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Effect of dissolved versus total zinc levels on bacterial zinc-dependent meropenem co-resistance in River Tyne sediments

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ABSTRACT

Metal pollution can increase in situ antibiotic resistance (AR), leading to elevated AR exposures and the potential evolution of new resistance. Metal-driven AR selection has been observed downstream of historic zinc (Zn) mining areas, such as the River Tyne catchment in Northeast England. The South Tyne was heavily mined until the early 20th century, leaving its waters and sediments with high Zn levels. Conversely, the North Tyne basin was unmined, providing a stark contrast in environmental Zn levels. Replicate sediment and water samples were collected six times from two North Tyne (Reaverhill, RH; Warks Burn, WB) and three South Tyne (Featherstone, FS; River West Allen, WA; River Nent, RN) sites, and phenotypic Zn, meropenem, and combined meropenem-Zn resistance were quantified relative to total and dissolved Zn levels and water quality conditions. In parallel, a 309-day batch experiment was performed on sediments from WB or WA, amended with inorganic Zn, and meropenem and Zn resistance were quantified over time. Sediment plating showed Zn-resistant and combined-resistant bacteria were significantly more abundant than meropenem-resistant bacteria in WA and RN ($p < 0.05$), suggesting Zn-dependent meropenem resistance prevailed at elevated Zn levels. Parallel batch studies, however, showed that only dissolved Zn levels correlated with phenotypic meropenem co-resistance, suggesting resistance was probably driven by dissolved rather than total Zn. Subsequent qPCR analysis on sediment samples showed Zn efflux and porin genes, especially *oprD*, associated with phenotypic Zn and combined Zn-meropenem resistance. Overall, results suggest meropenem co-resistance exists in the Tyne, but it is Zn-dependent, possibly linked to *oprD*-mediated uptake repression. However, dissolved Zn levels, not total Zn, drive meropenem resistance co-selection in the Tyne. In the future, dissolved and total metal levels should always be quantified in AR risk assessments on metal-polluted sites.

1. Introduction

Metal-contaminated land and water are a pervasive global problem, not one confined to a single river system or region. Metal pollution of agricultural and urban soils is estimated to impact between 900 million and 1.4 billion people globally (Hou et al., 2025), with historic and active metal mining directly contaminating about 479,200 km of river channels and 164,000 km^2 of floodplains worldwide, directly exposing at least 23 million people to potentially hazardous mining-associated metal releases (Macklin et al., 2023). Although the UK and continental Europe host some of the best-characterised legacy mining landscapes, comparable or more severe metal contamination exists in mining areas where regulatory oversight and remediation capacity are limited, such as in low- and middle-income countries (LMICs) in SE Asia, Africa, and the Americas (Caravanos et al., 2013). Elevated metals clearly can have negative ecotoxicological impacts on aquatic biota, including reduced growth, reproduction, and survival in fish and invertebrates (Eisler, 1993), but they also have other health consequences, such as the co-selection of antibiotic resistance (AR) in river environments (Gupta et al., 2023), which is not well understood at a mechanistic level.

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Previous studies have shown that the presence of transition metals, with or without antibiotic co-contamination, can exert selective pressure on bacteria within natural environments, including rivers and lakes (Summers et al., 1993; Alonso et al., 2001; Baker-Austin et al., 2006; Jain et al., 2009; Gupta et al., 2026). Metals co-select for AR genes (ARGs) and bacterial hosts and can contribute to AR levels across the wider environment (Foster, 1983; Alonso et al., 2001; Stepanauskas et al., 2006; Robins et al., 2022). Such ARGs can provide an ecological advantage for colonisation and survival of their hosts (Alonso et al., 2001), probably arising from the co-influence of metals and antibiotics on bacterial stress response and metabolism (Pal et al., 2015). However, specific mechanisms linking AR and different types of metal exposure are less understood, making it hard to generalise risks of metal-driven AR co-selection, including its persistence and potential for wider environmental transmission and spread.

Metal resistance (MR) in bacteria is primarily mediated by efflux of metal from cells, reduced metal uptake, sequestration, or conversion to less toxic forms (Bruins et al., 2000; Nies, 2003). MR mechanisms often overlap with AR mechanisms, suggesting bacterial cells may develop AR and MR in parallel when exposed to elevated metal concentrations (Baker-Austin et al., 2006). Selective pressure from transition metals also can induce efflux genes that eliminate both metals and antibiotics from the cell (Levy, 2002). As metals are not readily degraded and remain in the environment, they can maintain selective pressure on microbial communities for prolonged periods, often through the co-presence and local expression of MR and AR genes (Stepanauskas et al., 2006; van Uum et al., 2024). As such, metal-contaminated environments, including those impacted by Zn, Cr, and Pb, have been shown to harbour elevated numbers of AR strains and greater plasmid abundances (Rasmussen and Sorensen, 1998). Since MR and AR genes are often located on shared mobile genetic elements, increased gene mobilisation under metal stress can enhance the spread of AR genes among bacterial populations (Alonso et al., 2001; Pal et al., 2015), promoting the selection and proliferation of AR in environmental reservoirs (Summers, 2002; Jain et al., 2009). For example, evidence from biological wastewater treatment demonstrates significant links between Zn and ambient AR levels (Peltier et al., 2010), and other studies have shown that soil and sediment metal concentrations, particularly Zn and Cu, significantly correlate with specific AR genes at landscape levels (Knapp et al., 2011; Robins et al., 2022).

A recent study by Gupta et al. (2023) on the River Tyne reported higher levels of MR and AR bacteria and their associated resistance genes in water and sediment samples at high-metal sites relative to lower-metal sites. Despite such evidence, few studies have assessed how metal *bioavailability*, rather than the total metal burden, influences expressed and active phenotypic AR, and whether that resistance is retained when metals are reduced or removed. This is a critical issue when evaluating AR exposure risks if metal-contaminated sediments are disturbed or resuspended. Within this context, we hypothesize that bioavailable Zn might act as a selective agent, altering the permeability of bacterial membranes, thereby reducing meropenem uptake and inducing meropenem resistance.

Here, we assessed how total and dissolved Zn levels impact Zn resistance, meropenem resistance, and combined resistance in sediments from two geologically distinct sub-catchments of the River Tyne watershed: the North and South Tyne. The North Tyne has no history of mining and contains comparatively low concentrations of transition metals, whereas the South Tyne has exceedingly high metal levels owing to extensive historic mining activities in the nineteenth and early twentieth centuries (Macklin et al., 1994). Using this contrast, replicate sediment samples were collected from five sites across both rivers over a six-month period and characterised geochemically and microbiologically. Parallel laboratory experiments were performed to examine possible co-resistance mechanisms and evaluate the role of dissolved versus total Zn in governing phenotypic meropenem resistance and its persistence.

2. Materials and methods

2.1. Study area

The Tyne catchment, located in Northeast England, covers an area of approximately 2933 km^2 and comprises two geologically distinct sub-catchments: the North and South River Tyne (Fig. 1; Macklin et al., 1994; EA, 2008). As previously noted, the North Tyne catchment has no history of mining or significant industrial activity and has low ambient concentrations of Zn, Pb, and Cd, whereas the South Tyne has had extensive historic Pb-Zn mining, resulting in exceedingly high Zn levels in its floodplain riverbed sediments (Macklin et al., 1994; Gozzard, 2008).

The geology of the two sub-catchments differ markedly: the North Tyne is primarily underlain by the lower limestone and the Scremerston Group formations, whereas the South Tyne comprises Millstone Grit, Upper Limestone, and Middle Limestone, with significant non-metallic mineral deposits, including fluorspar, witherite, and barytes (EA, 2008). These stark geological contrasts influence metal-binding and associated geochemical activity in sediments from the two basins. As such, the sub-catchments provide an ideal natural system for contrasting MR and AR associated with different mineralogical assemblages and metal abundances in sediments and pore water, including the effects of differences in Zn partitioning between the solid and dissolved phases.

Within the above context, two sites were sampled from the North Tyne, i.e., Reaverhill (RH) and Warks Burn (WB), and three sites from the South Tyne: Featherstone (FS), River West Allen (WA), and River Nent (RN). Specific site locations are shown in Fig. 1.

2.2. Sample collection and preparation

Sediment and water samples were collected in duplicate from the five sites every four weeks for six consecutive months (June to October). Samples were collected approximately 0.5-1.0 m from the riverbank in sterile 50 mL containers (VWR) using circular plastic coring moulds with a depth of 3 cm. The moulds were also used to capture pore water around the sediments, thereby collecting water for dissolved metal analysis. River water samples were also collected from each site for metal and microbiological analysis. At each site, the water samples were filtered using 0.2 μm filters to measure dissolved metal concentrations.

Water and sediment cores were retained on ice until returned to the laboratory. Sediment aliquots were stored at 4 °C and −20 °C for metal and molecular biological analysis, respectively, and the remaining material was prepared for microbiological analysis on the day of collection. The onsite filtered water samples were acidified with 1 N HNO_3 and stored at 4 °C prior to analysis. In addition, pH, dissolved oxygen (DO, in mg/L), and river water temperature were measured at each site using a digital two-channel multi-meter (Hach-Lange, Salford, UK).

2.3. Quantifying meropenem and Zn resistance

All samples were analysed for meropenem and Zn resistance using classical spread-plating techniques (APHA et al., 2005) to examine the viable culturable proportion of the total bacterial community rather than isolating or identifying particular target species. Organisms were detached from sediments by mixing wet sediment with sterile 0.2% (w/v) sodium pyrophosphate decahydrate buffer solution ($Na_4P_2O_7{\cdot}10H_2O$; Sigma-Aldrich, St. Louis, MO, USA) at a 1:1 ratio (v/v) in sterile 50 mL Falcon tubes, shaken overnight at 20 °C at 112 rpm. The water samples and sediment extracts were serially diluted in sterile phosphate buffer solution (PBS) and plated in triplicate onto R2A agar (Sigma-Aldrich), a medium widely used for environmental microbial isolates (Reasoner and Geldreich, 1985), amended with 40.0 mg/L cycloheximide to inhibit fungal growth.

Selective media amendments were “no amendment” (the control), 2.0 mg/L meropenem only (Fluorochem Ltd., Hadfield, UK), 65.0 mg/L

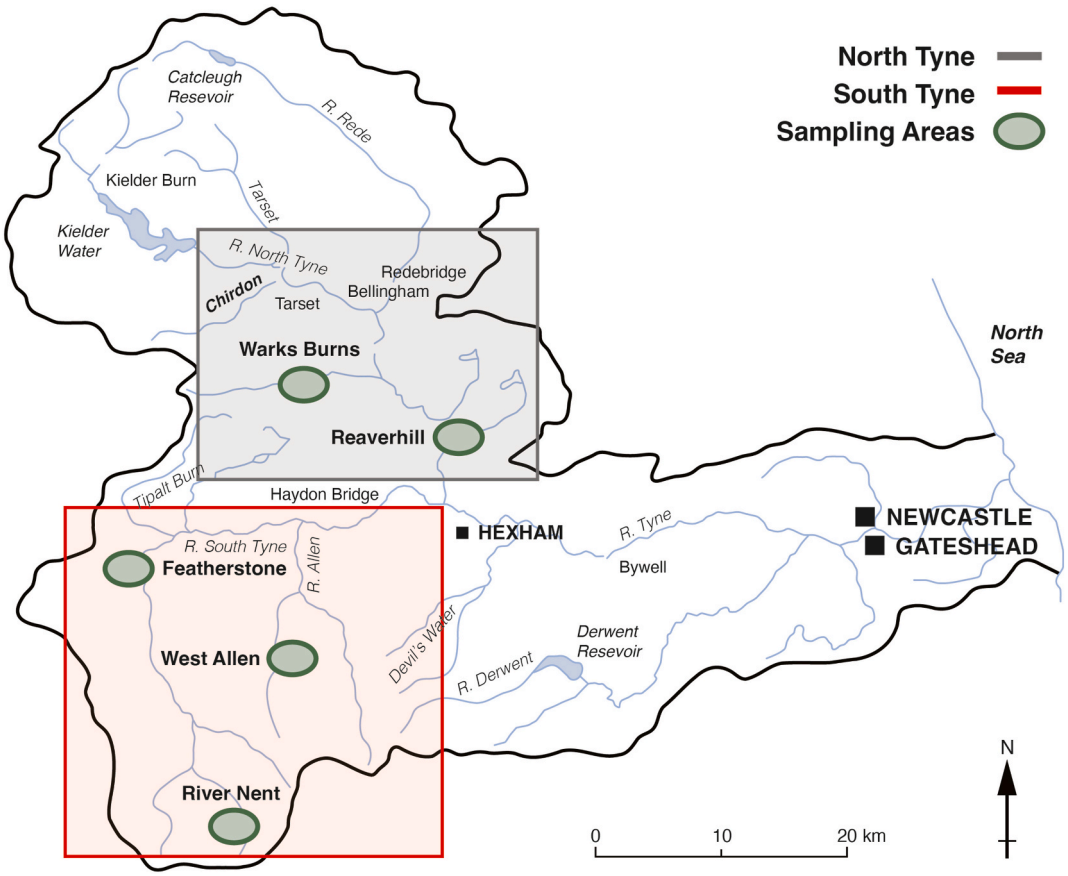


Fig. 1. Map of the Tyne basin, UK, outlining the North and South catchments and the sampling locations. Adapted from Archer (2008).

Zn only as $ZnSO_4$, and a combination of 2.0 mg/L meropenem plus 65.0 mg/L Zn. The 65.0 mg/L Zn amendment was selected based on a previous study of Zn resistance thresholds in environmental strains (Peltier et al., 2010).

The meropenem amendment of 2.0 mg/L was selected through empirical dose-ranging trials on environmental samples. It was found that standard clinical breakpoint concentrations (0.25 mg/L; Turner et al., 1999) resulted in over 60% of environmental isolates being classified as tolerant, and it was concluded that this concentration was insufficiently selective in this study. Sequential testing at increasing concentrations identified 2.0 mg/L as the most discriminating threshold for environmental meropenem resistance and was used consistently throughout.

All plates were incubated inverted for 3 days at 25 °C before colony enumeration. Resistance was expressed as the percentage of total colony-forming units (CFU) recovered on amended media relative to unamended controls, providing a direct and internally normalised measure of phenotypic resistance selection.

2.4. Metal analysis

All field samples were analysed for Zn. Sediments were dried overnight at 104 °C and acid-digested using 1 N HNO_3 (65%, Suprapur®, Merck) at 105 °C for 40 min (APHA, 2005). Digests were filtered through 0.2 μm cellulose acetate filters (VWR) and diluted at least tenfold for total metal analysis by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES; Vista MPX CCD, Varian). Dissolved metal concentrations in field water samples were measured on 0.2 μm-filtered, acidified aliquots. For batch reactor samples, dissolved Zn was similarly determined on the decant fraction following a 10-min settling period and passage through a 0.2 μm filter. Total Zn in reactor samples (see below) was determined on unfiltered aliquots after acidification. All glassware was acid-washed in 0.2% HNO_3, rinsed in deionised water, and ashed at 550 °C. Blanks and certified standards (1000 mg/L stock; VWR) were included in all analytical runs.

2.5. Batch studies contrasting dissolved vs total Zn on phenotypic meropenem resistance

To directly assess whether dissolved versus total Zn drives the selection of meropenem resistance, a 309-day bench-scale, batch reactor experiment was conducted to complement the field sampling. Four experimental conditions (two sediment types × two Zn amendment levels) were established in triplicate, providing 12 independent reactors for statistical comparison. Reactors were seeded with sediments from WB (low ambient Zn) or WA (high ambient Zn) and then amended with 2.0 mg Zn/L (Low) or 100 mg Zn/L (High) as $ZnSO_4$.

The reactors were 1-L cotton-stoppered Erlenmeyer flasks containing 500 mL of 20% R2A broth (diluted with deionised water), autoclaved at 121 °C for 15 min prior to sediment addition. Sediments from WB and WA (400 g each) were collected and sieved to < 2 mm, homogenised, and a sub-sample of 50 mg of damp sediment was used to seed respective reactors. Small sediment seeds were used to reduce native Zn carryover into the reactors, due to widely differing background sediment Zn levels. The High- and Low-Zn amendments were then added using $ZnSO_4$ stock solutions. "High-Zn" (i.e., 100 mg Zn/L) was based on estimates of exchangeable Zn in WA sediments from Gozzard (2008), who reported that approximately 5% of total sediment Zn was in the exchangeable fraction. This was used to set a field-grounded upper-bound exposure for WA sediments. "Low-Zn" conditions (i.e., 2.0 mg Zn/L) were chosen in proportion to the ratio of typical in situ Zn concentrations found in WA vs WB sediments.

In the experimental design, it was assumed that different sediment compositions, particularly legacy sulphide minerals and metal-binding phases in the mined WA sediments vs the geochemically simpler WB sediments, would result in different dissolved Zn levels from the same nominal amendment. This developed a range of dissolved-to-total Zn ratios across the four reactors, which is central to the study's experimental logic. Reactors were continuously mixed at 121 rpm and retained at 20 °C.

After seeding, initial samples were collected, and samples were then collected every 28 days on a fill-and-draw basis using a sterilised pipette. Reactors were allowed to settle for 10 min before sampling; 2 mL of the upper decant was taken for immediate microbiological plating on R2A agar with and without 2.0 mg/L meropenem. A separate 4 mL aliquot of the decant was taken for dissolved Zn analysis (filtered, 0.2 μm), and an unfiltered aliquot for total Zn quantification. After each sampling, reactor volumes were restored by adding deionised water.

2.6. DNA extraction and qPCR

DNA was extracted from sediment samples using Fast Soil DNA Spin Kits and a ribolyser (MP Biomedicals, Cambridge, UK) per manufacturer's instructions. Targeted genes included the efflux genes *czcA* (cobalt-zinc-cadmium resistance, RND-type; Roosa et al., 2014), *czcD* (CDF-type efflux; Roosa et al., 2014), *czrA* (cadmium-zinc resistance; Li et al., 2015), and *zntA* (P-type ATPase efflux; Xiong et al., 2015), as well as the outer membrane porin *oprD* (Dumas et al., 2006). Total bacterial abundance was estimated by 16S rRNA gene quantification (Yu et al., 2005) for normalisation. PCR reactions were performed in triplicate (20 μL volumes) using ssoFast/EvaGreen reagents (Bio-Rad) on a Bio-Rad iQ5 system. Annealing temperatures followed previously published protocols; thermal cycling comprised initial denaturation (95 °C, 2 min), followed by 36 cycles of denaturation (95 °C, 10 s), annealing (per gene, 10 s), and elongation (60 °C, 10 s). Post-analytical melt curves verified amplicon specificity. Standards spanning 10^1–10^8 copies were included ($r^2 > 0.99$; efficiency 92–109%), along with blanks.

2.7. Data analysis

All data were analysed using Minitab (Version 17). Normality checks confirmed that CFU, gene, and metal data were generally non-normally distributed. Mann-Whitney (MW) non-parametric tests were used to compare isolate, gene, and metal abundances between sites. Spearman's non-parametric bivariate correlation analysis was used to identify significant trends among resistance, metal, and gene data. Significance thresholds were set at $p \leq 0.05$. The p-values were adjusted using the Benjamini-Hochberg (BH) false discovery rate correction (q = 0.05), ensuring valid interpretation of the bivariate correlation data. Data were $\log_{10}$-transformed before calculating Spearman's rho to linearise relationships and reduce the influence of extreme values; as Spearman's rho is rank-based, the results are equivalent to those from non-transformed data, but the log-transformation step is noted for clarity.

3. Results and discussion

3.1. North and South Tyne water quality characteristics

Biotic and abiotic water quality conditions were measured monthly at each sampling site. Mean water temperatures ranged from 13.3 to 14.0 °C across the six-month campaign, and mean pH values from 7.16 to 7.86, consistent with regional rivers (Table 1). Dissolved oxygen at all sites ranged from 9.38 to 9.99 mg/L, indicating strongly oxidising conditions throughout.

3.2. Total and dissolved metal levels in the North and South Tyne basins

Consistent with previous data (Macklin et al., 1994; Gupta et al., 2022), total Zn levels were significantly higher (MW; $p \leq 0.05$) at WA and RN (3540 and 3560 mg/kg dry sediment, respectively) than at North Tyne sites RH and WB (53.6 and 18.4 mg/kg, respectively). The intermediate site, FS, had total sediment Zn of approximately 1550 mg/kg, which was significantly higher than both North Tyne sites (MW; $p \leq 0.05$; Table 1).

Dissolved Zn levels in the water phase were orders of magnitude lower than total levels in the sediments (Table 1), indicating the large majority of Zn is in the solid phase across the sites, which is typical of mining-impacted river sediments where metal-binding to organic matter, iron oxyhydroxides, and residual sulphide-bearing minerals restricts dissolved-phase metals (Manahan, 2010; Gozzard, 2008). However, dissolved Zn levels in pore water were significantly higher at FS, WA, and RN than RH and WB (MW; all $p \leq 0.05$; Table 1). Dissolved Zn was generally lower overlying river waters compared with pore water, suggesting that some metal remobilisation was occurring within the sediment columns.

3.3. Observed resistance across sites using different selective media

Microbial culturing of different sediment samples on Zn-amended, meropenem-amended, and combined-amended media showed significantly higher proportional Zn-resistant CFUs in sediments from high-Zn sites in the South Tyne (FS, WA, and RN) compared with the low-Zn North Tyne sites (RH and WB; $p < 0.05$; Fig. 2). This is consistent with other studies on the River Tyne (Gupta et al., 2023), which showed increased relative Zn-resistance in response to greater Zn exposures, which suggests the potential for antibiotic co-resistance due to shared cell defence mechanisms (Dickinson et al., 2019; van Uum et al., 2024). All culturing data are reported here as a percentage of total CFUs that are resistant, which is a direct, internally normalised measure of resistance selection, independent of absolute microbial densities.

In contrast to Zn-resistance, relative phenotypic meropenem resistance did not differ significantly across the five sites (MW; $p > 0.05$), even between sediments with widely different total Zn levels. However, relative levels of combined-resistant isolates (i.e., isolates on plates containing both meropenem and Zn) were significantly more abundant at RN than at RH and FS ($p < 0.05$), and at WA relative to RH ($p = 0.013$). These findings suggest that elevated Zn broadly promotes Zn- and combined-resistant phenotypes, although meropenem resistance appearance was only evident when Zn was present simultaneously in the plate culturing medium.

A similar pattern was seen in the river water samples, with the highest relative Zn- and combined resistance found at South Tyne sites (Fig. 2A), whereas no differential selection was seen for meropenem resistance alone. Previous reports indicate that Zn can alter bacterial efflux or porin activity, creating the conditions for co-resistance to antibiotics, but only when available Zn is present (Baker-Austin et al., 2006). Our sediment and water data are consistent with this interpretation.

3.4. Relationships between resistance phenotypes and Zn levels in field sediments

Spearman's non-parametric correlation analysis on pooled sediment data showed that relative Zn and combined resistance were significantly correlated ($p < 0.05$), whereas Zn resistance and meropenem resistance alone were not ($p > 0.05$) (Table 2). Neither total nor dissolved Zn correlated with meropenem resistance in isolate selection ($p > 0.05$). In contrast, Zn resistance significantly correlated with total and dissolved Zn (always $p < 0.05$), and Zn-resistance and combined-resistance correlated significantly with each other (sustained after BH correction). Combined resistance showed significant correlations with total and dissolved Zn at the nominal, uncorrected threshold ($p < 0.05$); however, neither of these relationships were as strong after BH correction. This weakening after correction likely reflects the limited number

Table 1
Measured total (mg/kg) Zn levels in sediments and dissolved (mg/L) Zn levels in sediment pore water and river water, and mean water quality parameters across the six-month sampling programme. Values are means ± standard deviation (n = 6).

Site	Sediment		River water			
	Total Zn (mg/kg)	Dissolved Zn (mg/L)	Dissolved Zn (mg/L)	pH	Temp. (°C)	O_2 sat. (%)
Reaverhill (RH)	53.6 ± 3.15	< 0.01	< 0.01	7.16 ± 0.22	14.0 ± 0.79	91.0 ± 2.13
Warks Burn (WB)	18.4 ± 0.10	< 0.01	< 0.01	7.18 ± 0.17	14.0 ± 0.82	90.3 ± 5.84
Featherstone (FS)	1550 ± 210	0.13 ± 0.03	0.10 ± 0.01	7.42 ± 0.28	13.9 ± 0.70	91.3 ± 4.96
West Allen (WA)	3540 ± 54.9	1.05 ± 0.01	0.88 ± 0.07	7.86 ± 0.22	14.4 ± 0.83	95.3 ± 3.68
River Nent (RN)	3560 ± 226	3.89 ± 0.85	1.22 ± 0.22	7.62 ± 0.19	13.3 ± 0.87	92.3 ± 2.17

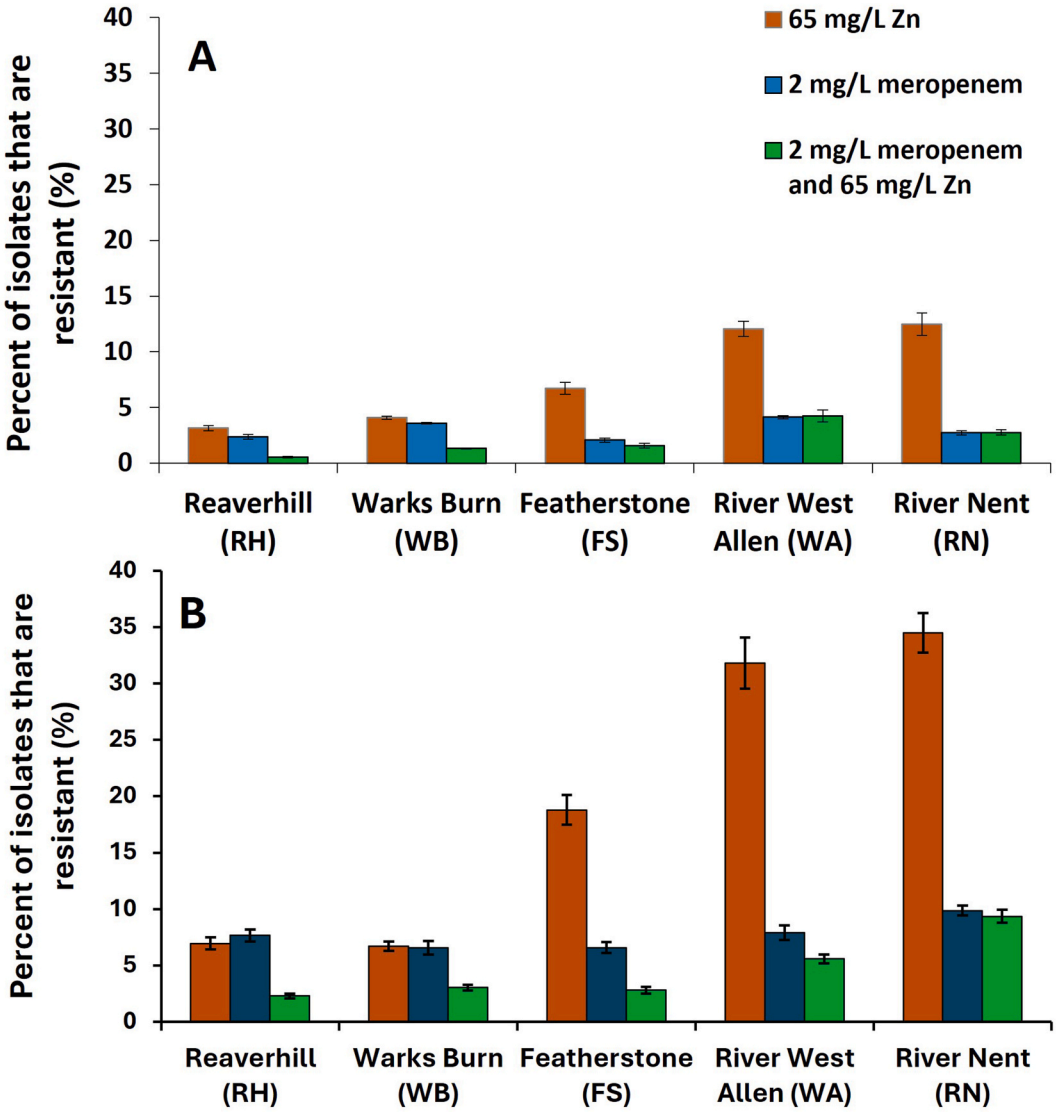


Fig. 2. Relative percentage (%) of colony isolates in the (A) water column and (B) sediments that are resistant to 65 mg/L Zn, 2 mg/L meropenem, and 2 mg/L meropenem combined with 65 mg/L Zn from each of the sites: Reaverhill (RH), Warks Burn (WB), Featherstone (FS), River West Allen (WA) and River Nent (RN). Values indicated are averages over six months. Standard deviation bars are shown.

of field sites and non-independence of the 45 pairwise comparisons, which reduces power to detect true effects at the field scale. Therefore, most of our field-level correlations are exploratory rather than confirmatory, and therefore, we rely on confirmatory results from the batch-reactor experiment (see Section 3.5). Our experimental design created a purpose-built dissolved Zn gradient across twelve replicated reactors providing a more statistically powered, controlled test of the dissolved Zn hypothesis, which detected statistically significant relationships.

With this qualification, results suggest that the observed phenotypic meropenem resistance is Zn-dependent, i.e., with low Zn in the growth environment, meropenem susceptibility largely disappears, even in colonies originating from highly Zn-contaminated sites. The reversible acquisition of meropenem resistance after Zn exposure and its subsequent loss upon Zn removal indicates a transient conditional physiological response rather than long-term genetic resistance. Results in Fig. 3 show that dissolved Zn and combined-resistance follow a broadly linear relationship, whereas dissolved Zn and Zn-resistance are non-linear and asymptotic, suggesting a threshold of influence, such as the

Table 2
Spearman's non-parametric bivariate correlation coefficients between total and dissolved Zn, five Zn resistance-associated genes, and Zn, meropenem, and combined resistant isolate abundances for river sediment samples collected on the River Tyne. Bold red indicates nominal (uncorrected) statistically significant correlations ($p \leq 0.05$). However, the p-values were adjusted using the Benjamini-Hochberg false discovery rate correction ($q = 0.05$); correlations marked with * remained statistically significant after this correction.

	Total Zn	Dissolved Zn	*zntA*/16S	*czcD*/16S	*czcA*/16S	*czrA*/16S	*oprD*/16S	Zn Resistance	Meropenem Resistance
Dissolved Zn	**0.932** [a] **(0.001)***								
zntA/16S	0.164 (0.650)	0.367 (0.297)							
czcD/16S	-0.418 (0.229)	-0.375 (0.285)	-0.018 (0.960)						
czcA/16S	0.571 (0.084)	0.608 (0.062)	0.433 (0.211)	0.116 (0.751)					
czrA/16S	0.182 (0.614)	0.439 (0.204)	**0.659 (0.038)**	0.310 (0.383)	0.539 (0.110)				
oprD/16S	**0.632 (0.050)**	**0.712 (0.021)**	**0.774 (0.009)**	-0.136 (0.374)	0.552 (0.098)	0.466 (0.174)			
Zn Resistance	**0.806 (0.005)***	**0.882 (0.001)***	0.596 (0.069)	-0.152 (0.676)	**0.729 (0.017)**	0.584 (0.077)	**0.863 (0.001)***		
Meropenem Resistance	0.055 (0.881)	0.150 (0.679)	0.596 (0.069)	0.394 (0.260)	-0.006 (0.987)	0.389 (0.266)	0.419 (0.228)	0.394 (0.260)	
Combined Resistance	**0.661 (0.038)**	**0.775 (0.008)**	0.547 (0.102)	-0.248 (0.489)	0.274 (0.444)	0.456 (0.185)	**0.729 (0.017)**	**0.818 (0.004)***	0.524 (0.054)

Notes: [a] Correlation coefficient (r) with associated p value in brackets.

possible induction of Zn-related defence genes.

3.5. Relative meropenem resistance versus Zn availability in batch reactors

Field data show that dissolved Zn relates more strongly with Zn- and combined resistance phenotypes compared with total Zn (Table 2), but since dissolved and total Zn are themselves highly correlated across the field sites ($p < 0.05$), field data alone cannot disentangle the relative contributions of dissolved vs total Zn to resistance selection. Therefore, a controlled laboratory experiment was performed to examine this question. Inorganic $ZnSO_4$ additions at 2 and 100 mg/L were provided to WB (low ambient total Zn) and WA (high ambient total Zn) sediments in batch reactors, which were maintained and sampled for total and dissolved Zn and meropenem resistance over 309 days.

As shown in Table 3, the same nominal Zn amendment produced markedly different dissolved Zn levels depending on sediment type, yet all significantly decreased the available dissolved Zn concentrations (62-99%). The 100 mg/L Zn addition to WB sediments yielded almost double the mean dissolved Zn compared to the same addition to WA sediments (1.35 ± 0.35 vs 0.70 ± 0.37 mg/L). At the low Zn amendment, WA sediments retained dramatically more dissolved Zn than WB sediments (0.76 ± 0.32 vs 0.07 ± 0.02 mg/L). This differing behaviour is consistent with general principles of Zn geochemistry and Zn interactions with charged sedimentary surfaces, such as iron (oxyhydr)oxides and phyllosilicates (Stumm and Morgan, 1996). In the WB sediments, the apparent saturation effect is typical for sorption to metal-binding minerals (Stumm and Morgan, 1996) and indicates a limited abundance of potential solid-phase Zn binding sites in these sediments.

In contrast, the low dissolved Zn concentration found in the high-Zn WA sediments (Table 1) suggest only marginal disturbence by inorganic Zn additions, which is consistent with equilibrium Zn (co-) precipitation in carbonate phase(s) observed previously in North Tyne sediments (Gozzard et al., 2011). The resulting experimental design, therefore, successfully created four distinct dissolved-to-total Zn ratio scenarios across the 12 reactors, enabling a direct test of the dissolved Zn hypothesis.

Phenotypic meropenem resistance over time in the reactors is shown in Fig. 4. The high-Zn WB sediments displayed the greatest increases in relative meropenem resistance, whereas low-Zn WB sediments showed the smallest increases, consistent with dissolved Zn controlling resistance levels. Plotting dissolved and total Zn against meropenem resistance across all twelve reactors helps visualise this trend (Fig. 5): dissolved Zn and phenotypic meropenem resistance significantly correlate ($r = 0.628$, $p = 0.029$), whereas no correlation exists between total Zn and meropenem resistance ($r = -0.298$, $p = 0.354$). These results provide direct experimental evidence that dissolved Zn, not total Zn, is the operative driver of phenotypic meropenem co-resistance in Tyne sediment environments.

3.6. Relationships between Zn efflux and porin genes and resistant phenotypes in Tyne sediments

To explore the possible genetic basis of the observed Zn-dependent meropenem-resistance patterns, qPCR was used to quantify five Zn-resistance-associated genes in the sediment samples. Zn is an essential micronutrient in all organisms at low concentrations (Choudhury and Srivastava, 2001) but can become highly toxic at elevated levels, and bacteria have evolved multiple defence systems to regulate intracellular Zn (Gupta et al., 2026). These include: the resistance-nodulation-division (RND) efflux systems, encoded by *czcA* (cobalt-zinc-cadmium, an inner membrane transporter that extrudes metals from the cytoplasm (Nies, 2003)); *czcD* (a cation diffusion facilitator, CDF-type, responsible for Zn efflux across the cytoplasmic membrane (Paulsen and Saier, 1997)); the *czrA* system (an efflux transporter linked to cadmium and zinc resistance (Hassan et al., 1999)); and the P-type ATPase, *zntA*, responsible for active Zn efflux in Gram-negative organisms (Thelwell et al., 1998). Further, the outer

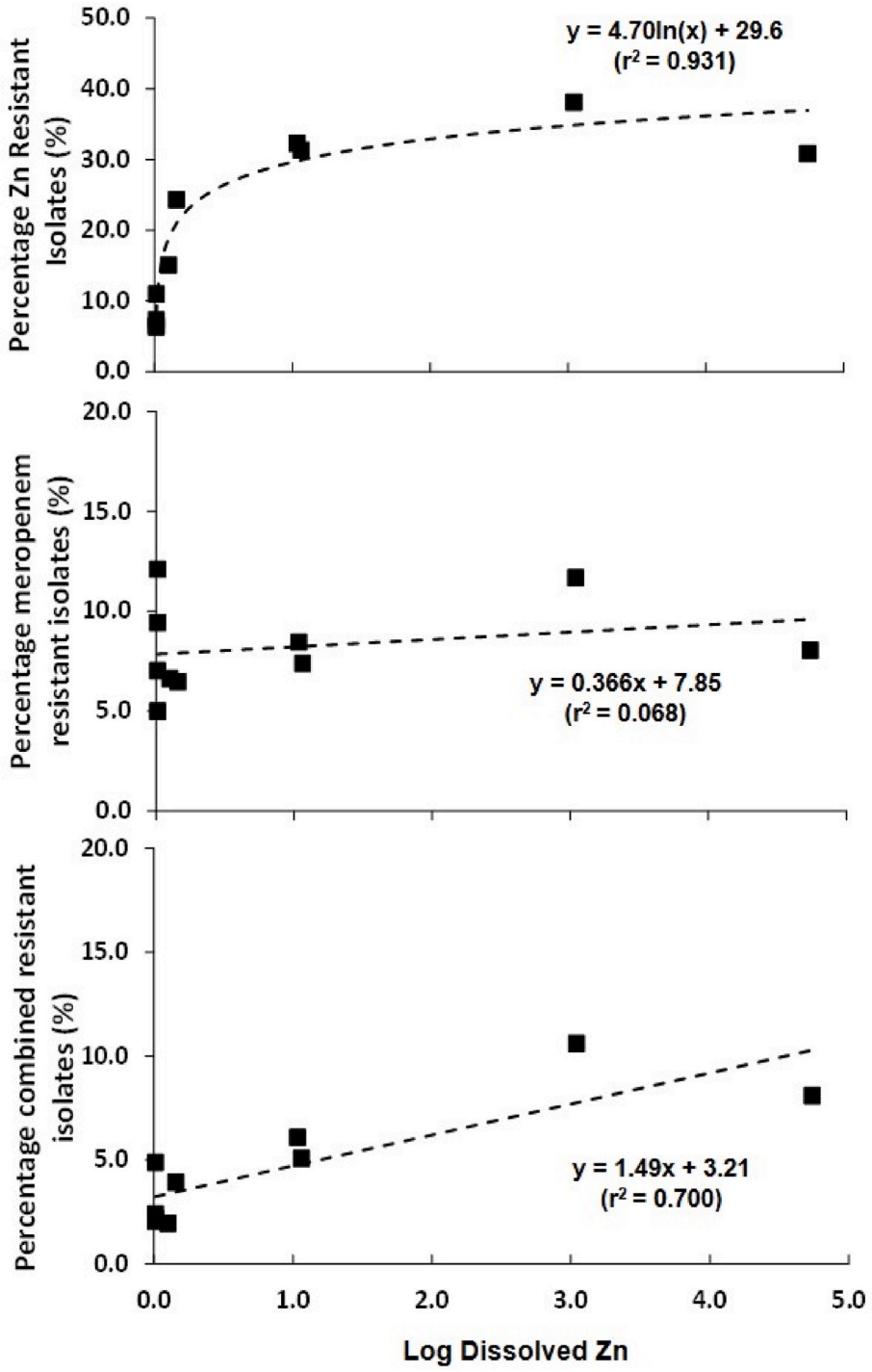


Fig. 3. Relationships between the relative percentage (%) of Zn, meropenem, and combined resistant colony isolates, and dissolved Zn in River Tyne sediments collected across the six-month sampling programme.

membrane porin, *oprD*, normally mediates carbapenem uptake but is blocked by elevated intracellular Zn exposure, thereby conferring Zn-dependent meropenem resistance (Perron et al., 2004; Bedenić et al., 2014). A schematic overview of these systems is shown in Fig. S1.

Relative abundances of these genes (normalised to 16S rRNA) are summarised in Fig. S2. Higher *czcA*/16S levels were observed in South Tyne sediments (FS and WA) compared with North Tyne sediments (WB; $p < 0.010$), and *oprD*/16S levels were elevated at FS, WA, and RN relative to RH ($p < 0.001$). Correlation analyses (Table 2) showed that *czcA*/16S and *oprD*/16S correlated significantly with phenotypic Zn resistance at the uncorrected threshold ($p < 0.05$). However, of these, only the *oprD*/16S-Zn resistance correlation remained statistically significant after BH correction. Critically, *oprD*/16S was the only gene to correlate significantly with combined resistance at the nominal threshold ($p < 0.05$) but showed no correlation with meropenem resistance alone ($p > 0.05$).

Although this *oprD*-combined resistance association did not retain statistical significance after BH correction, we retain it in the discussion as it aligns with the biochemical pathway reported in *Pseudomonas aeruginosa*, where *oprD*-mediated porin closure under Zn exposure decreases meropenem uptake (Perron et al., 2004; Bedenić et al., 2014). Further, the relationship is supported by our batch-reactor findings,

Table 3
Mean total and dissolved Zn (mg/L) in batch reactor slurries seeded with Warks Burn (WB) and West Allen (WA) sediment at low (2 mg/L) and high (100 mg/L) $ZnSO_4$ amendments. Values are means from triplicate reactors sampled over 309 days (n = 12 per treatment). Standard deviations are shown.

	Warks Burn (WB)		West Allen (WA)	
	Total Zn (mg/L)	Dissolved Zn (mg/L)	Total Zn (mg/L)	Dissolved Zn (mg/L)
Low Zn Amendment (2 mg/L)	31.9 ± 9.58	0.07 ± 0.02	1240 ± 91.7	0.76 ± 0.32
High Zn Amendment (100 mg/L)	158 ± 6.22	1.35 ± 0.35	1300 ± 94.7	0.70 ± 0.37

which show that higher dissolved Zn induces phenotypic meropenem resistance. These patterns imply that *oprD*-mediated porin closure under Zn stress is a plausible mechanism for the type of Zn-dependent meropenem co-resistance observed in Tyne River sediment samples. However, it is unknown whether Zn-induced porin regulation represents a primary defensive strategy among diverse environmental bacteria or is limited to specific taxa, such as *Pseudomonas aeruginosa*.

Taken together, field and laboratory data show that phenotypic meropenem resistance in River Tyne sediments may be conditional as a function of dissolved Zn levels – speculation supported by significant correlations with different Zn efflux and porin genes, and the loss of meropenem resistance when dissolved Zn is reduced. These findings are consistent with the literature on metal-antibiotic co-resistance mechanisms (Baker-Austin et al., 2006; Gupta et al., 2023; van Uum et al., 2024) and reinforce the important conclusion that metal *bioavailability* – not total metal level - is a more critical exposure metric for metal-related antibiotic resistance risk assessments (Robins et al., 2022).

Although meropenem resistance was reversible with Zn removal, bacteria may quickly reacquire a resistant phenotype upon renewed Zn exposure. This has practical implications for catchment water quality management: in undisturbed mining-impacted sediments where dissolved Zn levels are low, the risk of persistent meropenem-resistant strains is correspondingly low, but occasional or localised inputs of bioavailable Zn could rapidly reactivate resistance.

The dissolved Zn concentrations found here can be compared to the minimal co-selective concentrations (MCSCs) for Zn published previously. Specifically, Arya et al. (2021) derived an *Escherichia coli* specific MCSC for bioavailable Zn of 1.6 mg/L. The dissolved Zn levels determined here at the most polluted site, River Nent (3.89 ± 0.85 mg/L in sediment, 1.22 ± 0.22 mg/L in pore water; Table 1), and in the highest dissolved Zn batch reactors (up to 1.35 ± 0.35 mg/L; Table 3), are in the same order of magnitude as this threshold, and in pore water at RN, slightly below. This implies that our observed co-resistance selection data are consistent with Arya et al.'s model and broadly consistent with metal co-selection thresholds evaluated by Seiler and Berendonk (2012).

4. Conclusions

This study assessed phenotypic Zn-, meropenem-, and combined-resistance in sediments from the North and South Tyne over six months. Results showed significantly higher abundances of Zn-resistant and combined-resistant organisms at South Tyne sites FS, WA, and RN

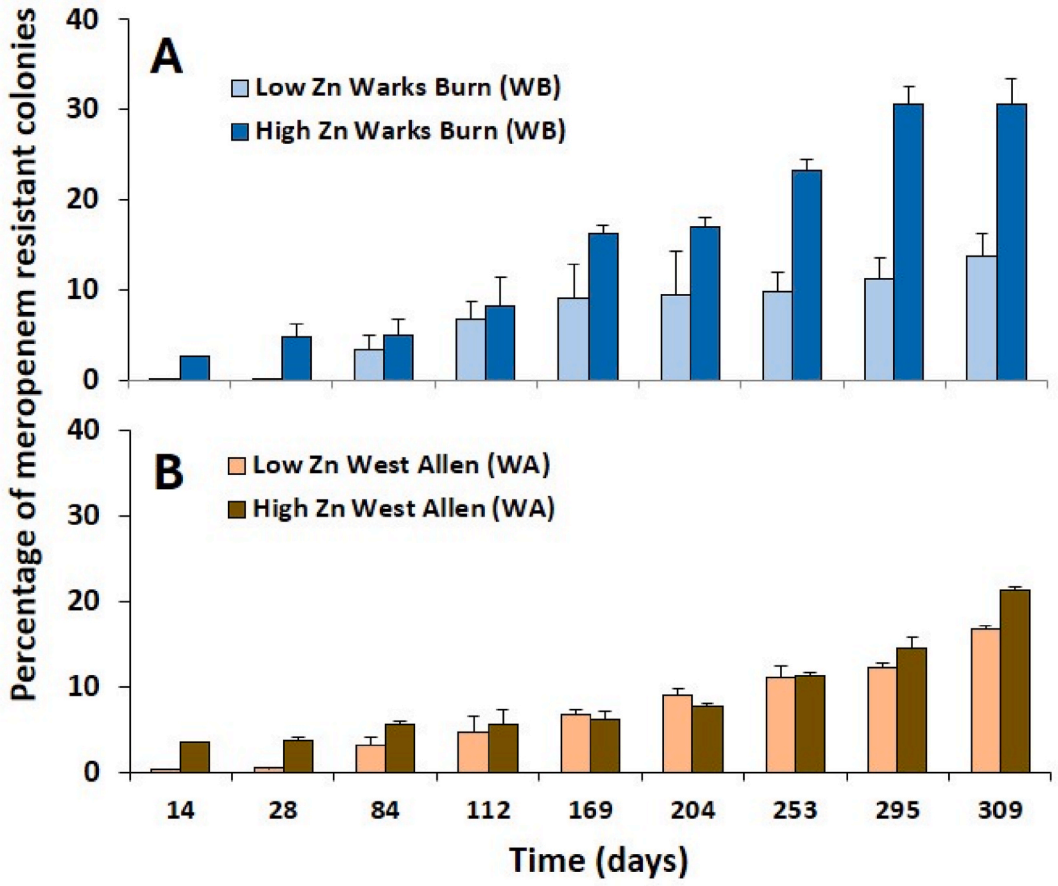


Fig. 4. Relative percentage (%) of colony isolates resistant to 2 mg/L meropenem over 309 days in batch reactors seeded with (A) Warks Burn (WB) and (B) West Allen (WA) sediments. Low and High refer to reactors with 2 and 100 mg/L Zn amendments (as $ZnSO_4$). Values are means from triplicate independent reactors sampled over time. Standard deviation bars are shown.

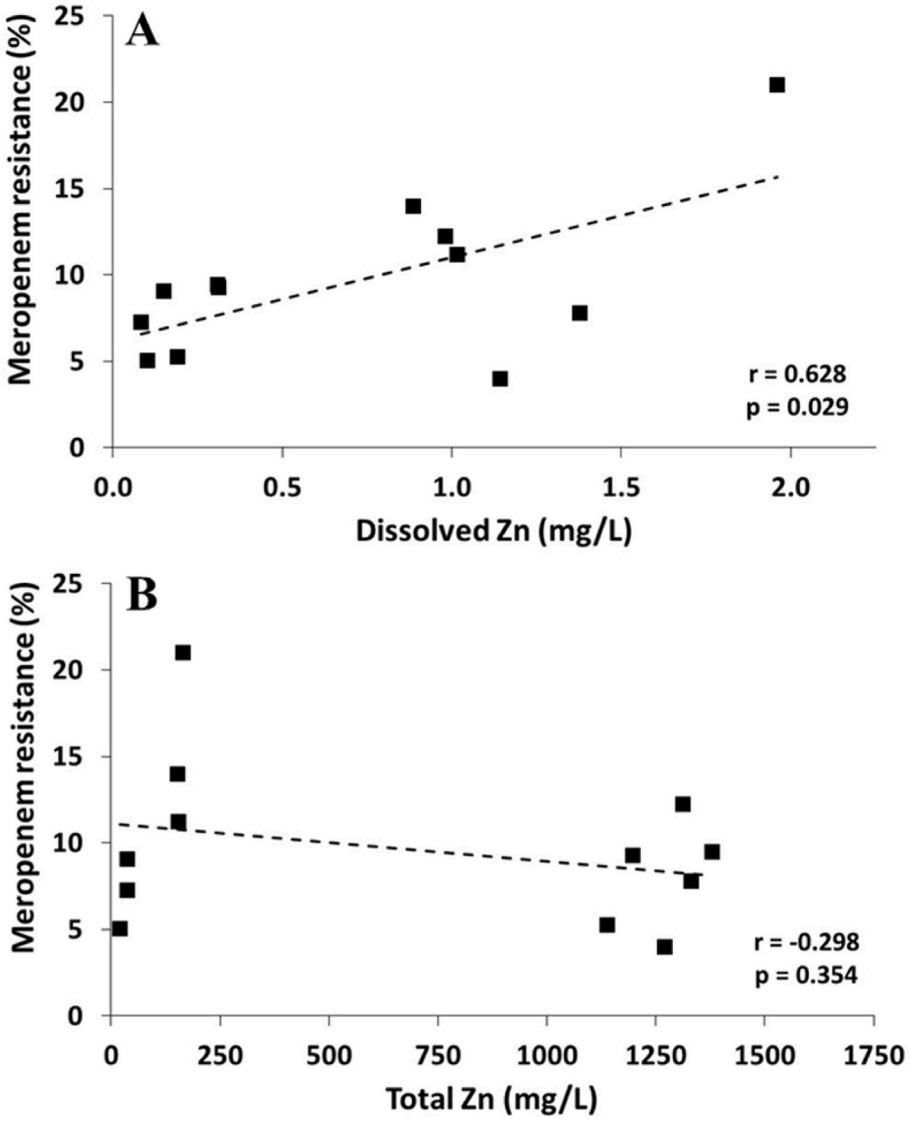


Fig. 5. Relationship between mean total and dissolved Zn levels in the batch reactors and the relative percentage (%) of meropenem-resistant colony isolates over 309-day exposures.

relative to North Tyne sites. Crucially, meropenem resistance was only higher when Zn was in the growth medium, suggesting that observed phenotypic meropenem resistance in River Tyne sediments is conditional, i.e., only higher when elevated dissolved Zn is also present. This speculation in consistent with our long-term batch experiment results, which showed dissolved Zn drives meropenem-resistance selection, with dissolved Zn only correlating significantly with meropenem-resistant phenotypes ($r = 0.628$, $p < 0.05$). Further, the loss of meropenem resistance when dissolved Zn is low suggests a conditional and reversible mechanisms. In fact, qPCR analysis showed a strong correlation between *oprD*/16S and phenotypic Zn resistance, which was maintained after BH correction. Its association with combined resistance, which was the strongest of the five genes examined, did not survive the correction and deserves further study because it is consistent with proven mechanisms in *Pseudomonas aeruginosa*.

Overall, results here carry a practical message for risk assessment: monitoring programmes for Zn-polluted rivers such as the Tyne should prioritise the dissolved, bioavailable Zn fraction rather than total sediment Zn burdens when evaluating antibiotic co-resistance risk. Findings also highlight the continued value of culture-based phenotypic methods, which were able to detect conditional, reversible co-resistance that would be very difficult to detect using current genetic methods alone. However, it must be noted that a limitation of our study is that the link between Zn exposure, *oprD*, and reversible meropenem resistance is based on phenotypic observations and correlation analyses, instead of direct molecular validation. Therefore, future research should combine methods and analyse the expression of *oprD* during Zn exposure and after Zn removal to clarify the basis of observed reversible phenotypes and expanding the dissolved-vs-total metal exposure approach to other metals and antibiotics. The ultimate goal is to identify general drivers of metal-associated AR in mining-impacted and other environments - essential information for better-informed metal-associated AR risks assessments.

CRediT authorship contribution statement

Catherine L. Hands: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sonia Gupta:** Writing – review & editing, Formal analysis. **Anke Neumann:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Charles W. Knapp:** Writing – review & editing, Methodology, Formal analysis. **S.Z. Ahammad:** Writing – review &

editing. **Jason Snape:** Writing – review & editing, Supervision, Funding acquisition. **David W. Graham:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: David W Graham reports financial support was provided by UK Research and Innovation Natural Environment Research Council. Catherine Hands reports financial support was provided by AstraZeneca UK Limited. David Graham reports a relationship with United Nations Environment Programme that includes: board membership. David Graham reports a relationship with International Centre for Antimicrobial Resistance Solutions that includes: consulting or advisory. David Graham reports a relationship with Chinese Academy of Sciences that includes: consulting or advisory. David Graham reports a relationship with UK Research and Innovation Medical Research Council that includes: funding grants. If there are other authors, they 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2026.129055.

Data availability

Data will be made available on request.

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