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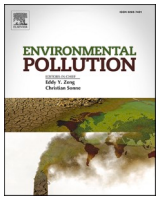

The plastisphere can protect *Salmonella* Typhimurium from UV stress under simulated environmental conditions☆

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ABSTRACT

Plastic waste is found with increasing frequency in the environment, in low- and middle-income countries. Plastic pollution has increased concurrently with both economic development and rapid urbanisation, amplifying the effects of inadequate waste management. Distinct microbial communities can quickly colonise plastic surfaces in what is collectively known as the 'plastisphere'. The plastisphere can act as a reservoir for human pathogenic bacteria, including *Salmonella* enterica sp. (such as *S.* Typhimurium), which can persist for long periods, retain pathogenicity, and pose an increased public health risk. Through employing a novel mesocosm setup, we have shown here that the plastisphere provides enhanced protection against environmental pressures such as ultraviolet (UV) radiation and allows *S.* Typhimurium to persist at concentrations ($>1 \times 10^3$ CFU/ml) capable of causing human infection, for up to 28 days. Additionally, using a *Galleria Mellonella* model of infection, *S.* Typhimurium exhibits greater pathogenicity following recovery from the UV-exposed plastisphere, suggesting that the plastisphere may select for more virulent variants. This study demonstrates the protection afforded by the plastisphere and provides further evidence of environmental plastic waste acting as a reservoir for dangerous clinical pathogens. Quantifying the role of plastic pollution in facilitating the survival, persistence, and dissemination of human pathogens is critical for a more holistic understanding of the potential public health risks associated with plastic waste.

1. Introduction

Infectious diseases cause approximately 13.7 million deaths per year, with bacterial pathogens responsible for 7.7 million (56%) of these (Murray et al., 2022). The environment is now considered a major reservoir for such organisms, with bacterial pathogens commonly released into this setting through sewage spills, illegal dumping of sewage waste, open defecation, and agricultural run-off (Albini et al., 2023). Once in the environment, microorganisms are subjected to multiple environmental stressors, including extremes of temperature, pH, salinity, humidity, and UV radiation (DasSarma and DasSarma, 2018). Bacteria have evolved several strategies that allow them to tolerate and overcome these pressures; in addition to the general bacterial stress response, this includes the induction of specific transporters (including osmotic and salt transporters), regulators of metabolic functions, and regulators of virulence mechanisms. However, the predominant protector against environmental stress is the production of biofilm. In the environment, most microorganisms exist within a biofilm (Watnick and Kolter, 2000), which is a dense collection of microbial cells encased in an extracellular polymeric substance (EPS) matrix. The close proximity of microbial cells to one another provides significant advantages for communication strategies and for generating genetic diversity through the transfer of mobile genetic elements (Yin et al., 2019).

It is well established that plastics are ubiquitous in the environment, and rapidly become colonised with both prokaryotic and eukaryotic biofilm communities, which are collectively known as the 'plastisphere' (Zettler et al., 2013). There is growing evidence that the plastisphere can harbour both human pathogenic bacteria and viruses (Metcalf et al., 2022; Moresco et al., 2022; Silva et al., 2023), acting as a reservoir and ultimately aiding pathogen dissemination. Research has demonstrated that dangerous clinical pathogens can colonise the plastisphere and can persist for lengthy periods under simulated environmental conditions, ultimately posing a threat to human health by remaining pathogenic (Metcalf et al., 2023; Ormsby et al., 2023; Ormsby et al., 2024b).

Adverse environmental conditions are likely to become exacerbated

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by climate change, with projections indicating that more extreme weather will bring more frequent floods and droughts, enhance ocean acidification, increase temperatures, and expose the earth's surface to higher levels of UV radiation (IPCC, 2019). Although, the plastisphere can offer some level of protection against temperature, humidity, and salinity stressors (Metcalf et al., 2023; Ormsby et al., 2023), whether this novel habitat can also offer protection against the harmful effects of UV exposure has never been tested. Climate change projections indicate that levels of UV radiation that the Earth's surface is exposed to, are likely to increase considerably over the coming decades, with predicted increases of 3% (tropics) and 8% (mid-latitudes) by 2100 (Lamy et al., 2019; Bernhard et al., 2023). Understanding the ability of bacterial pathogens to withstand UV exposure is essential, as UV radiation can induce bacterial mutations, drive bacterial evolution and result in the rapid acquisition of adaptive traits. Evidence has even suggested that the evolution of UV-resistance in bacteria can coincide with increased antimicrobial resistance (AMR) (Álvarez-Molina et al., 2020).

The global prevalence of AMR is a major threat to human health., The high burden of infectious diseases is expected to disproportionately affect low- and middle-income countries (LMICs) (Sulis et al., 2022). In LMICs, there is often inadequate treatment of sewage, including hospital effluent, which is routinely discharged directly into the environment (Kookana et al., 2020; Zerbo et al., 2021). Communities regularly resort to dumping waste directly in uncontrolled urban dump sites, which are frequently located near inhabited areas (Maturi et al., 2022). These waste sites are known reservoirs for human faecal pathogens (Krystosik et al., 2020) and include large volumes of plastic. Therefore, there is a significant public health risk for those individuals who directly interact with such waste (Kretchy et al., 2020). Importantly, *Salmonella* spp., which are the most frequently isolated bloodstream pathogen in hospitalised patients in Africa (Uche et al., 2017; Stanaway et al., 2019), can persist and retain their pathogenicity on the surfaces of plastic under simulated conditions of environmental waste piles (Ormsby et al., 2024a); on the surface of plastics in soils (Woodford et al., 2024a); and attached to plastics on the surface of edible crops (Woodford et al., 2024b)

With the projected rise in UV radiation, understanding the ability of the plastisphere to protect from UV exposure is crucial for our understanding of how plastic pollution facilitates the persistence and dissemination of human pathogens in the environment. Therefore, here, we aimed to: (1) quantify the ability of S. Typhimurium to persist on plastic waste under environmentally realistic conditions of UV exposure; and (2) determine the pathogenicity of *S.* Typhimurium following its recovery from the plastisphere after exposure to UV-stress.

2. Materials and methods

Bacterial strains and growth conditions. African *S.* Typhimurium strain of MLST ST313, D23580, was used in this study. D23580 was recovered from a paediatric blood sample in Blantyre, Malawi, in 2004 (Kingsley et al., 2009). Prior to use in this study, D23580 was grown in Luria-Bertani (LB) broth (Invitrogen, UK) at 37 °C with shaking at 120 rpm, unless otherwise stated. D23580 is chloramphenicol resistant, so following recovery from the surfaces of plastic or glass, isolates were grown on LB agar supplemented with chloramphenicol (25 μg/ml) and colony forming units (CFU) enumerated.

Formation of a natural biofilm on the surfaces of glass and low-density polyethylene (LDPE). Natural biofilms were formed on glass (untreated glass microscope slides [Academy, UK] and clear LDPE (Magnum, UK) housed in bespoke high-grade stainless-steel frames and submerged in river water at ambient room temperature (ca. 18–21°C), as previously described (Ormsby et al., 2023). The absence of chloramphenicol-resistant bacteria was confirmed in the river water prior to use (data not shown). Conductivity (149 μS), pH (7.3), and turbidity (10.2 NTU) of the river water were measured using a portable probe (Combo pH and EC, Hannah Instruments Ltd., UK) and a HI-88703-02 Bench Top Turbidity Meter (Hanna Instruments Ltd., UK).

Inoculation of LDPE and glass. Both LDPE and glass were inoculated as previously described (Ormsby et al., 2024a). Briefly, individual replicate colonies (n = 4) of *S.* Typhimurium strain D23580 were grown overnight in LB. Overnight cultures were then diluted 1:100 into fresh, pre-warmed LB (to 37 °C) and grown to an OD600nm of 0.7 (this equated to approximately 5×10^8 CFU/mL). While these cultures were growing, the frames containing the LDPE and glass were removed from the tanks. Each large LDPE square in the frame (4 cm × 4 cm) was subsequently cut into four individual squares (2 cm × 2 cm), gently rinsed with PBS to remove any cells that were only loosely adhering, and placed into individual glass jars (50 ml; Fig. 1a). Individual squares of glass which had been precut (2 cm × 2 cm) prior to their insertion into the metal frame, were rinsed in PBS, and placed into individual glass jars. All jars were left at room temperature for 4h to allow evaporation of any remaining water from the surfaces of the plastic and glass squares.

Once cultures had reached an OD_{600nm} of 0.7, 15 ml were pelleted by centrifugation (4000 rpm, 10 min, 4 °C). Human faecal material (which had been stored frozen, and subsequently sterilised by autoclaving after thawing) was added to four replicate flasks containing 100 ml of river water at a concentration of 10 mg/ml and thoroughly homogenised by vortexing (this faecal suspension is subsequently referred to as 'FS'). Bacterial pellets of *S.* Typhimurium were then resuspended in 35 ml from each replicate flask of FS. Three hundred microlitres of the bacterial FS was pipetted onto each square of LDPE or glass at a concentration of approximately 2.48×10^7 bacterial cells, which was comparable between isolates and replicates (data not shown). After 24h, the LDPE and glass squares in half of the jars were completely submerged under 30 ml of river water. Of the total 288 jars (72 dry LDPE; 72 submerged LDPE; 72 dry glass; 72 submerged glass), half (144 jars) were covered with opaque black plastic (henceforth referred to as 'No UV-exposed'), and the remaining jars were left uncovered (and are referred to as 'UV-exposed').

UV exposure and persistence of *S.* Typhimurium. Bespoke PVC frames were assembled to house white (13W Slimline T5 Fluorescent Batten; [Lamps Especiales, Barcelona, Spain]) and full spectrum lights (24W Arcadia ProT5 Dragonlamp 14% UV-B; [Arcadia, UK]), in suspension above the glass jars (Fig. 1c). Lights were suspended approximately 30 cm from the plastic and glass samples, generating a UV index (UVi) of 8. Frames were completely covered in opaque black plastic to ensure no light from external sources could reach the jars. The lights were controlled by timers (24 Hour Plug-in Compact Timer Plug Socket, [HBN, UK]) which produced a photoperiod of 4 h white light; 4 h UV; 4 h white light; and 12 h darkness. Temperatures were recorded every 1 h for the duration of the experiment using an i-Button temperature logger chip (iButtonLink, WI, 176 USA; Supplementary Fig. S1).

One, 2, 3, 6, 8, 10, 14, and 21-days after inoculation (DAI) of the plastic and glass squares, replicates of each material were removed from individual glass jars and transferred to a sterile 12-well plate. Three hundred microlitres of sterile PBS was added to each square and left at room temperature, for 15 min. Using a sterile plastic loop, the biofilm was disrupted by gentle agitation. From this suspension, 20 μl was serially diluted in PBS and plated onto LB-chloramphenicol for subsequent *Salmonella* enumeration. Periodically, confirmatory PCR was conducted on isolates to ensure their identity as *Salmonella* spp., as described previously (data not shown; Ormsby et al., 2024a).

Challenge of *Galleria mellonella* with *S.* Typhimurium. *G. mellonella* larvae (Livefood, Axbridge, UK), were prepared as previously described (Ormsby et al., 2024a). To determine the minimum infectious dose, a ten-fold dilution series of a pure culture of *S.* Typhimurium strain D23580 was generated to challenge *Galleria* larvae at concentrations between 10^3 to 10^{12} CFU. Ten microlitres of each dilution were injected into each *Galleria* larva as described previously (Ormsby et al., 2023). Following challenge, larvae were placed in an incubator at 37 °C and survival assessed for 72 h, with larvae considered dead when they no longer responded to touch stimuli. Experiments were

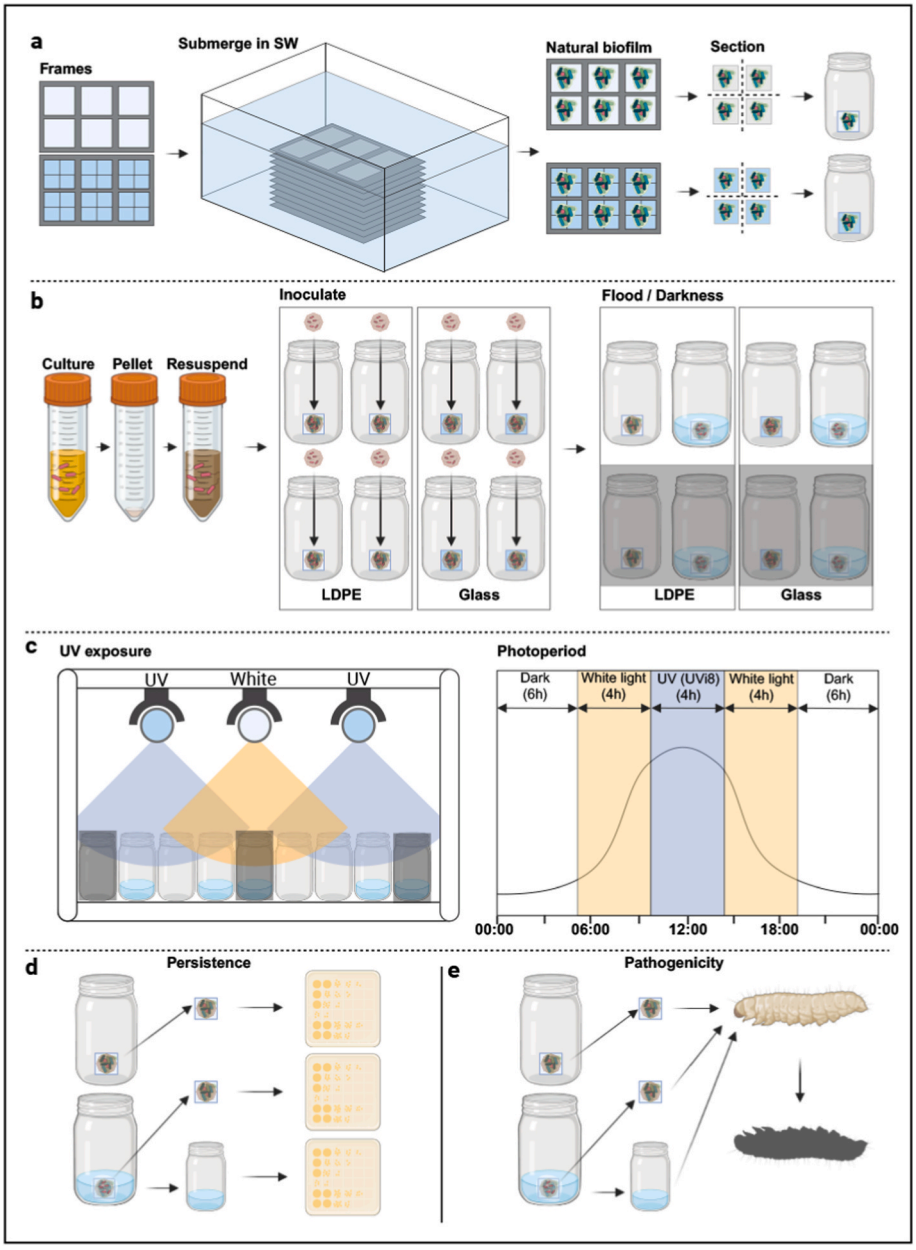

Fig. 1. Schematic overview of the sampling procedure to determine *S.* Typhimurium persistence following UV stress. Steel frames housing low-density polyethylene (LDPE) or glass were submerged in river water for 7 d to allow a natural biofilm to form on the material. Individual squares were excised from the frame and added to individual glass jars (a). Cultures of *S.* Typhimurium strain D23580 were added to a faecal suspension (FS) in river water and pipetted onto each individual square of LDPE or glass. Half of the jars had 30 ml of river water added to submerge the squares of plastic and glass, and the other half left dry; half of all jars were covered to block all light, and half left uncovered to allow UV exposure. Jars containing were randomly organised and exposed to a photoperiod of 4h white light, 4h UV, and 4h white light, followed by 12h darkness (**c**). At defined timepoints, samples were removed, and persistence determined by serial dilution and CFU analysis (**d**). Pathogenicity was determined using a *Galleria mellonella* infection model (**e**). The figure was generated using BioRender (https://biorender.com/).

conducted in biological triplicate. For all challenge experiments, an inoculation of PBS was used as a negative control (n = 10 larvae per experiment) to account for mortality caused by the procedure. To assess the pathogenicity of *S.* Typhimurium following recovery from the LDPE and glass, 21-day samples were recovered from the material in PBS as described above and were injected directly into *Galleria* larvae. This allowed examination of the pathogenicity at exact concentrations on each material at this timepoint, and a comparison with the expected pathogenicity as calculated by a concentration killing curve.

Statistical analyses. Statistical analyses were conducted using GraphPad Prism Software. A two-way ANOVA with Holm-Šídák's multiple comparisons post-test was used to compare persistence over time, with P values < 0.05 considered significant. Die-off rates were calculated as previously described (Ormsby et al., 2024b).

3. Results

Influence of UV on the persistence of *S.* Typhimurium on the surfaces of plastic and glass. Following desiccation, *S.* Typhimurium was able to persist on both plastic and glass for at least 28 days (Fig. 2). At the 10-day timepoint, significantly ($p < 0.01$) fewer cells of *S.* Typhimurium were recovered from plastic exposed to UV (5.5×10^3 CFU/ml) compared to plastic that had no UV exposure (2.3×10^5 CFU/ml). However, on days 14, 21, and 28, there were no significant

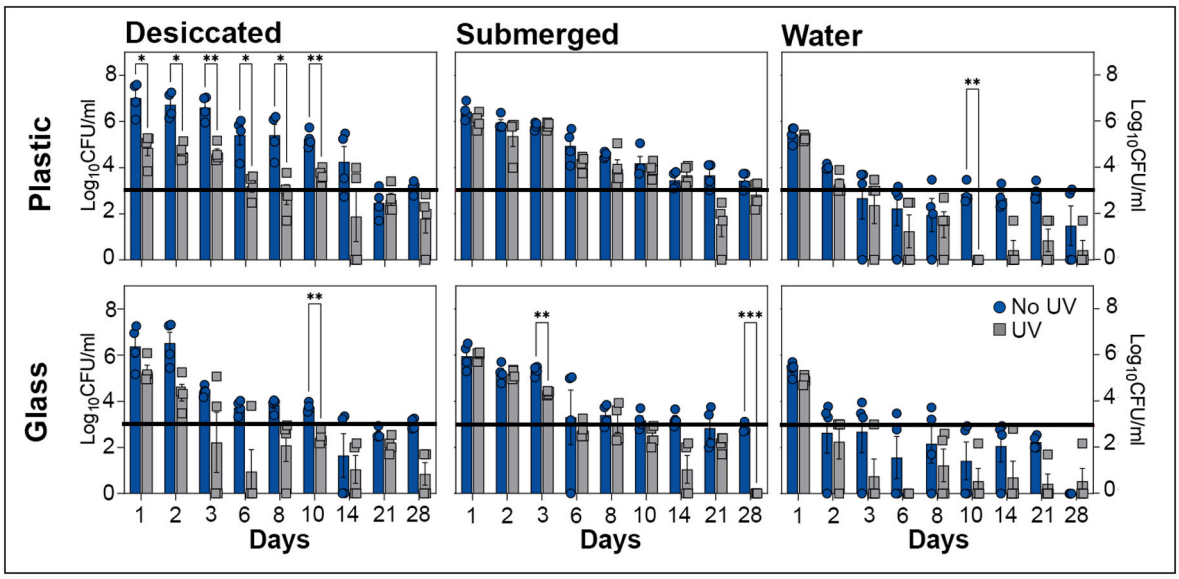

Fig. 2. The effect of UV exposure on the persistence of *S.* Typhimurium on plastic and glass. Bars represent the mean (±SE) concentration of *S.* Typhimurium, and individual data points represent distinct biological replicates ($n = 4$). Significant differences in *S.* Typhimurium concentration between non-UV and UV treatments were tested by two-way ANOVA with Holm-Šídák's multiple comparisons post-test (*P < 0.05; **P < 0.01). The solid black line indicates the minimum reported infectious dose for *Salmonella* (1×10^3 CFU).

differences in *S.* Typhimurium concentrations on the surface of UV-exposed and no-UV exposed plastics. Log-linear regression models to determine modelled linear decline rate constants (K) and decimal reduction times (D-values) indicated that the concentration of *S.* Typhimurium on the surface of plastics exposed to UV declined at a slower rate (D-value: 7.6 days) than on the surface of non-UV exposed plastics (D-value: 6.2 days) (Table 1; Fig. S2).

There was no significant difference in *S.* Typhimurium persistence on UV-exposed glass compared to non-UV-exposed glass (except at day 10; $p < 0.05$). However, by day 28, the concentration of *S.* Typhimurium that remained on the glass (25.5 CFU/ml) following UV-exposure was 10-fold less than on plastic (250.3 CFU/ml), which was also reflected in the decline rate (non-UV exposure D-value: 5.9 days; UV-exposed D-value: 1.1 days).

On the surface of plastic submerged under water, there were no significant differences in *S.* Typhimurium concentrations between UV-exposed and non-UV-exposed plastics (Fig. 2). However, significantly fewer cells of *S.* Typhimurium were recovered from the surface of glass exposed to UV relative to non-UV-exposed glass at day 3 ($p < 0.01$) and day 28 ($p < 0.01$). Log-linear regression models show that the concentration of *S.* Typhimurium on non-UV exposed surfaces declined at a slower rate than on UV-exposed surfaces for both plastic (D-Value: 9.5 days vs. 6.8 days) and glass (D-Value: 7.1 days vs. 4.1 days).

Table 1
Linear decline rates and decimal reduction times for *S.* Typhimurium.

	Treatment		K (day^{-1})		D-value (days)	R^2
			Mean	SEM		
Plastic	No UV	Desiccated	0.376	0.026	6.2	0.769
		Submerged	0.250	0.025	9.5	0.698
		Water	5.470	2.568	0.9	0.927
	UV	Desiccated	0.459	0.150	7.6	0.696
		Submerged	0.370	0.071	6.8	0.757
		Water	6.282	1.956	0.5	0.983
Glass	No UV	Desiccated	0.641	0.230	5.9	0.708
		Submerged	0.936	0.685	7.1	0.705
		Water	2.514	1.155	2.4	0.881
	UV	Desiccated	2.208	0.296	1.1	0.719
		Submerged	0.666	0.149	4.1	0.840
		Water	2.979	1.130	1.1	0.957

Concentrations of *S.* Typhimurium transferred from either plastic or glass into the water were not significantly affected by UV exposure (with the exception at day 10 in the water that had contained the plastic; $p < 0.01$); however, the quantity of *S.* Typhimurium cells was consistently lower following UV-exposure, with mostly only single replicates recovered at each timepoint (Fig. 2). Log-linear regression models showed that *S.* Typhimurium deteriorated rapidly in the water following both UV exposure (i.e., transferred from plastic: 0.5 days; and from glass: 1.1 days) and non-UV exposure conditions (i.e., transferred from plastic: 0.9 days; and from glass: 2.4 days).

***S.* Typhimurium survival at infectious concentrations and retention of pathogenicity following UV stress.** Following desiccation, *S.* Typhimurium survived on the surfaces of plastic at concentrations higher than the reported minimum infectious dose, for at least 21 days under both UV-exposed and non-UV exposed conditions (Fig. 2). At day 28, the mean concentration of *S.* Typhimurium on the desiccated surface of plastic exposed to UV (250.5 CFU) had fallen below the minimum accepted infectious dose of 1×10^3 cells. *S.* Typhimurium survived above the minimum infectious dose on the submerged plastic for the duration of the experiment (however, at day 21 the mean concentration was just below: 112.8 CFU). In the water (following release from the plastic), the mean concentration of *S.* Typhimurium on the surface of plastic that had been exposed to UV was below the minimum infectious dose by day 6 (150.5 CFU), while *S.* Typhimurium not exposed to UV was still recovered at an infectious dose by day 28 (1.2×10^3), albeit in only a single replicate.

S. Typhimurium was not recovered at an infectious dose from the desiccated glass that had been exposed to UV beyond day 6; or from the submerged glass exposed to UV beyond day 10. Non-UV exposed glass-associated *S.* Typhimurium was recovered at an infectious dose for the whole duration of the experiment. In the water (following release from the glass), the concentration of *S.* Typhimurium following UV-exposure was on the threshold of the minimum infectious dose by day 2 (1×10^3 CFU in 3 out of 4 replicates), but conclusively below it by day 6. In the water with no UV-exposure, the concentration of *S.* Typhimurium was below the infectious dose by day 8.

A standard curve determined the concentration-dependent pathogenicity of *S.* Typhimurium in a *Galleria mellonella* infection model (Fig. 3); as the concentration of *S.* Typhimurium increased, the

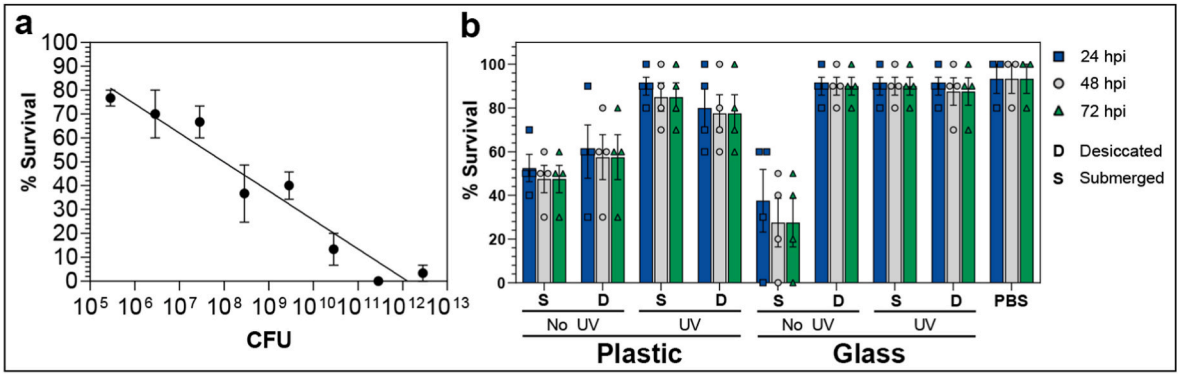

Fig. 3. Virulence of *S.* Typhimurium in a *Galleria mellonella* infection model following recovery from plastic and glass after UV-exposure. (**a**) A concentration-dependent killing curve was generated following infection with increasing concentrations of *S.* Typhimurium isolate D23580, with larval survival measured 72 h post-inoculation (hpi). Data points represent the mean ($n = 10$ *G. mellonella* larvae) of three independent biological replicates (±SE). (**b**) Cells of *S.* Typhimurium recovered from plastic and glass at day 21 were injected directly into *G. mellonella* larvae, and the survival monitored at 24, 48, and 72 hpi. *Galleria* larvae were injected with PBS as a control.

percentage survival of *Galleria* larvae decreased. This allowed the generation of a standard curve (Y = intercept + Slope*log(X); Y-intercept = 147.1; Slope = −12.14). Isolates of *S.* Typhimurium recovered from the surfaces of plastic and glass at day 21 were injected directly into *G. mellonella* larvae, which allowed a realistic assessment of their pathogenicity (i.e., in terms of concentration and metabolic state). In all cases, *S.* Typhimurium retained pathogenicity and was able to kill the *Galleria* larvae, with *S.* Typhimurium recovered from the non-UV exposed materials being the most lethal (Fig. 3b). In general, *S.* Typhimurium recovered from plastic remained pathogenic at lower concentrations than those recovered from glass, with only *S.* Typhimurium from the non-UV exposed submerged glass killing more *Galleria* than its plastic counterpart. This did not appear to be due to the infectious dose, as in several cases, the infectious doses were comparable. Utilising the equation from the standard curve, the expected mortality rate of the recovered concentrations could be calculated and compared to the observed killing. In all cases, the concentrations of *S.* Typhimurium injected into the *Galleria* larvae should not have been sufficient to induce death in the *Galleria*; however, as observed, the concentrations injected did cause mortality in *Galleria* larvae (Table 2).

4. Discussion

In this study, we have demonstrated that the plastisphere can provide some level of protection from the major environmental stress of UV exposure. Importantly, our results have indicated that *S.* Typhimurium can have greater pathogenicity following its recovery from the plastisphere, suggesting that the plastisphere may select for more virulent variants within a population. The effect of UV radiation on microorganisms at both community and cellular levels can have a significant impact, influencing community diversity and causing damage to lipids, DNA, and proteins (Pérez et al., 2017). Our study has revealed that the plastisphere can provide protection for *S.* Typhimurium against UV exposure, with the pathogen surviving for considerably longer when associated with plastic than when free-living in the water following its dissociation from the plastisphere.

With the Intergovernmental Panel on Climate Change (IPCC) projections indicating that climate change parameters, including UV radiation, are likely to worsen over the coming decades (IPCC, 2019), which will have significant implications for public health. The ability of *S.* Typhimurium to withstand UV exposure whilst in the plastisphere is likely due to the UV radiation being unable to fully penetrate the top layers of cells of the plastisphere biofilm, with recent work highlighting the capacity of a dense biofilm matrix formed on plastics to 'shield' the underlying plastic from UV-degradation (Nelson et al., 2021). Biofilms are an essential defence mechanism for bacteria and provide resistance to many physical forces including extreme temperatures, salinity, humidity, antibiotics (Shree et al., 2023), and UV radiation (de Carvalho, 2017). The physicochemical surface properties of plastic and glass will impact the attachment of primary colonisers and subsequent biofilm development (Teughels et al., 2006; Rummel et al., 2017). Hydrophobic surfaces such as plastics are more conducive to bacterial attachment and biofilm development compared with hydrophilic surfaces, such as glass (Donlan, 2002). The density and integrity of the plastisphere biofilm on the surface of plastic in this study, was therefore likely greater than that on the glass, allowing greater binding and incorporation of *S.* Typhimurium into the biofilm structure, and hence greater protection from UV stress. In this study, biofilm structure and composition were not quantified. Additionally, the structure and diversity of biofilm communities colonising plastic and glass will vary. Many plastisphere studies report 'plastic-specific taxa' not identified in biofilm on other materials, e.g. species in biofilms on LDPE compared to biofilms on glass (Pinto et al.,

Table 2
Comparison between expected and actual pathogenicity of *S.* Typhimurium following recovery from the plastisphere.

Conditions			Dose (CFU)		Predicted survival[a]		Actual Survival	
			Mean	SEM	Mean (%)	SEM	Mean (%)	SEM
Plastic	**No UV**	Desiccated	5.9	3.5	133.7	8.9	57.5	10.3
		Submerged	74.1	32.4	100.5	7.6	47.5	6.3
	UV	Desiccated	8.3	5.8	130.2	8.1	77.5	8.5
		Submerged	1.1	0.7	145.5	6.3	85.0	6.5
Glass	**No UV**	Desiccated	4.4	1.4	130.6	3.2	90.0	4.1
		Submerged	21.1	13.0	123.8	12.2	27.5	11.1
	UV	Desiccated	1.6	0.7	144.2	4.9	87.5	6.3
		Submerged	1.8	0.5	142.4	4.6	90.0	4.1

[a] Y = intercept + Slope*log(X); Y-intercept = 147.1; Slope = −12.14 (Calculated from Fig. 3a).

2019). Therefore, the association between *S.* Typhimurium and the microbial community in the plastisphere may have been more facilitative; however, in an environmental context, *Salmonella* spp. can readily adhere to, and persist on, biofilm associated with both plastic and glass (Ormsby et al., 2024a). While in this study we have focused on an African strain of *S.* Typhimurium, future work must look to include bacteria from different lineages to see how widespread the observations made here are amongst different bacterial species.

When exposed to solar radiation, the rate of hydrocarbon emission from LDPE is higher than from other types of plastics (Royer et al., 2018), which may influence the composition of the associated plastisphere community and change the dynamics of competition, or increase synergistic interactions, with *S.* Typhimurium. In the short-term, exposure to UV radiation is unlikely to modify glass or cause chemical release (unless the glass has been treated); however, the surface of glass will reflect UV radiation rather than absorb it. This intensified UV will increase the amount of UV exposure for biofilm communities on the surface of the glass, leading to a greater rate of die-off for potential pathogens in the plastisphere.

Following dissociation from the surfaces of both plastic and glass, isolates of *S.* Typhimurium rapidly died upon entry to the water, suggesting that it is the biofilm community/structure that protects from UV stress, rather than an inherent ability of *S.* Typhimurium to survive UV radiation directly (Guo et al., 2019; Jeong and Ha, 2019; Sharma et al., 2023). Water has well-established abilities to scatter UV-light (Christensen and Linden, 2003; Zhang et al., 2019), although it is unlikely that this occurred substantially in our study, as materials were submerged in only a relatively small depth of water (3 cm). In water in the environment (containing dissolved organic matter, metals etc.) UV-A and UV-B can penetrate up to 10 cm deep, depending on the turbidity (Huovinen et al., 2003). Biofilms are composed of over 70 % water, which is essential for biofilm function – maintaining osmotic pressure, dissolving nutrients, and allowing macromolecular transport (Quan et al., 2022). Being submerged under water would therefore inhibit the desiccation of the biofilm and maintain a healthier and more secure structure than in a desiccated environment, and ultimately improve the likelihood of survival.

While the surrounding environment plays a significant role in persistence, the phase of growth of *S.* Typhimurium in the biofilm may ultimately have been responsible for the enhanced survival observed. *S.* Typhimurium die-off is much more rapid when exposed to UV radiation during exponential growth (which would be occurring in the water) than when in stationary phase (such as in a biofilm) (Child et al., 2002). This is thought to be due to gene expression of RpoS, the stationary phase/stress sigma factor. RpoS is responsible for the control of multiple systems, including those affecting pathogenesis, metabolism, biofilm formation, and nutrient deprivation (Schellhorn, 2020). This further highlights the importance of the biofilm matrix in protecting from harmful UV radiation, and quantifying levels of expression of RpoS when associated with different substrates would provide valuable information on stress responses of pathogens in the plastisphere.

Informal waste pickers often collect materials from waste piles, including plastics, to be recycled, resold, or repurposed (Kretchy et al., 2020). Although most of these items will be at the surface of the waste pile, and therefore exposed to the greatest levels of UV stress, the persistence of human clinical pathogens on desiccated plastics means there is a considerable risk to those humans that interact with such waste. *S.* Typhimurium on both plastic and glass after 21 days of UV/No-UV exposure in this study, were recovered at concentrations capable of causing infection. The infectious dose of *S. enterica* strains is reported to be fewer than 1×10^5 CFU (to cause enteric fever) and 1×10^3 CFU for non-typhoidal salmonellosis (Teunis et al., 2010; Gharpure et al., 2021). However, infectious doses can be considerably lower in compromised individuals or if the level of stomach acidity has been reduced. In such cases, 1 CFU is infectious (Taitt et al., 2004). Most importantly, however, the *S.* Typhimurium isolate employed in this study showed an enhanced pathogenicity in *G. mellonella* following its recovery from the plastisphere, even following UV-exposure. The *Galleria* model has proven reliable for the study of *Salmonella* pathogenesis (Bender et al., 2013; Luiz de Freitas et al., 2021), with the model reflecting the pathogenesis of specific isolates, rather than inoculum dose (Ormsby et al., 2024a). Nutrient levels within the plastisphere may play a role in selecting fast-growing virulent strains, or strains adapted to use resources more efficiently, with our study suggesting that the protection afforded by the plastisphere against environmental stress may also facilitate the selection for fitter, more virulent variants. The heightened durability of plastic in the environment offers opportunities for pathogens to repeatedly encounter and colonise plastic waste, which together with the lightweight and buoyant properties of plastic allowing for enhanced distribution, indicates significant environmental and human health implications.

Although the plastisphere will inhibit UV penetration to some extent, variants within the microbial population that have acquired mutations in genes affecting DNA repair and replication will survive more readily (Alcántara-Díaz et al., 2004) and hence become more dominant in the population. While the focus of this study was to establish observable effects based on bacterial survival within the plastisphere, understanding the genetic modifications induced by UV exposure of pathogens while in the plastisphere could greatly enhance our understanding of pathogen-plastisphere interactions and the protection afforded. Experimental evolution of UV-resistance in *E. coli* has demonstrated increased resistance to desiccation and starvation compared to controls (Goldman and Travisano, 2011); and there is also evidence for UV driving selection of antimicrobial resistance (AMR) (Álvarez-Molina et al., 2020). Therefore, the influence of interactions of the plastisphere with environmental stressors on the simultaneous generation of AMR now needs a concerted research effort to more fully understand the role of the plastisphere in driving resistance.

5. Conclusions

The human health risks associated with plastic waste extend beyond the aesthetic, as environmental plastic pollution is now well-established as a reservoir for potentially dangerous clinical pathogens. This study has demonstrated that by associating with plastic debris, pathogenic organisms can evade environmental stressors, potentially disseminate widely through the environment, and retain (and even enhance) their pathogenic potential. The increased discharge of both plastics and pathogens into the environment, against a backdrop of climate change conditions (including increased UV radiation), highlights the pressing need to understand the role of plastics in facilitating the survival, selection, and dissemination of pathogens in the environment, and subsequently inform health legislation, control infection, and reduce the public health risk.

Environmental implications

Plastics are ubiquitous in the environment and are often colonised by human pathogens, representing a significant human health risk. The properties of plastic polymers facilitate their enhanced distribution through the landscape and ultimately increase the risk of human exposure. Pathogens associated with the plastisphere will be exposed to diverse environmental conditions, which are projected to become more extreme through climate change. Our data indicates that the major human pathogen *S.* Typhimurium can survive and retain virulence, on plastic waste following realistic periods of UV-exposure. This demonstrates that the plastisphere heightens the survival of human pathogens in the environment and increases the exposure risk to humans from dangerous clinical pathogens.

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CRediT authorship contribution statement

Michael J. Ormsby: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Luke Woodford:** Writing – review & editing, Data curation. **Hannah L. White:** Writing – review & editing, Data curation. **Rosie Fellows:** Writing – review & editing, Data curation. **Richard S. Quilliam:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124464.

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