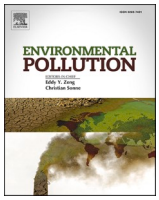
Environmental Pollution 366 (2025) 125518

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol



Fluvial flooding and plastic pollution – The delivery of potential human pathogenic bacteria into agricultural fields☆

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ARTICLE INFO

Keywords:
Agricultural pollution
Environmental pathogens
Enteric pathogens
Water contamination
Microbial pollution
Plastisphere

ABSTRACT

The frequency of plastic debris entering agricultural land is likely going to increase due to increased discharge into surface waters and more frequent flood events. Microbial biofilm on the surfaces of plastic pollution (known as the 'plastisphere') in freshwater environments often includes human pathogenic bacteria capable of causing disease. Pathogens have been detected on the surface of plastics in freshwater environments, but it is yet to be determined whether plastic debris can also transport pathogens into agricultural fields during flooding. Therefore, this study quantified the presence of viable pathogenic bacteria on the surface of plastic pollution at five agricultural fields along two rivers. All visible plastic debris, including sewage-associated plastic waste, were collected along a perpendicular 100 m transect from the riparian zone into each field. All plastic pieces were screened for five target bacteria *(Escherichia coli*, intestinal enterococci, *Salmonella* spp., *Campylobacter* spp., and *Klebsiella* spp.) using selective media, and positively identified colonies subsequently tested for antimicrobial resistance. In all five fields, there were higher volumes of plastic in the areas closer to the river, with 75% ± 24% of plastic collected within 30 m from the riverbank. Overall, 49% of all plastic collected in agricultural fields was colonised by phenotypically positive colonies for at least one or more target bacteria, with resistance to commonly prescribed antibiotics detected among several of these target bacteria. Therefore, the transport of contaminated plastic debris from fluvial floodwater into agricultural fields could pose an as yet unquantified risk of introducing potentially harmful bacteria into agricultural systems and the ultimately into the food chain.

1. Introduction

Plastic pollution has become abundant in riverine systems due to the mismanagement of waste, wastewater discharge, and urban and agricultural run-off (Rinasti et al., 2022; Montecinos et al., 2022; Treilles et al., 2021; Campanale et al., 2020). Once in the aquatic environment plastic debris is rapidly colonised by a range of microorganisms that form a diverse biofilm community known as the "plastisphere" (Zettler et al., 2013). The plastisphere provides a distinct habitat compared to the surrounding environment and offers microorganisms (including human pathogens) protection from environmental stressors, (Li et al., 2024; Metcalf et al., 2023). Enteric pathogens are frequently discharged into the environment in treated and untreated wastewater (Puljko et al., 2022; Passerat et al., 2011), and can associate with the plastisphere or bind directly to plastic particles (Metcalf et al., 2023). Plastic debris downstream from wastewater treatment plants (WWTPs) therefore, are colonised by a greater abundance and diversity of enteric pathogenic bacteria compared with upstream plastisphere communities (Silva et al., 2023). In addition to microplastics (plastics <5 mm), sewage-associated plastic waste such as wet wipes, sanitary products, cotton bud sticks can enter waterways in wastewater discharge during combined sewage overflow (CSO) or spillage events, and act as environmental reservoirs for faecal bacteria and genes for antimicrobial resistance (Metcalf et al., 2022;Ormsby et al., 2023).

Previous research has focused on the transport of plastic from freshwater to marine environments; however, rivers can also act as a sink for plastic pollution, e.g., by entrainment by vegetation or becoming trapped in sediment (Emmerik et al., 2023; Gallitelli et al., 2024a; Gallitelli et al., 2024b). During periods of heavy rainfall, trapped plastic can become remobilised by fast flowing water and transported back into the terrestrial environment during flood events (Emmerik et al., 2023; Valyrakis et al., 2024). During flooding, larger plastic debris is likely to become trapped within the riparian zone (Cessarni et al., 2022); however, smaller lightweight plastics can be transported onto

☆ This paper has been recommended for acceptance by Dr. Meththika Vithanage.

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https://doi.org/10.1016/j.envpol.2024.125518
Received 4 July 2024; Received in revised form 5 December 2024; Accepted 7 December 2024
Available online 10 December 2024

floodplains (Rolf et al., 2022). As flooding is expected to increase with projected climate change (Ramachandran et al., 2019; Yu et al., 2021), the dissemination of plastic pollution from aquatic to terrestrial environments is likely to increase.

Importantly, the fertile soils of floodplains are often used as farmland, and the consequences of flooding in agriculture are well documented, e.g., waterlogging of soil, destruction of crops, farm buildings and equipment, and the impact on the livelihoods of farmers (Kim et al., 2023; Rayburg et al., 2023; Sam et al., 2021). However, the abundance of plastic accumulating in fields from fluvial flooding and whether these plastics are colonised by enteric bacteria has not yet been established. Pathogen-free food production environments are vital for food safety as contamination of soil, irrigation water, and production and processing equipment can often lead to outbreaks of foodborne illness with serious

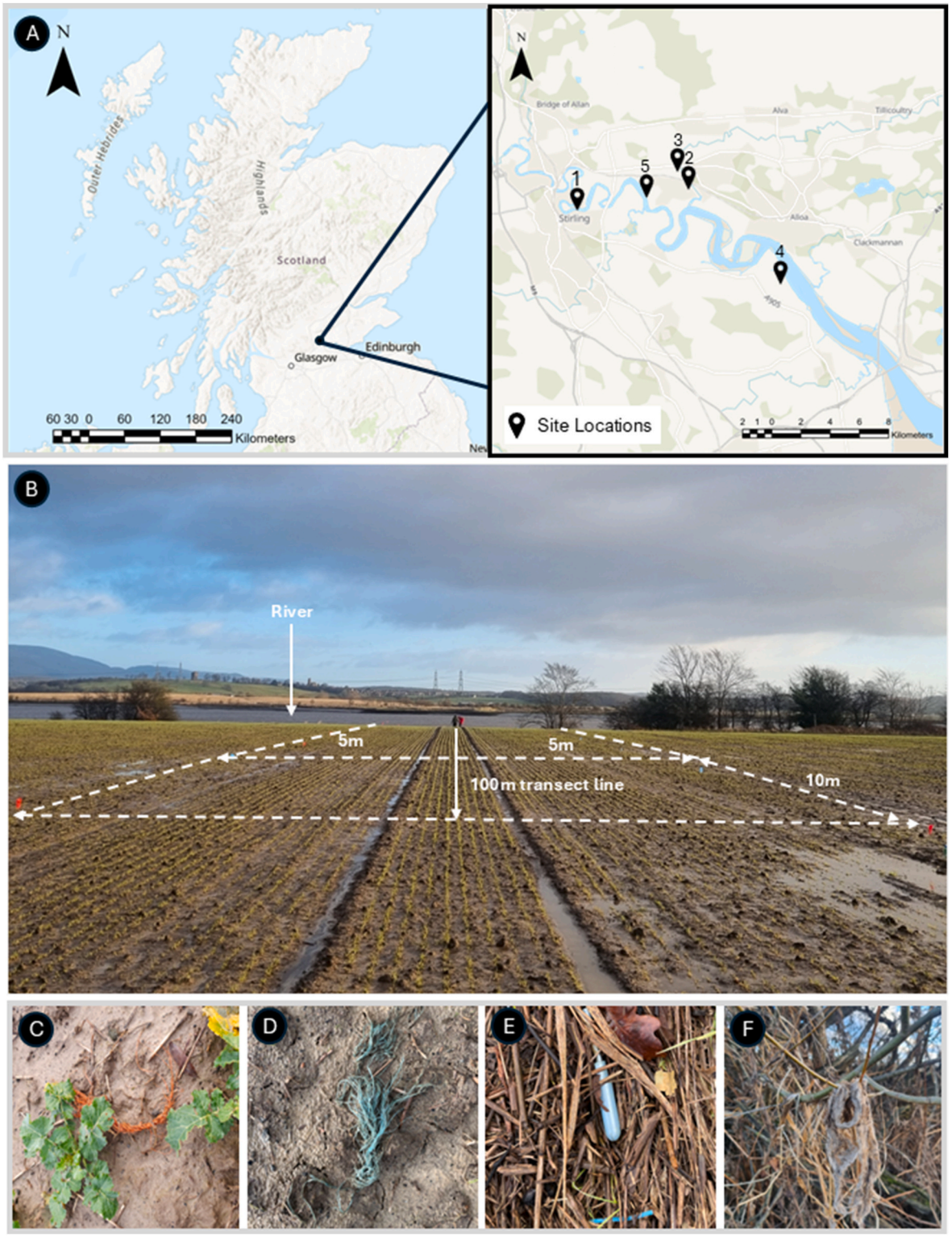

Fig. 1. Location of sampling sites (A), and an example of the sampling method showing one 10 m by 10 m quadrat (B). Examples of plastic types recovered from agricultural field sites: fishing gear embedded in soil (C and D), and sewage associated waste on riverbank (E and F).

impacts on human and animal health. The transport of plastics into agricultural fields could be a pathway for introducing pathogens into agricultural systems (Quilliam et al., 2023), for example, once delivered into a field, potential pathogens colonising plastic debris could continue to persist due to the protection afforded by the plastisphere, and even be directly transferred to crops or livestock (Woodford et al., 2024a). Therefore, the aims of this study were to, (1) quantify the spatial distribution of macroplastic debris (those >5 mm) from the riverbank into agricultural fields, and (2) determine colonisation by five potentially enteric bacterial pathogens on the surface of plastic sampled from agricultural flood-prone fields.

2. Materials and methods

2.1. Site locations and sampling

The low-lying catchments of the River Forth and River Devon, situated in central Scotland (Fig. 1), are dominated by agricultural land susceptible to flooding (SEPA, 2015; LUC, 2012). Five agricultural fields from these catchments were used as sampling sites, with sites 1, 4 and 5 situated on the River Forth (Site 1, 56°08′00.2″N 3°57′26.1″W; Site 4, 56°05′22.0″N 3°47′04.1″W; Site 5, 56°07′40.5″N 3°54′51.0″W) and Sites 2 and 3 on the River Devon (Site 2, 56°08′06.6″N 3°51′28.0″W; Site 3, 56°08′28.2″N 3°50′57.3″W). All sites were part of working arable farms, apart from site 2 which was used for rearing sheep.

At each site, a 100 m transect was divided into ten 100 m^2 quadrats using a GPS (Garmin, USA) and marked out with flags (Fig. 1B). Samples were collected by systematically walking up and down within each quadrat collecting all visible pieces of plastic on the soil surface. Each piece of plastic was placed in a separate sterile sample bag using sterile forceps. Where plastic did not fit into the sample bag, a section was cut from it using sterile scissors.

2.2. Isolation of target bacteria

All plastic debris was returned to the laboratory in cool boxes and processed within 4 h for colonisation by five target pathogens (*E. coli*, *Salmonella, Klebsiella, Campylobacter* spp. and intestinal enterococci). These pathogenic bacteria were selected based on their association with faecal contamination and their potential to cause enteric diseases in humans (Cho et al., 2020; Farhadkhani et al., 2020; Krawczyk et al., 2021). Each piece of plastic collected from the field was cut into approximately 16 cm^2 pieces and added to sterile glass vials containing 10 ml of 1 % phosphate buffered saline (PBS) and five glass beads (diameter 4 mm). All vials were vortexed at 1600 rpm for 30 s to disrupt the biofilm on the surface of the plastics. Concentrations of target bacteria were quantified by membrane vacuum-filtration by adding 1 ml of the wash solution and 1 ml of PBS to five different 0.45 μm cellulose acetate membranes (Merck, Germany) and vacuum-filtered (Microsart E. Jet Liquid Transfer Pump, Sartorius, Germany). Each membrane was subsequently transferred onto the surface of one of five selective media: Slanetz and Bartley (Oxoid, UK), Membrane Lactose Glucuronide Agar (Oxoid, UK), Klebsiella ChromoSelect (Merck, Germany) supplemented with Klebsiella Selective Supplement (Merck, Germany) Campylobacter Blood Free Agar (Oxoid, UK) supplemented with CCDA supplement (Oxoid, UK) and Salmonella Shigella Agar (Oxoid, UK). Plates were inverted and incubated according to the manufacturer's instructions and colony forming units (CFU) enumerated.

2.3. Antimicrobial susceptibility testing

The three sites (Sites 1, 3 and 5) with the most quadrants containing phenotypically positive colonies were chosen for antimicrobial susceptibility testing using M14 g negative mast rings (Mast Group, UK), to give an overview of AMR prevalence in the plastisphere across the agricultural field. For each 100 m^2 quadrat, representative triplicate colonies of *E. coli*, intestinal enterococci, *Campylobacter* and *Klebsiella* spp. (when present), were added to 10 ml of Luria-Bertani (LB) broth and incubated at 37 °C overnight. Overnight cultures (100 μL) were then added to 3 ml molten agar (0.75%) and poured onto LB agar and left to set. Sterile forceps were used to place a mast ring on the surface of each plate before incubating at 37 °C overnight. Where there was no zone of inhibition around the antibiotic disk, the bacterial colony was determined to be resistant to the antibiotic at the concentration administered by the mast ring.

2.4. Polymer characterisation

All plastic polymers collected from agricultural fields were characterised by Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy and divided into three categories Polyethylene (PE), Polypropylene (PP) and Other (O). In addition to FTIR, plastic debris In addition to FTIR, plastic debris were visually characterised as being either sewage, or non-sewage, related, for example, items such as wet wipes, cotton bud sticks or sanitary products were characterised as sewage-associated plastics.

3. Results

3.1. Plastic abundance, spatial distribution, and polymer type

A total of 195 pieces of plastic were collected across the five sites with an average of 39 (±14) pieces per site. Between 48% (Site 5) and 97% (Site 3) of plastic was collected within 30 m of the riverbank (Fig. 2), i.e., quadrats 1–3, and although the relative abundance of each polymer type (PE, PP, or O) varied between sites this was not consistent (Table S1). No other types of non-plastic litter were present within any of the sample zones. There was visual evidence of flooding at all five sites, with sewage-associated plastic waste (wet wipes, cotton bud sticks and sanitary products) collected in fields within 30 m from the river at four of the sites (Fig. 1). Site 3 had the highest abundance of sewage-associated plastic waste (19%) of which 50% of those samples were positive for enteric bacteria (Table S2). No sewage-associated waste was detected at Site 5; however, fishing net was found embedded into agricultural soil up to 90 m from the river, and all four samples of fishing net were contaminated with at least one of the target bacteria.

3.2. Bacterial colonisation of plastic debris and AMR

Phenotypically positive colonies of all five target bacteria were isolated (Fig. 3), with 48% of all plastic pieces positive for at least one of the

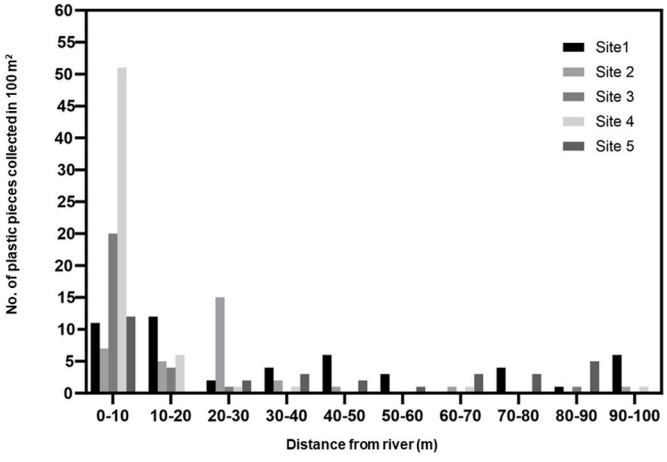

Fig. 2. Spatial distribution and abundance of plastic waste in agricultural fields.

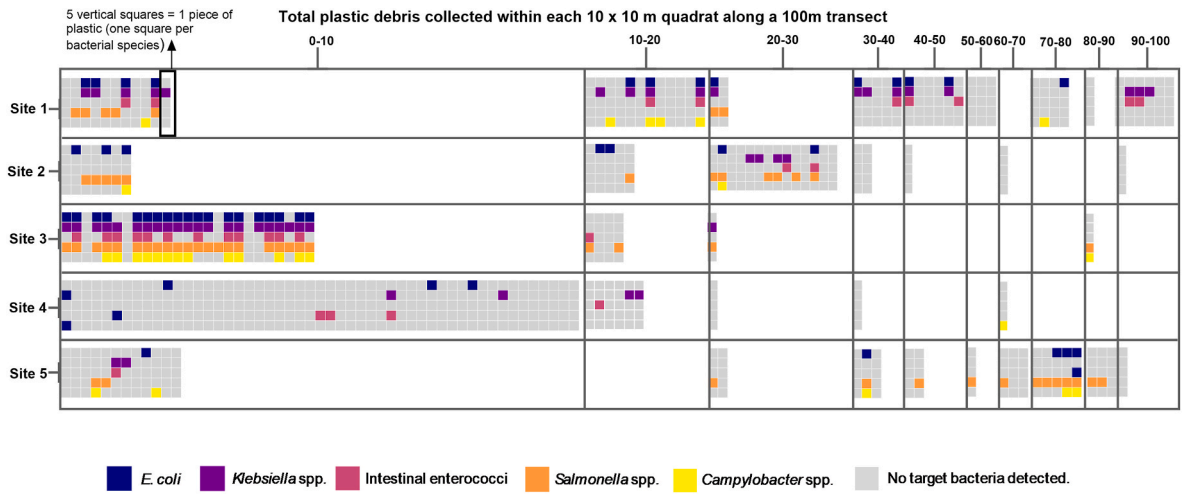

Fig. 3. Target enteric bacteria isolated from the surface of plastics sampled from flood prone agricultural fields.

target bacteria, although colonisation rates differed greatly between the five sites. Site 3 had the highest percentage of plastic colonised (84%) and Site 4 had the lowest (25 %). The faecal indicator organisms (FIOs), *E. coli* and intestinal enterococci were the most frequently isolated bacteria on plastic debris (Sites 2, 3, and 4), while presumptive *Klebsiella* spp. were most frequently isolated at Site 1. Across the three field sites tested, there was evidence of resistance to the antibiotics Cephalothin, Sulphatriad, and Ampicillin in some of the isolates. This was most commonly within the first 60 m from the river with the acceptation of site 5 where resist colonies were observed up to 90 m from the riverbank (Fig. 4).

4. Discussion

In this study, presumptive *E. coli*, *Campylobacter*, *Salmonella*, *Klebsiella* and intestinal enterococci were isolated from the surfaces of plastic debris found in agricultural land adjacent to two rivers (Fig. 3). At all five sites there was a correlation between plastic abundance and proximity to the river, suggesting a novel transportation pathway for human pathogens to enter agricultural land during flood events, which could increase the potential for food and waterborne disease especially in ready to eat crops such as leafy greens (Woodford et al., 2024a).

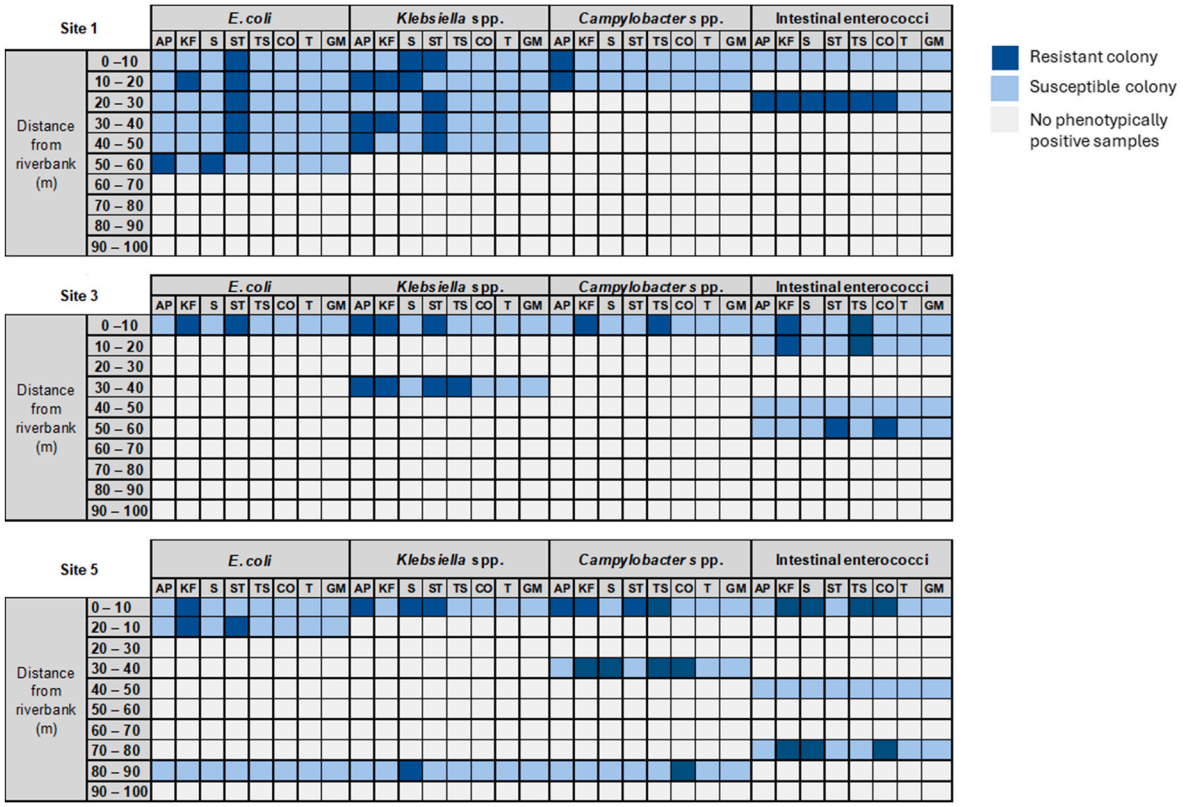

Fig. 4. Antibiotic susceptibility testing of presumptive E. coli, Klebsiella spp. Campylobacter spp. and intestinal enterococci isolated from plastic collected from agricultural fields prone to flooding. AP = Ampicillin 10 μg, KF =Cephalothin 5 μg, CO =Colistin Sulphate 25 μg,GM = Gentamicin 10 μg, S= Streptomycin 10 μg, ST= Sulphatriad 200 μg, T = Tetracycline 25 μg, TS= Cotrimoxazole 25 μg.

4.1. Spatial distribution of plastic debris in agricultural fields

The spatial distribution of plastic can vary considerably between floodplains and have different accumulation patterns such as a deposition line parallel to the water or more general patterns of clustering due to environmental topography (Tasseron et al., 2024).In this study the highest abundance of plastic in agricultural fields was within 30 m of the river, similar to other studies where between 90 and 58% of macroplastic debris were deposited within 5 m of the water line (Tasseron et al., 2024) A significant proportion of plastic debris, e.g., wet wipes and sanitary products, transported in flooded river water were likely immobilised by riparian vegetation (Cesarini and Scalici, 2022). On riverbanks of central Italy, for example, 93.9% of macroplastic (including sanitary waste), was immobilised by riparian vegetation, trees, shrubs and reeds (Gallitelli et al., 2024b). However in this study there were also plastics detected further into the field, e.g., fragments of fishing net, suggesting significant inland transport of plastics in flood waters, which could also be coupled with subsequent wind dispersal (Rezaei et al., 2019). Although this study solely focused on macroplastics (>5 mm), it is very likely that high concentrations of microplastics (<5 mm) will also get deposited during flooding and due to their size and lightweight properties are likely to be disseminated more uniformly throughout the field following flooding. For example, in the flood plains of the river Rhine, the top layer of soil (<5 cm) is contaminated by over 51,000 particles of microplastics kg^{-1} (Rolf et al., 2022); all of which will be colonised by autochthonous soil and/or freshwater microbial communities.

4.2. Colonisation of plastic waste by potential pathogens

It is unclear how long the plastic debris collected in this study had been at each of the field sites and when they had been colonised, i.e., whether they were already colonised when they were deposited into the field or if they became colonisation once in the field. During flooding with river water, planktonic pathogens, or pathogens binding to particulate organic matter, will also be transported into agricultural fields and deposited on to the soil (Castro-Ibáñez et al., 2015) Biofilm on soil particles does not offer the same environmental protection compared to the plastisphere (Woodford et al., 2024b), and importantly, enteric pathogens such as *E. coli* can survive and retain their virulence within the plastisphere for a minimum of 28 days (Ormsby et al., 2024). Once in the field, plastic debris can become recolonised following contact with, e.g., manure, livestock faeces, or by being submerged in subsequent floodwater, and due to the recalcitrant properties of plastics could remain in the soil for a significant period (Qi et al., 2020; Wang et al., 2022). With time, plastic particles move vertically through soil where they can become adsorbed onto the surface of plant roots (Yu et al., 2021). This may provide an opportunity for pathogens to leave the plastisphere biofilm, e.g., in response to chemotactic stimuli in root exudate, and either bind to the surface of roots or become internalised into the plant (Quilliam et al., 2023). Similarly, enteric pathogens can be transferred from plastic mulch, which is common in agricultural settings, directly onto the leaves of ready-to-eat crops (Woodford et al., 2024a).

Genes for antimicrobial resistance are actively expressed within the plastisphere and can be transferred through the plastisphere community by horizontal gene transfer and plasmid acquisition resulting in the soil plastisphere being a hotspot for the evolution of antimicrobial resistance (Zhu et al., 2022; Rillig et al., 2024; Ormsby et al., 2024). Globally, antimicrobial resistance is considered a significant threat to human and animal health with associated human deaths expected to reach 10 million per year by 2050 (Murray et al., 2022). However, whilst this study demonstrated the presence of enteric bacteria resistant to commonly prescribed antibiotics including Ampicillin and Cephalothin, the minimum inhibitory concentration was not determined, and so it is not known whether these isolates would impact treatment of possible infections transmitted from these agricultural sources.

4.3. Environmental and agronomic management

Given the high yield potential of fertile soils adjacent to rivers it is not reasonable or practical to prohibit farmers from growing crops in these areas. However, significant volumes of sewage-associated plastics are being discharged into rivers through either sewage spill events or deliberate release of untreated effluent, whilst high volumes of microplastics are still being discharged in treated wastewater effluent (Besley and Cassidy, 2022; Lee et al., 2021). Our study has indicated a potential health risk associated with agricultural land near flood prone rivers, which demonstrates that measures must be put in place to prevent sewage contaminated plastic reaching food producing land. Considering the abundance of plastic within riparian areas, putting aside larger buffer zones comprised of native vegetation could act as a natural barrier to plastic debris reaching agricultural fields (Cesarini and Scalici, 2022). Similarly, stone, sand and soil bunding near riverbanks could be used to stop the lateral movement of flood water and the transfer of plastic beyond the river channel (Srivastava et al., 2023). However, the maintenance and modification of agricultural land that would be needed to reduce plastic pollution from flooded rivers entering food production areas comes at a cost, and currently there are no clear guidelines of who should pay for this. Clearly, it is in the best interests of farmers and landowners to not have their fields contaminated with plastic debris (particularly if colonised by potential pathogens), specifically if these areas are being used for producing food. Although local community organisations are often effective at developing short-term projects to clean up local community areas, this is not a sustainable solution for stopping plastic debris being transported into agricultural fields; in the long-term, major improvements to waste management processes need to be made at source.

5. Conclusion and future perspectives

Fluvial flooding can impact agricultural land, destroy crops and erode soils; however, little is known about the hidden risks of the co-pollutants that it carries. This study has demonstrated the link between potential pathogens colonising plastic debris in agricultural fields and fluvial flooding. Such plastic may pose a threat to public health if in contact with crops or livestock on agricultural land however, it is critical that the significance of this potential risk is quantified in environmentally and agronomically relevant terms. It was not known how long the plastic collected in this study had been in these agricultural fields, or when the field was last flooded, therefore, quantifying the role of the plastisphere for facilitating the persistence of potential pathogens in soil together with the mechanisms of biofilm detachment needs to be identified to establish potential routes of human pathogens from the plastisphere into the food chain.

Despite implementing potential mitigation strategies, the transport of plastic from flooding rivers into agricultural land is likely to continue unless plastic pollution is targeted at its source. In the UK, single use plastics such as cotton buds have been banned, yet these products were frequently found in the riparian zone in this study suggesting a legacy effect in the environment. Single use plastics are often used for safe hygiene practices such as personal care items and therefore information about, and even enforcement of, proper disposal methods should be implemented to prevent wet wipes and cotton buds accumulating in sewerage networks or being dumped in undesignated landfill. Education programmes and new infrastructure could be funded by plastic taxes, the ‘polluter pays’ principle, or ‘pay as you throw’ approaches to increase commercial and community responsibility. Importantly, conservative estimates report that agriculture uses at least 12.5 million tonnes of plastic annually, which will contribute to plastic pollution in fields (FAO, 2021; Wang et al., 2022), and so farmers should be empowered to make informed choices about sustainable alternatives to commonly used

plastic such as seed coatings and mulch films. Therefore, a multi-stakeholder collaborative effort is needed from governments and policy makers to create and enforce legislation on the production and management of plastic waste so that it does not end up in agricultural and food-producing systems.

CRediT authorship contribution statement

Chloe J. Pow: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Rosie Fellows:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Hannah L. White:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Luke Woodford:** Writing – review & editing, Formal analysis. **Richard S. Quilliam:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Acknowledgments

This work was supported by the UKRI Natural Environment Research Council (NERC) as part of the GCRF SPACES project [grant number NE/V005847/1] and the Plastic Vectors Project, "Microbial hitchhikers of marine plastics: the survival, persistence & ecology of microbial communities in the 'Plastisphere'" [grant number NE/S005196/1]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.125518.

Data availability

Data will be made available on request.

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