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Baseline

Persistence of ‘wet wipes’ in beach sand: An unrecognised reservoir for localised *E. coli* contamination

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ABSTRACT

The flushing of wet wipes down toilets leads to blockages of sewerage systems. This, together with unregulated sewage discharge, often results in increasing numbers of wet wipes washing up onto beaches. However, it is unclear how long wet wipes can persist on the beach and whether they pose a prolonged public health risk if contaminated by *E. coli.* In this mesocosm study, three types of wet wipes (plastic containing, and home and commercially compostable) colonised with *E. coli* were buried in beach sand and their degradation, tensile strength, and concentration of *E. coli* was quantified over 15 weeks. Wet wipes containing plastic remained largely intact for 15 weeks, whilst both compostable wet wipes fragmented and degraded. Importantly, *E. coli* persisted on all three wet wipe types, representing localised reservoirs of *E. coli* in the sand, which could present a human health risk at the beach.

1. Introduction

Reports of illegal sewage discharges are becoming more common, although governments and water companies are failing to make the appropriate changes to prevent future sewage spills or discharge events. For example, in the UK, despite the implementation of fines and stricter environmental regulations (e.g., UK Environment Bill 2021), illegal sewage discharges continue even during periods of drought (Stallard et al., 2023). Furthermore, the disposal of wet wipes in toilets causes sewerage blockages, that also leads to an increase in sewage spill events (Water UK, 2023). Collectively, this results in an increasing number of wet wipes washing up onto our beaches, with a recent survey reporting 63 wet wipes for every 100 m of beach in Scotland, an increase of 150 % compared with 2021 (Marine Conservation Society, 2022). This has negative consequences for beach aesthetics and tourism and has attracted significant media attention (Heany, 2023; Taggart, 2023), but may also pose a sanitary risk to human health.

Wet wipes are multipurpose non-woven textiles, used for personal hygiene and disinfection, and are composed of several different polymer fibres and chemical additives (e.g., lotions, antimicrobial agents, and preservatives) (Das and Pourdeyhimi, 2014). These polymer fibres include synthetic plastics (e.g., polyethylene and polyester) and cellulose from either a natural source (e.g., wood pulp, cotton, and bamboo) or chemical regeneration (e.g., viscose) (Pantoja Munoz et al., 2018; Allison et al., 2023). In 2021, 90 % of wet wipes contained plastics (Zhang et al., 2021), which can accumulate and persist in the environment (Ó Briain et al., 2020; Rapp et al., 2020). However, as public awareness improves, compostable non-plastic wet wipe alternatives have become increasingly available (although many brands of compostable wipes still contain plastics (Ó Briain et al., 2020)). To be considered compostable, wet wipes must fulfil certain criteria (e.g., BS EN 13432 compostability standard); a ‘commercially compostable’ wipe must decompose in an industrial composting facility (at 58 °C) within 180 days, whilst a ‘home compostable’ wipe must decompose at ambient temperature (20–30 °C) within 365 days (British Plastics Federation, 2023). However, under natural environmental conditions, there is evidence of compostable products, including wet wipes, failing to fully degrade within these timeframes (Manfra et al., 2021; Allison et al., 2023).

The presence of plastic-associated sewage waste is becoming more prevalent at beach environments (Metcalf et al., 2022), yet the persistence and/or degradation of wet wipes in beach sand is yet to be quantified. Wastewater treatment plants (WWTPs) are known hotpots of human bacterial, viral, and fungal pathogens, which can harbour antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs) (Conco et al., 2022). MGEs including plasmids and bacteriophages can facilitate the transfer of antimicrobial resistance and virulence genes, which can increase in frequency in microplastic associated bacteria

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compared to free-living bacteria (Arias-Andres et al., 2018; Metcalf et al., 2024). Having a water content of >90 %, wet wipes are highly susceptible to microbial colonisation (Salama et al., 2021), and due to their larger area for colonisation compared to microplastics could become enriched with harmful bacteria on their transition through WWTPs (Pham et al., 2021). Once discharged or spilled from the WWTP, pathogens colonising microplastics can survive the transition through the freshwater-marine continuum to beach environments (Metcalf et al., 2023), and it has previously been shown that potential bacterial pathogens are more often associated with wet wipes compared to seaweed (Metcalf et al., 2022); however, it is unclear whether these potential pathogens could continue to survive on wet wipes when buried in beach sand. We are also yet to understand how different wet wipe types degrade in beach sand and how this will influence pathogen survival and dispersal within the environment. As beach environments are a main route for human exposure to potential pathogens colonising wet wipes, it is timely that we increase our understanding of the public health risks associated with this. Therefore, this study aimed to determine the physical degradation of wet wipes (plastic-containing and compostable), and the persistence of *E. coli* on their surfaces when buried in beach sand. To address this, we have used a culture-dependent approach to determine how long *E. coli* can remain viable on wet wipe surfaces in beach sand environments, and quantified tensile strength measurements of the wet wipes as a proxy for degradation.

2. Materials and methods

2.1. Mesocosm set-up

Wastewater effluent, seawater, and sand were collected from sites within the river Forth catchment (wastewater effluent, Dunblane [56.184°N, −3.963°W]; seawater and sand, Kirkaldy [56.117°N, −3.146°W]) in Scotland, UK between 10th–17th May 2023. Samples were stored at 4 °C and used within 24 h. Salinity and pH were measured with a salinity refractometer (RGBS) and a HI 2209 pH meter (Hanna Instruments, UK), respectively (Table S1). To determine background *E. coli* concentrations, water samples (100 mL, $n = 4$) were vacuum filtered through 0.45 μm cellulose acetate membranes (Merck, Germany). To extract background *E. coli* in the sand samples, 20 g sand was added to 20 mL sterile phosphate buffered saline (PBS) and vortexed (1500 rpm, 10 min); the samples were left to settle before 1 mL of the supernatant was vacuum membrane filtered as above. Membranes were aseptically transferred onto the surface of selective media (membrane lactose glucuronide agar [MLGA]; Oxoid, UK). Colony forming units (CFU) were enumerated after incubation at 37 °C for 24 h (Table S1). To determine the sand dry weight, four replicate sand samples were placed into a drying oven (Swallow Oven, UK) at 75 °C for 24 h.

Three commercial wet wipe brands were selected with differing categories of advertised compostability (A: non-compostable, containing plastic; B: home compostable; C: commercially compostable). All wet wipes were cut into replicate 7 × 7 cm squares and passed through a series of treatments to simulate their journey from the bathroom to the beach (Fig. 1), i.e., flushed down the toilet, discharged from the WWTP into seawater, and finally washed up onto beach sand. Initially, wet wipes were placed into a bucket containing 10 L of tap water containing 250 g sterile human faeces supplemented with an additional inoculum of *E. coli* (1×10^3 CFU/ml). Wet wipes were stirred continuously for 2 min to simulate being flushed down the toilet, before being moved into glass beakers containing 2 L of fresh effluent discharged from a WWTP with an additional inoculum of *E. coli* (5×10^3 CFU/ml). All beakers were subsequently incubated on an orbital shaking incubator for 48 h (15 °C, 80 rpm). Wet wipes were then transferred into glass beakers containing

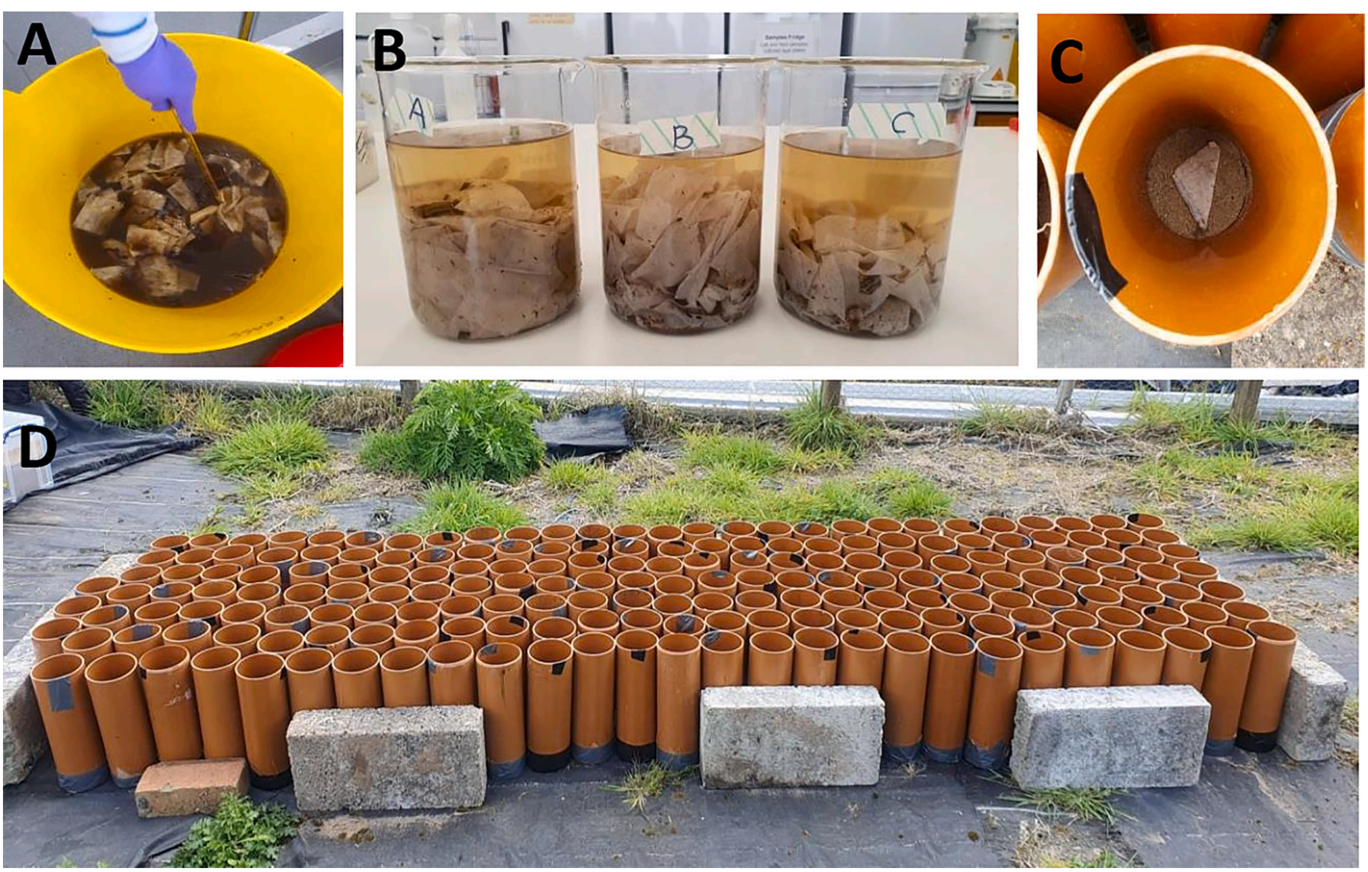

Fig. 1. Mesocosm set-up. Wipes were passed through a simulated toilet flush (A), incubated in effluent followed by seawater (B), before being placed into the sand mesocosm tubes (C, D).

2 L seawater and incubated for a further 24 h (15 °C, 80 rpm), before being transferred into environmental beach sand mesocosms (18th May 2023; Fig. 1). The sand mesocosms were constructed from drainpipes (11 cm circumference; Tool Station, Bridgwater, UK) cut into 30 cm long sections and filled with sand to 10 cm deep. Frost hessian fabric (EU Fabrics, Birmingham, UK) was used to cover the bottom end of the tube to prevent loss of sand but allow drainage. Each wet wipe was folded in half (to form a triangle), and added to each pipe and covered with a further 5 cm of beach sand. I-Button temperature logger chips (iButtonLink, WI, 176 USA, $n = 4$) were placed into mesocosms to monitor the temperature throughout the study. Rainfall and temperature data for the duration of the study were obtained from the closest Met Office weather observatory at Grangemouth, Scotland (56.017°N, −3.700°W; Met Office, 2023).

2.2. Sample processing

At weekly timepoints (for 15 weeks), four of each wet wipe type were randomly selected, and removed from the mesocosm using sterile forceps. The loosely adhering sand was shaken off and each wet wipe transferred into a sterile glass vial containing 20 mL PBS. Vials were then vortexed at 1500 rpm for 5 min and *E. coli* enumerated as described above and previously (Metcalf et al., 2022). The wet wipes were removed from the glass vials and left to dry overnight. The following day, a 50 N digital force meter (Vogueing Tool, Hubei, China) was used to quantify tensile strength by measuring the force required to break each wipe.

2.3. Statistical analysis

Statistical analyses were conducted using R Studio version 3.3.2 (R Core Team, 2016). Analysis of variance (ANOVA) with Tukey's post-hoc test was used to compare the tensile strength and *E. coli* concentrations between the three different types of wet wipes. All data were tested for distribution and homogeneity of variance (Shapiro-Wilk and Levene's) before parametric tests were used. Where assumptions were not met, non-parametric Kruskal-Wallis tests were used. Data is reported as mean ± SE. P values ≤0.05 are considered significant.

3. Results

3.1. Wet wipe degradation

Although all wet wipes became darker over time (Fig. 2), the plastic-containing wet wipes ("wipe A"), remained intact compared to both of the compostable wipes ("wipes B and C") which fragmented and degraded during the 15-week course of the experiment (Fig. 2); wipe C had completely degraded by week 10 whilst wipe B had degraded by week 14. Plastic-containing wipes started with a lower tensile strength than the compostable wipes (Fig. 3; ANOVA, $F_{2,9} = 26.32$, $p < 0.001$). However, unlike the compostable wet wipes whose tensile strength decreased over time as the wipes degraded (Fig. 3; ANOVA; Wipe B: $F_{13,}$

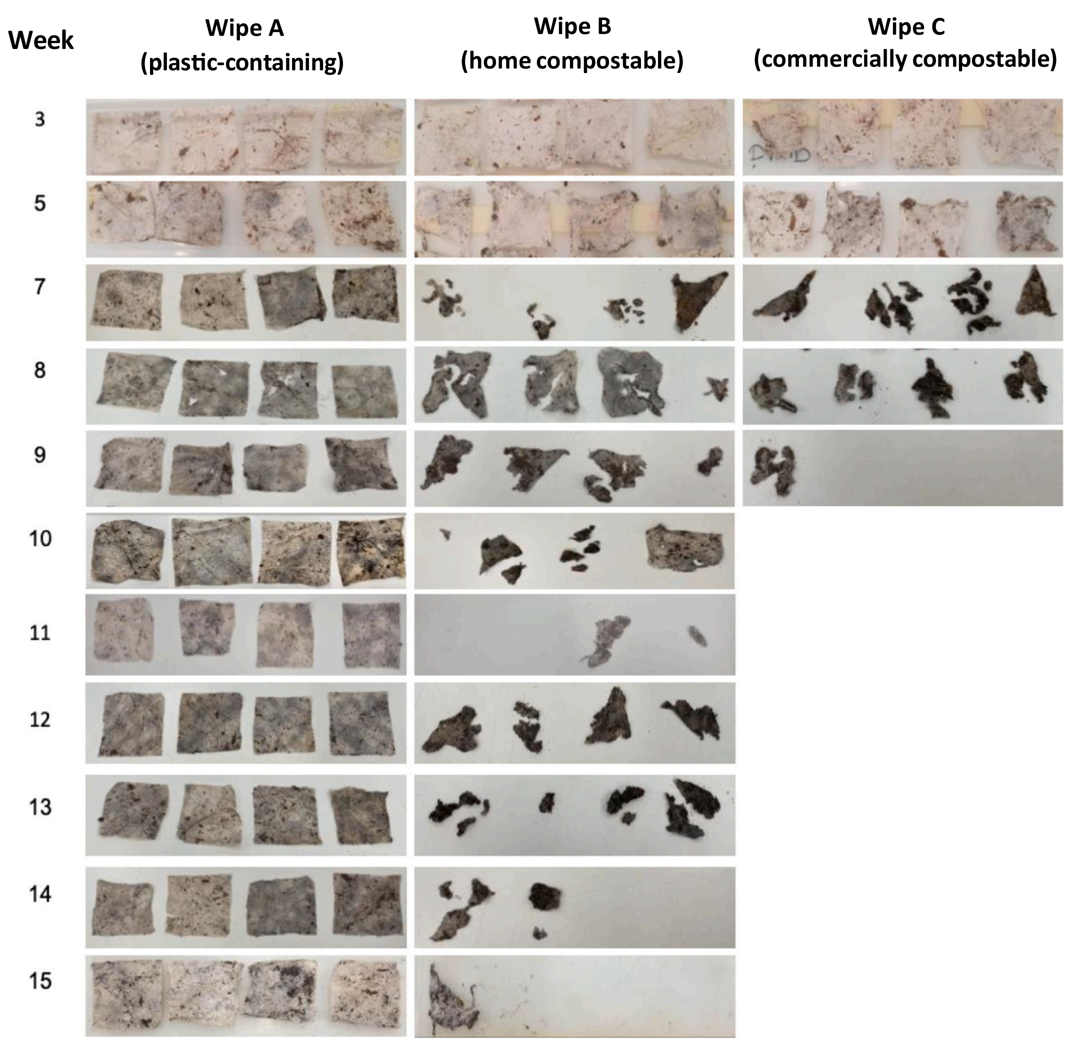

Fig. 2. Visual assessment of wet wipe degradation in beach sand.

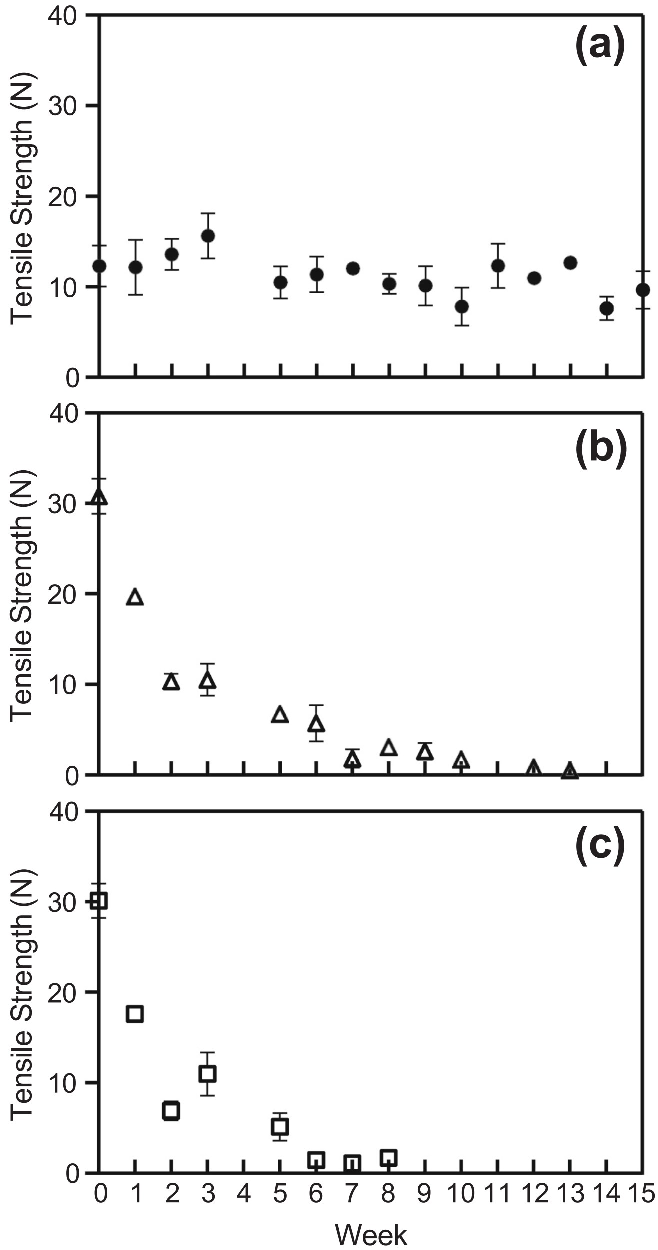

Fig. 3. Tensile strength of: (a) plastic-containing wipes (black circles); (b) home compostable wipes (white triangles}; and (c) commercially compostable wipes (white squares}. Each datapoint represents the mean calculated from four replicates, ± standard error. The final measurements for home compostable (b) and commercially compostable (c) wipes were at weeks 13 and 8 respectively.

$_{38} = 58.64$, $p < 0.001$; Wipe C: $F_{7,\ 24} = 39.52$, $p < 0.001$), the tensile strength of the wet wipes containing plastic remained constant throughout the experiment (ANOVA, $F_{14,\ 44} = 1.206$, $p = 0.305$). The increase in fragmentation of wipes B and C after week five (Fig. 2), may be linked to the increased rainfall from week 5 (Fig. S1). The first four weeks of the study were warm and dry, with only four days of recorded rainfall in this period (7.5 mm in total). In comparison, the subsequent four weeks of the study (weeks 5–8) were wet; a total of 140 mm rain fell within this period (Fig. S1).

3.2. Survival of E. coli on the surfaces of wet wipes buried in beach sand

Background *E. coli* concentrations for water used in the simulated flush were highest in the effluent (10^4 CFU/100 mL; Table S1), and wet wipes became colonised by *E. coli* during the inoculated simulated toilet flush and transfer to WWTP effluent, which persisted during the incubation in seawater. Concentrations of *E. coli* in the sand mesocosms decreased with time for all three wet wipe types (Fig. 4; ANOVA, Kruskal-Wallis; Wipe A: $F_{15,\ 48} = 17.41$, $p < 0.001$; Wipe B $H(13) = 47.47$, $p < 0.001$; Wipe C: $F_{7,\ 24} = 17.36$, $p < 0.001$). *E. coli* on wipe A showed an exponential biphasic decay curve, with the most rapid decrease occurring within the first three weeks. Between weeks 3 and 7, *E. coli* concentrations increased; this corresponded with an increase in temperature and rainfall in the mesocosms at weeks 4 and 5 (Fig. S1). *E. coli* was able to withstand high temperatures in the mesocosms (max temperature 48.5 °C recorded during weeks 4 and 5). This was followed

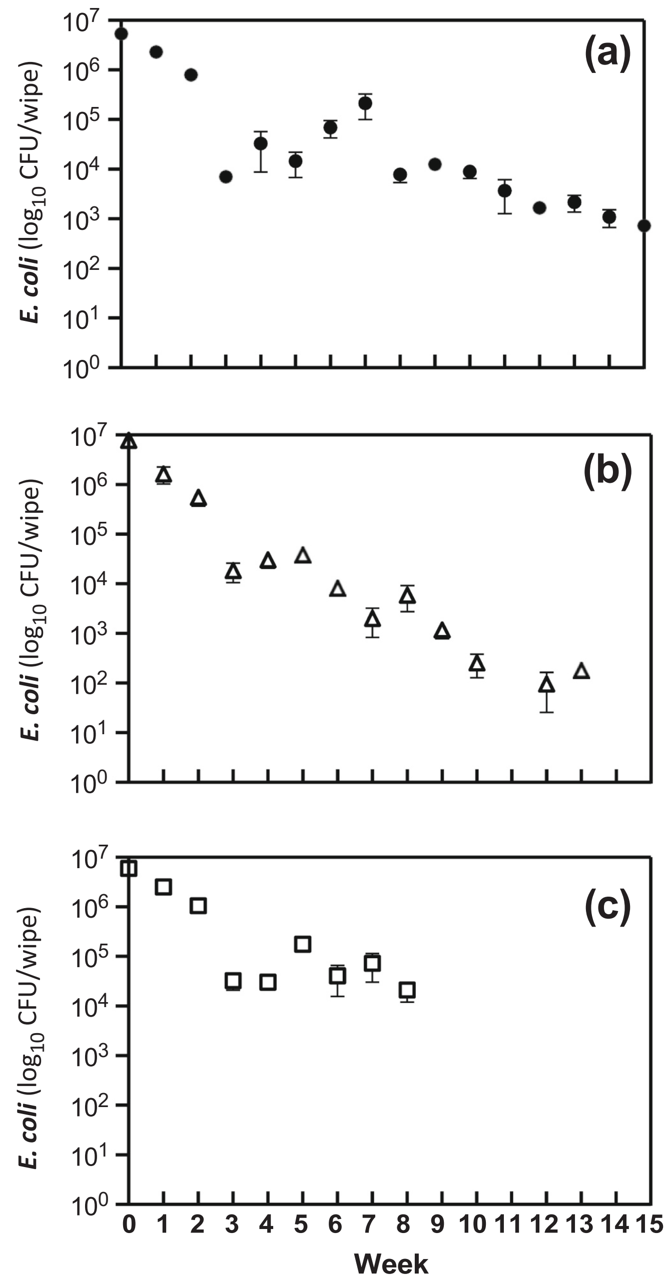

Fig. 4. Concentration of *E. coli* on: (a) plastic-containing wipes (black circles); (b) home compostable wipes (white triangles); and (c) commercially compostable wipes (white squares). Each data point represents the mean calculated from four replicates, ± standard error. The final measurements for home compostable (b) and commercially compostable (c) wipes were at weeks 13 and 8 respectively, after which they had degraded.

by a rapid decrease in *E. coli* concentration for wipe A between weeks 7 and 8, when there was increased rainfall saturating the sand (66 mm of rain fell within this week; Fig. S1). After week 7 the die-off rate of *E. coli* on wipe A plateaued and remianed constant for the rest of the experiment. *E. coli* on wipe C showed a similar biphasic decay curve, with concentrations plateauing between weeks 3 and 8 after an initial rapid die-off. In contrast, *E. coli* concentrations followed a steady decline over time on wipe B with concentrations decreasing by 5 log CFU/wipe between weeks 0 and 13. After week 14, *E. coli* still remained on wipe B, but was excluded from analysis due to an absence of replicates (Mean CFU per wipe: Week 14 = 1.90×10^2, $n = 2$; Week 15 = 3.20×10^2, $n = 1$). At later timepoints, wipes B and C had significantly lower surface area available for colonisation compared to wipe A (Fig. 2).

4. Discussion

In this study, we have demonstrated that wet wipes containing plastic remained largely intact when buried in beach sand during a 15-week time course, whilst both types of compostable wet wipes fragmented and degraded. *E. coli* can persist on all three types of wet wipe when buried in beach sand, with a concentration of 10^3 per wipe remaining after 15 weeks on those wet wipes containing plastic. This as yet unquantified reservoir for potential pathogenic bacteria at the beach could pose a significant public health risk, and highlights the need for (i) increased public awareness of incorrect flushing of all wet wipe types, (ii) improved management of wastewater discharge and spills, including more effective regulation, and (iii) a greater impetus for policy change concerning wet wipes, particularly those that contain plastic.

The wet wipes containing plastic showed little degradation during the study, suggesting they can persist for long periods in beach sand environments. Previous studies had demonstrated that *E. coli* can persist on the surfaces of plastics in the environment for at least 28 days (Metcalf et al., 2023; Ormsby et al., 2023), but here we have shown that *E. coli* can continue to persist and survive for at least four months. However, despite the plastisphere and wet wipe surfaces providing a protective environment for *E. coli* (Li et al., 2024), concentrations decreased with time. This is likely due to the non-optimal survival and growth conditions within the mesocosm; the optimal temperature for *E. coli* growth is 37 °C under aerobic conditions (Jang et al., 2017). Several environmental factors may also have influenced the survival and die-off rate of *E. coli*, including temperature, pH, rainfall, solar radiation, and moisture (Williams et al., 2005; Petersen and Hubbart, 2020). The moisture content of the sand will have changed due to fluctuating air temperatures and rainfall: low moisture content is associated with decreased cell survival and growth limiting conditions (Underthun et al., 2018), whereas increases in sand moisture following rain can resuscitate dormant cells (Beversdorf et al., 2007). In this study, the rainfall from week 4 would have increased the sand moisture and likely resuscitated any desiccated or water-stressed cells, which would have been further promoted by the corresponding increase in temperature. However, as the sand became saturated, *E. coli* cells would have been washed off the wet wipe and leached through the column and out of the bottom of the mesocosm.

Wet wipes are responsible for 75 % of sewerage blockages which, in the UK costs around £100 million a year to clean up (Water UK, 2023). Blocked sewerage systems lead to an increase in spill events where raw sewage can be released directly into the environment, contaminating bathing waters and causing serious illness (Slack et al., 2022). In 2021, around 90 % of wet wipes contained plastics (Zhang et al., 2021); however, more recently alternatives are becoming more widely available, and several retailers have already banned the sale of wet wipes containing plastics (DEFRA, 2023). Additionally, following much debate in parliament, the English government has proposed a ban on wet wipes containing plastic by 2024 (DEFRA, 2023); however, there appears to be little evidence of a wider global ban on wet wipes containing plastics.

As public awareness increases, alternative plastic-free wet wipes are increasingly becoming available. In the last decade the market size of compostable wet wipes has tripled (> US$3 billion in 2022; Allison et al., 2023). The degradation processes of wet wipes in aquatic environments are already well understood (reviewed in Allison et al., 2023); however, the degradation process when buried in beach sand will be different, with factors, such as temperature, sand moisture, and the autochthonous microbial communities influencing the rate of degradation. Abiotic hydrolysis, a degradation process where molecular chains (e.g., polyesters, cellulose) are broken down when water reacts with the material's surface, is likely to increase following rainfall (Speight, 2017). The differing conditions (e.g., particle type and size, salinity, moisture, temperature) in beach sand compared to commercial composting facilities may have facilitated more rapid degradation. For example, salinity would have been higher in the beach sand, which can influence microbial decomposition rates (Morrissey et al., 2014). The moisture content may also have been higher than in a commercial composting facility due to the high rainfall (i.e., at week 8 the sand was completely saturated), increasing microbial activity and the degradation rate. Under appropriate composting conditions (e.g., in an industrial composting facility), the physical fragmentation of cellulose fibres in wet wipes can be rapid; however, molecular degradation in the environment is a much slower process due to physicochemical manufacturing properties and non-optimal breakdown conditions (Allison et al., 2023). This results in both cellulose and plastic microfibres persisting in the environment (Ó Briain et al., 2020), where they can enter the food chain with the potential to transport harmful contaminants (Kwak et al., 2022). For example, microfibres from wet wipes in the River Thames, London have been linked to decreasing populations of Asian clams (McCoy et al., 2020).

Despite wet wipes being treated with a number of chemical additives (e.g., malic acid, sodium hydroxide) and antimicrobial agents (e.g., sodium benzoate, benzalkonium chloride) (Salama et al., 2021), *E. coli* was still able to survive and persist on all three wet wipe types. However, such chemical additives and antimicrobial agents are likely to be significantly diluted or washed off during toilet flushing and transfer through the WWTP, facilitating the persistence of *E. coli* on wet wipes as they enter the environment. In a recent survey, 88 % of people said they were aware wet wipes harmed the environment, but 22 % still admitted to flushing them down the toilet anyway (Water UK, 2023), resulting in 2.9 billion wet wipes entering WWTPs in the UK every year (DEFRA, 2023). Although compostable wet wipes do degrade with time, they can still persist in beach environments for up to 14 weeks. Therefore, compostable wet wipes could still pose a public health risk after washing up on beaches by acting as a localised source of *E. coli* contamination. As these wet wipes fragment and breakdown, potential pathogens associated with them could be released into the sand where they could continue to persist long after the wet wipe has degraded (Weiskerger et al., 2019).

5. Conclusion

We have demonstrated that wet wipes can persist in beach sand, which could pose a heightened human health risk at the beach, depending on the wet wipe material. Wet wipes continue to be popular consumer products, with 1.36 million tons being produced in 2020 (Hadley et al., 2023). But there continues to be frequent confusion among consumers regarding appropriate wet wipe disposal, resulting in wet wipes being incorrectly flushed down toilets and causing sewerage blockages and spills. Therefore, there is a pressing need to increase public education and awareness to prevent the incorrect disposal of wet wipes down the toilet, together with improved wastewater management and environmental regulations. Collectively, this will help to ensure that wet wipes are prevented from entering the environment, which would reduce their occurrence at beaches and the introduction of potentially harmful pathogens into the beach environment.

CRediT authorship contribution statement

Rebecca Metcalf: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Rosie Fellows:** Data curation, Investigation, Writing – review & editing. **Hannah L. White:** Conceptualization, Data curation, Investigation, Writing – review & editing. **Richard S. Quilliam:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.116175.

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