrio*, have been found colonising nearly all plastic polymer types (Table S1). Bacterial pathogens are also detected on the surfaces of non-plastic ‘control’ materials, e.g., glass and wood (Table 1), with *Vibrio* spp. detected on the majority of these materials. This suggests that both the plastic polymer and the control material can influence the pathogens which bind to it. There is also evidence to suggest that the properties of the bacterial species themselves can influence which materials they preferentially attach to. For example, some strains of pathogenic *E. coli* and *Enterococcus faecalis* have high surface free energy, resulting in weaker adhesion forces to hydrophobic surfaces such as plastics, whereas *Salmonella typhimurium* and *Pseudomonas putida*, have low surface free energy and preferentially attach to more hydrophobic surfaces (Zhang et al., 2015; Song et al., 2020). Importantly, most pathogenic species colonising plastic surfaces have not been simultaneously tested with a non-plastic control material, which makes it difficult to discern the comparative risk of environmental plastic pollution for disseminating human pathogens.

4. What is an appropriate ‘control’ substrate for plastisphere studies?

Although it is well documented that the community composition of the plastisphere is often significantly different to the surrounding environment due to planktonic/biofilm species preferences (Xue et al., 2020; Martínez-Campos et al., 2021), this does not provide a functional ‘control’ that gives us a narrative on the relative role of environmental plastic pollution. Natural organic materials, such as leaves and wood, and inorganic materials, such as glass and rubber, are also important materials for the transport of freshwater and marine organisms (Kesy et al., 2019; Miao et al., 2019), and human pathogens have previously been identified within the biofilms of materials such as glass, wood and feathers (Islam et al., 2007; Sun et al., 2020; Pham et al., 2021). Although more recent studies have begun to include control materials (Kesy et al., 2019; Sun et al., 2020; Martínez-Campos et al., 2021), for the majority of plastisphere studies, there is still a lack of comparison between microbial communities binding to plastic surfaces compared with the surfaces of other substrates in the environment (Table 2). This makes it difficult to draw useful conclusions on whether the composition and survival dynamics of pathogens and plastisphere communities differ from the biofilms colonising other substances, and whether

environmental plastics play any more of a significant role in facilitating the survival and dispersal of human pathogens than other materials. The source and dispersal routes of plastic pollution often significantly differ from potential control materials. For example, there are several opportunities for plastics to encounter high concentrations of human pathogens, e.g., when they pass through WWTPs or are exposed to hospital waste; in contrast, potential control materials are less likely to be exposed to these sources, and therefore, plastics may be entering the environment already colonised with significant populations of human pathogens.

Of the studies that reported pathogen enrichment on plastic surfaces, 62% showed higher pathogen abundances on plastic compared to control materials (Table 2), and often include species of *Vibrio* and *Pseudomonas* (Wu et al., 2019; Sun et al., 2020). Plastics in the environment can provide a novel niche, with distinct properties and characteristics that promote pathogen colonisation (Sun et al., 2020). Some pathogens, including *Vibrio*, are known to be secondary opportunistic colonisers dependent on primary colonisers already present in the biofilm (Datta et al., 2016; Foulon et al., 2016). As plastics support distinct microbial communities compared to control materials (Kirstein et al., 2019; Miao et al., 2019; Oberbeckmann and Labrenz, 2020), the composition of the plastisphere plays an important role in the potential colonisation of pathogens (Wu et al., 2019).

Evidence on whether pathogens preferentially colonise plastics over other materials in the environment remains contradictory (e.g. Oberbeckmann and Labrenz, 2020; Song et al., 2020). These inconsistencies are likely due to the variable environmental conditions of each study, with environmental factors often having a stronger influence on plastisphere formation and diversity than the type of polymer (Basili et al., 2020; Kesy et al., 2021; Zhang et al., 2021a). Organic materials in the environment, e.g., seaweed and wood, can provide a more readily available source of nutrients compared with plastics (Takemura et al., 2014; Quilliam et al., 2014; Song et al., 2020), but the higher durability of plastics compared to organic materials, increases the potential for dissemination and transport of microbial colonisers. However, to understand the relative risk of pathogen persistence in the plastisphere, studies that include environmentally relevant control materials are urgently needed to determine whether plastic pollution really does increase the opportunity for pathogen transport and transmission in comparison to colonisation of other substrates in the environment.

A range of different organic and inorganic controls have been used in plastisphere studies (Table 2). The most commonly used organic control is wood (10 of 46 studies), whilst glass is the most commonly used inorganic control (23 of 46 studies), with the majority of studies preferring an

inorganic material as a control substrate (35 of 46 studies). To ensure a similar available colonisation area, the control material needs to be of a very similar size, shape and texture as the plastic particle being quantified. Size, shape and colour of materials are important to control for because not only can they influence available surface area, buoyancy and transport, biofilm community structure and the abundance of potential pathogens, such as *Vibrio* and *Pseudomonas* (Mughini-Gras et al., 2021; Zhang et al., 2021b), but also any subsequent potential ingestion, e.g., by bivalve species (Bowley et al., 2020). Many plastisphere studies have reported higher microbial diversity on control substrates compared to plastic surfaces (Table 2), which implies that the availability of a surface to colonise is probably more important for driving microbial diversity than the composition of the surface itself.

To date, the majority of plastisphere research has focused on individual environmental matrices with a particular emphasis on the marine environment (e.g., Bowley et al., 2020). This conceptual compartmentalisation of the environment masks our understanding of how plastisphere communities behave as they are transported between different environments within the landscape. The “plastic cycle” transports plastics between different abiotic (and biotic) compartments as they are transferred through terrestrial, freshwater, and marine environments (Bank and Hansson, 2019; Rochman, 2018). This needs to be considered when selecting an appropriate control as the transport mechanisms of both the plastic and the control material are likely to be affected by their interaction with the conditions in each specific environmental matrix. For example, stream and river ecosystems are characterised by continuous downstream movement, whilst marine ecosystems have varying tidal flows and currents; therefore, the specific behaviour of plastic and control materials is likely to vary in these contrasting environmental matrices (Boyle and Örmeci, 2020).

Although plastisphere communities differ between environmental matrices, there are relatively few studies that have considered plastisphere communities in both freshwater and marine environments (Kettner et al., 2017; Kettner et al., 2019; Oberbeckmann et al., 2018); and only one that has physically moved plastic particles between these two environments (Song et al., 2020). Whereas the transition of plastics between terrestrial and aquatic environments e.g., from runoff and erosion of contaminated soil or when plastics are washed up on beaches, has so far been ignored in the literature. The contrasting environmental conditions and surrounding autochthonous microbial communities of terrestrial vs aquatic (freshwater and marine) environments will strongly influence the composition and diversity of plastisphere and biofilm communities (e.g., in terms of the species involved with primary biofilm formation and subsequent succession of the community), before they are delivered to the new environmental matrix.

Environmental factors, such as nutrient availability, temperature, and salinity, impose differential selective forces and can significantly influence plastisphere composition and structure (Li et al., 2019; Pinto et al., 2019; Zhang et al., 2021a). For example, the higher nutrient concentrations found around WWTPs in freshwater environments can increase bacterial richness and diversity of biofilm communities of both plastic and wood compared to coastal environments (Oberbeckmann et al., 2018). Yet, relatively little is known about how pathogenic bacteria in the plastisphere are affected as they transition between environmental matrices. The survival and abundance of certain pathogenic species decrease as particles transition from freshwater to saltwater environments. Higher abundances of the taxonomic groups Enterobacteriaceae and *Vibrio* were found colonising plastics in freshwater locations compared to marine locations (Oberbeckmann et al., 2018), whilst the survival of *E. coli* decreased as plastic and control particles transitioned along a salinity gradient (Song et al., 2020). Interestingly, Song et al. (2020) detected higher abundances of pathogens on the control particles (tyre wear and wood) compared to the HDPE plastic particles, suggesting that pathogenic bacteria were less likely to survive the transition between environmental matrices on plastics compared to other materials. Determining how the colonisation and persistence of pathogens in the plastisphere changes as it transitions between environmental compartments will help determine the risk of pathogen transfer and transmission on

microplastics and other materials as they are transported within and between environments.

Several different organic and inorganic substrates have previously been used as control materials (Fig. 1). In addition to providing a surface to colonise, organic materials can also provide a nutrient source and are often associated with higher diversity and heterotrophic growth, whereas inert inorganic substrates are more associated with autotrophic growth (Tobias-Hünefeldt et al., 2021). This suggests that heterotrophic human bacterial pathogens are likely to be more abundant on organic substrates, which may explain why pathogen abundances can be enriched on control materials, such as wood and chitin (Table 2). However, organic materials are not always as buoyant as plastics (Fig. 1) and decompose more quickly (e.g., straw and coconut husks); thus, plastics have the potential to transport and disseminate microbes for longer and further than natural organic materials (Thiel and Gutow, 2005; Keswani et al., 2016; Laverty et al., 2020). Although plastic is the main constituent of anthropogenic litter, other inorganic materials, such as metal, glass and ceramics make up a proportion of the anthropogenic litter across all environments within the landscape (Addamo et al., 2017; Nelms et al., 2017). Importantly, the use of a single control material will not control for all variables of any particular plastic polymer; therefore, some studies have used both organic and inorganic controls, which gives more useful information by providing several comparisons (Ogonowski et al., 2018; Muthukrishnan et al., 2019; Tobias-Hünefeldt et al., 2021). Most potential control materials will be found within most environmental matrices, although there are some which are only found (or are much more abundant) in certain environmental compartments or geographical locations (e.g., seaweed, pumice). The selection of a control material needs to be environmentally relevant to the environmental matrices being studied, but the provenance of the material is also an important factor as in natural systems the material will have already been colonised in the preceding matrix.

5. Material properties affecting microbial colonisation

The development and composition of biofilm communities is influenced by a range of biotic and abiotic driving factors (Harrison et al., 2018). Both physical and chemical differences between plastic polymers and potential control materials can influence microbial adhesion and community composition (Renner and Weibel, 2011). Physicochemical properties of surfaces are most influential during the primary stages of colonisation and the importance of these properties decreases as the biofilm matures (Datta et al., 2016; Ogonowski et al., 2018). Therefore, the intrinsic properties of the material supporting plastisphere communities are likely to be most influential at points where plastics are first released into the environment (Harrison et al., 2018). Virgin plastic polymers and other control materials, such as glass and ceramic, have smooth surfaces, whilst materials such as wood have rougher surfaces that increase the surface area and potential sites available for bacterial attachment, whilst also providing protection against shear forces (Bollen et al., 1996; Yoda et al., 2014; Zheng et al., 2021). As a result, increased surface roughness can enhance bacterial adhesion and diversity but is also likely to increase persistence of any attached bacteria due to increased protection. Surface roughness is highly variable, and differs between plastic polymers and control materials (Table S2), but can also change rapidly as materials are colonised, for example, Bhagwat et al. (2021) demonstrated that the accumulation of biomolecules, together with the formation of conditioning films, increased the surface roughness of plastic surfaces over 24 h.

Different materials have varying levels of buoyancy, hydrophobicity, surface charge and roughness (Table S2); all of which can influence microbial adhesion and biofilm formation, and subsequent transport and dissemination (Ogonowski et al., 2018; Cai et al., 2019; Gong et al., 2019). These properties need to be taken into consideration when deciding upon an appropriate control for plastisphere studies and will need to be relevant to the properties of the specific plastic polymer being used. Density is important for facilitating environmental transport and ultimately fate, e.g., by determining how particles disperse and/or sink in the aquatic environment

Table 2
Recent plastisphere studies that have used a control material.

Plastic type ^a	Size (mm)	Control	Size (mm)	Environment	Method	Experimental design ^b	Higher diversity	Difference in community composition		Pathogen enrichment on plastic	Reference
								Plastic vs. Control	Plastic vs. Matrix		
PVC, PA, PE, PS	0.03–1.5	Glass	0.03	Terrestrial (soil)	16S rRNA	Mesocosm, field deployed	No difference	Difference	Difference	–	(Zhu et al., 2021)
PE, PP	3–4	Cobblestone, wood	30–50	Freshwater	16S rRNA	Mesocosm	Control	Difference	–	–	(Miao et al., 2019)
PVC	3	Rock, leaves	2–4	Freshwater	16S rRNA	Mesocosm	Plastic	Difference	Difference	Yes	(Wu et al., 2019)
PE, PS	0.085–0.106	Sand	0.088–0.105	Freshwater	16S rRNA	Mesocosm	Plastic	Difference	–	Yes	(Pham et al., 2021)
PLA, PHB, PCL, PET, POM, PS, LDPE	3–5	Glass	2–8	Freshwater	16S rRNA	Field deployed	No difference	Difference	Difference	–	(Martínez-Campos et al., 2021)
PE, PS	–	Glass	–	Freshwater	16S rRNA	Mesocosm	Control	Difference	Difference	–	(Parrish and Fahrenfeld, 2019)
LDPE, HDPE, PP, PC, PS	–	Glass	–	Estuary	Selective media	Field collected; field deployed	Plastic	No difference	Difference	Yes	(Lavery et al., 2020)
PLA, LDPE	3–5	Glass	4	Estuary	Microscopy	Field deployed	–	–	–	–	(Richard et al., 2019)
PC	–	Steel	20–50	Estuary	16S rRNA	Field deployed	–	Difference	Difference	–	(Jones et al., 2007)
PS, PP, PVC, PCL	3–4	Wood	–	Estuary	Metagenomic	Field deployed	No difference	Difference	Difference	Yes	(Bhagwat et al., 2021)
Undetermined	–	Cardboard, leaves, glass, aluminium, ceramic tiles	–	Marine	16S rRNA	Field collected	No difference	Difference	–	–	(Hodlein et al., 2014)
PET, PHA	3–4	Ceramic	3–4	Marine	Metagenomics	Mesocosm, field deployed	–	No difference	Difference	–	(Pinnell and Turner, 2019)
PA	–	Chitin	–	Marine	16S rRNA	Mesocosm	No difference	No difference	Difference	No	(Key et al., 2017)
Undetermined	1–4	Feathers	1–4	Marine	Microscopy, 16S rRNA	Field deployed	No difference	No difference	Difference	Yes	(Sun et al., 2020)
PP, PE, PLA	300	Glass	300	Marine	16S rRNA	Mesocosm	No difference	Difference	Difference	–	(Cheng et al., 2021)
PVC	5 × 10	Glass	5 × 10	Marine	16S rRNA	Field deployed	Control	–	–	–	(Dang et al., 2008)
LDPE	–	Glass	–	Marine	16S rRNA	Field deployed	No difference	Difference	Difference	–	(Enri-Cassola et al., 2020)
PS	–	Glass	–	Marine	Microscopy	Field deployed	–	Difference	–	–	(Hung et al., 2008)
PS	0.25–0.4	Glass	0.25–0.4	Marine	16S rRNA	Mesocosm	–	Difference	Difference	No	(Key et al., 2016)
HDPE, LDPE, PP, PS, PET, PLA, SAN, PESTUR, PVC	50 × 50	Glass	50 × 50	Marine	16S rRNA, 18S rRNA	Mesocosm	–	Difference	–	–	(Kirstein et al., 2018)
LDPE, HDPE, PP, PS, SAN, PESTUR, PLA, PET, PVC	–	Glass	–	Marine	16S rRNA	Mesocosm	Plastic	Difference	–	–	(Kirstein et al., 2019)
PMMA	170 × 100	Glass, steel	170 × 100	Marine	16S rRNA	Field deployed	–	Difference	Difference	–	(Lee et al., 2008)
PET	–	Glass	–	Marine	16S rRNA	Field deployed	No difference	Difference	Difference	–	(Oberbeckmann et al., 2014)
PET	–	Glass	–	Marine	16S rRNA, 18S rRNA	Field collected	–	No difference	Difference	–	(Oberbeckmann et al., 2016)
PE, PP, PS	2–2.5	Glass, Cellulose	0.2/0.0063 × 0.13	Marine	16S rRNA	Mesocosm	No difference	Difference	Difference	–	(Ogonowski et al., 2018)
LDPE, HDPE, PP, PVC	40 × 40 × 0.5	Glass	10 × 10 × 1	Marine	16S rRNA	Field deployed	No difference	Difference	Difference	–	(Pinto et al., 2019)

PS, PE, PVC PMMA	– 75 × 25	Glass Glass, ceramic, wood	– –	Marine Marine	Microscopy 16S rRNA, 18S rRNA	Laboratory Field deployed	– Control	– Difference	– –	(Snoussi et al., 2009) (Tobias-Hünefeldt et al., 2021)
Undetermined HDPE, PLA	– 3	Glass, metal, fabric, rubber Glass	– 18	Marine Marine	Microscopy, 16S rRNA	Field collected Field deployed	Plastic No difference	Difference No difference	Difference Difference	(Woodall et al., 2018) (Zhang et al., 2021a)
PE, PP, PS PS	5 × 5 60	Glass Granite	5 × 5 300	Marine Marine	Microscopy 16S rRNA	Field deployed Field deployed	– Control	– –	– –	(S. Zhao et al., 2021) (Chung et al., 2010)
PP, HDPE, BDA	3 × 4	Gravel	3 × 4	Marine	16S rRNA	Field deployed	Control	Difference	Difference	(Agostini et al., 2021)
LDPE, HDPE, PS, PP, PET, PU, PLA	–	Latex, rope, steel	–	Marine	16S rRNA	Mesocosm	Plastic	Difference	–	(Gerritse et al., 2020)
PP, PA, PVC	–	Paint, cellulose	–	Marine	16S rRNA	Field collected	–	Difference	Difference	(Tagg et al., 2019)
Undetermined PVC	100 × 50	Sand, seaweed Steel, titanium	– 100 × 50	Marine Marine	Selective media, 16S rRNA	Field collected Field deployed	– –	– –	No Difference	(Quilliam et al., 2014) (Rajeev et al., 2019)
PVC	4	Steel, silica	4	Marine	16S rRNA	Field deployed	Control	Difference	–	(Wang et al., 2020)
PS	40 × 50	Volcanic pumice	–	Marine	Microscopy	Field deployed	Plastic	Difference	–	(Bravo et al., 2011)
PE, PP, PET, PS	–	Wood	–	Marine	16S rRNA, 18S rRNA	Field collected	–	Difference	Difference	(Debras et al., 2017)
PE, PS	3	Wood	–	Marine	16S rRNA	Mesocosm	Control	No difference	Difference	(Kesy et al., 2019)
HDPE, PS	3–5	Wood	–	Marine, freshwater	18S rRNA	Field deployed	No difference	Difference	Difference	(Kettner et al., 2017)
PE, PS	3–5	Wood	–	Marine, freshwater	18S rRNA	Field deployed	Control	Difference	Difference	(Kettner et al., 2019)
PET, PE	3	Wood, steel	600 cm ²	Marine	16S rRNA	Field deployed	Control	Difference	Difference	(Muthukrishnan et al., 2019)
HDPE, PS	3	Wood	–	Marine, freshwater	16S rRNA	Field deployed	–	Difference	Difference	(Oberbeckmann et al., 2018)
HDPE	4	Wood, tyre wear	4	Marine, freshwater	Selective media	Field deployed	–	–	No	(Song et al., 2020)

^a PP-polypropylene, PE-polyethylene, HDPE-high density polyethylene, LDPE-low density polyethylene, PS-polystyrene, PB-polybutylene, PVC-polyvinyl chloride, PLA-poly(lactic acid), PA-polyamide, BDA-HDPE with oxo-bio-degradable additive BDA, PET-poly(ethylene-terephthalate), SAN-styrene-acrylonitrile, PESTUR-polyurethane-prepolymer, PHB-poly-3-hydroxybutyrate, PCL-polycaprolactone, POM-polyoxymethylene, PHA-poly(hydroxyalkanoate), PMMA-poly(methyl methacrylate), PCL-polycaprolactone.

^b Experimental design classifies studies depending on whether samples were collected from the field (field collected), placed out into the field under controlled settings (field deployed) or placed in a controlled mesocosm within the laboratory (mesocosm).

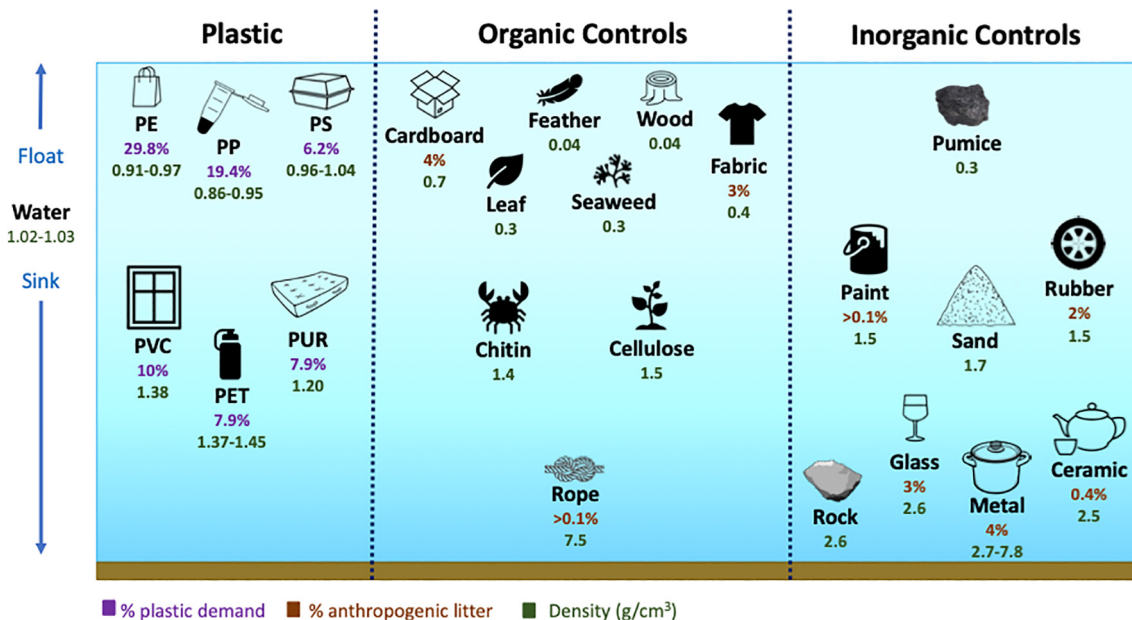


Fig. 1. Potential organic and inorganic control materials for plastisphere studies. Plastic polymers: PE-polyethylene, PP-polypropylene, PS-polystyrene, PVC-polyvinyl chloride, PET-polyethylene terephthalate, PUR-polyurethane.

(Horton et al., 2017; Erni-Cassola et al., 2019). Biofilm formation can increase the density of buoyant plastics and cause them to lose their buoyancy (Chubarenko et al., 2016; Lagarde et al., 2016; Wright et al., 2020); however, Amaral-Zettler et al. (2021) recently showed how there is a size tipping point, above which microbial colonisation alone fails to induce particle sinking. Therefore, even after microbial colonisation, most microplastics are likely to remain floating on or near the water surface. Three of the most abundant plastic polymers (polyethylene, polypropylene and polystyrene) are less dense than water and float on the surface leading to greater dissemination than plastic polymers which are less buoyant and become incorporated into the sediment. Consequently, there is a move to study more buoyant plastic polymers, which have the ability to transport pathogens for longer and over further distances. Although some control materials can also float (Fig. 1), others are denser than plastic and so behave differently in water, e.g., glass and ceramics, and are perhaps less relevant for demonstrating the potential for long distance transport than a control material that floats. However, several studies have used metal cages to sink both the plastic and control samples (Sun et al., 2020; Martínez-Campos et al., 2021). Although this ensures that the materials remain at the same level within the water column and removes the effects of differing buoyancies, it does not replicate the actual movement and conditions experienced by the materials, which are unlikely to remain in one location over extended periods of time.

Hydrophobicity can also influence bacterial adhesion and community composition, with hydrophobic surfaces being more attractive to bacteria (Rummel et al., 2017; Ogonowski et al., 2018; Martínez-Campos et al., 2021). A contact angle (i.e., the angle at a solid-liquid interface) $> 90^\circ$ indicates a hydrophobic surface (Law, 2014), and plastics usually have a contact angle of between 83° and 93° (Cai et al., 2019), whilst control substances such as wood and glass have lower contact angles and are therefore often hydrophilic (Iglauer et al., 2014; Papp and Csiha, 2017). Thus, the hydrophobic surfaces of plastics are likely to be more attractive to bacteria than hydrophilic control surfaces. At neutral pH, most bacteria possess an overall negative charge due to the presence of peptidoglycan and therefore are more attracted to surfaces with a positive surface charge (Zhu et al., 2015; Kovačević et al., 2016; Guo et al., 2018). In addition to influencing initial bacterial attachment, surface charge can also affect subsequent bacterial attachment at later stages of biofilm development (Kao et al., 2017; Shen et al., 2020), which is important for secondary colonisers such as some pathogenic species of *Vibrio* (Datta et al., 2016). Many plastics have

a negatively charged surface, with an average zeta potential of -10 mV (Cai et al., 2019), whereas potential control materials, including wood and glass, have a more negative zeta potential (Gu and Li, 2000; Muff et al., 2018). Zeta potential changes with pH (Xu et al., 2014), meaning bacterial attraction and adhesion will differ as a result of the material moving through the different environmental matrices of the landscape.

Once released into the environment, plastics become fragmented and degraded over time due to mechanical, photo-, chemical-, and biodegradation (Gewert et al., 2015), leading to increased surface roughness through the formation of pits and ridges (Zettler et al., 2013). Therefore, surface roughness is a property that not only differs between materials but can also spatially and temporally vary across a given surface. Surface roughness, chemical composition, colour, and the surface charge of plastics change as they age (Liu et al., 2020; Luo et al., 2020; Su et al., 2021). These age-related properties will subsequently influence the persistence and potential dissemination of human pathogens within these plastisphere communities. Biofilm formation is greater on aged plastics (Rummel et al., 2017; Kaiser et al., 2017), with the pathogenic potential and abundance of antimicrobial resistance genes (ARGs) also enhanced on aged microplastics compared to virgin microplastics (Su et al., 2021). As plastics become more weathered, they begin to release additives, e.g., phthalates and Bisphenol A (Luo et al., 2020; Wu et al., 2021), which can either be used as a microbial nutrient source or can be toxic to plastisphere communities. However, this intrinsic physicochemical property of weathered plastic polymers in the environment is difficult to experimentally control for with the types of control materials discussed above (Table S2), and the effect of compounds leaching from plastics on the potential transport of pathogens within the plastisphere has not yet been considered.

Although there is no 'perfect' control for studies on human pathogens in the plastisphere, the trade-off for selecting which variables will be controlled for will be determined by the study's specific objectives and research question. Density is suggested as the most important factor to consider when selecting a control material, as it can significantly influence both the transport and environmental fate of materials. Glass is the most commonly used control material in plastisphere studies because like plastics, glass also persists in the environment, and is not an immediate source of nutrients. However, glass is perhaps not the most appropriate choice of control material: glass has very smooth surfaces compared to plastics, and its higher density, which causes it to sink, limits its ability to transport and transfer pathogens within aquatic environments. With buoyant environmental

plastic pollution being the most abundant type of plastic, similarly buoyant control materials are perhaps most relevant as these materials have the potential to transport human pathogens for longer and over further distances.

6. Conclusion

With plastic demand continuing to grow and production expected to quadruple by 2050 (Suaria et al., 2016), the volume of microplastics entering the environment will also rise, increasing the environmental surfaces available for colonisation by human pathogens, and therefore increasing the potential risk of exposure of humans and other species to harmful pathogens in the plastisphere. To fully understand this risk, it is essential that appropriate controls are included in future experiments and environmental surveys to determine whether plastic pollution does increase the opportunity for pathogen transport and transmission compared to binding to other substrates. However, it is clear there is no single control substrate relevant for all plastisphere studies, but it is important to take into account a number of factors relating to the plastic polymer, the control material, and the environmental conditions before deciding on the most relevant control to use. With infectious diseases responsible for 22% of annual deaths worldwide (Lozano et al., 2012), we must continue to study the potential of plastics to act as novel vectors of disease (Wißmann et al., 2021), particularly as the longevity of plastic in the environment could facilitate the increased persistence and dissemination of pathogens compared to more accepted environmental pathways.

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CRediT authorship contribution statement

Rebecca Metcalf: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **David M. Oliver:** Writing – review & editing. **Vanessa Moresco:** Writing – review & editing. **Richard S. Quilliam:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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