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Linking micropollutant gradients and multigenerational microplastic exposure to insecticide tolerance in field populations of *Anopheles gambiae* s.l

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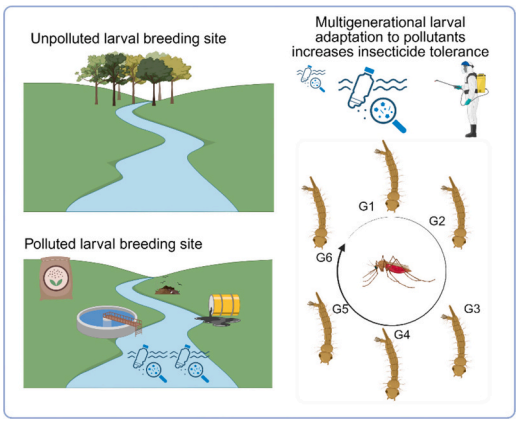
HIGHLIGHTS

- Urban freshwater mosquito breeding sites are frequently polluted
- Field-collected larvae from polluted water show insecticide tolerance in adults
- Larvae rapidly adapt to repeated generational exposure to microplastics and insecticides
- Repeated microplastic exposure increased phenotypic insecticide tolerance in adults
- Polluted urban breeding habitats may contribute to the development of insecticide resistance

GRAPHICAL ABSTRACT



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ABSTRACT

Urban freshwater systems increasingly serve as breeding habitats for *Anopheles gambiae*; however, the effects of prolonged larval exposure to complex waterborne pollutants remain insufficiently characterised. Therefore, larvae were collected from two mosquito breeding sites with pronounced spatial differences in baseline concentrations of heavy metals (Pb, Cd, Hg, As), pesticides (organochlorines/organophosphates), inorganic nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}), and microplastics (MPs). Principal Component Analysis demonstrated a pronounced difference in the overall pollutant profiles of the two breeding sites, with substantially greater contamination at the downstream site. The influence of repeated exposure to MPs and the insecticide deltamethrin (either on its own or with MPs) on field populations of larvae was quantified in laboratory experiments. Larval survival and development were recorded over six generations, and insecticide tolerance of adult mosquitoes derived from these larvae quantified by WHO resistance bioassay tests. Initially, MP exposure reduced larval survival and delayed development; however, after several generations, larvae exhibited increased survival, accelerated development, and enhanced adult tolerance to deltamethrin. These responses were more pronounced in

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mosquitoes originating from the more polluted site, suggesting potential pre-existing environmental conditioning, although differences in sibling-species composition between sites cannot be excluded as a contributing factor. Exposure to combined MPs and insecticide resulted in higher survival and shorter development times compared to insecticide exposure alone. Collectively, these findings provide evidence that repeated MP exposure, in conjunction with environmental pollution, can progressively increase phenotypic insecticide tolerance in *An. gambiae*. Polluted urban breeding habitats may therefore contribute to reduced insecticide susceptibility, emphasizing the need to incorporate environmental pollution considerations into vector-control and insecticide-susceptibility management strategies.

1. Introduction

Anopheles gambiae sensu lato contains some of the most efficient malaria vectors in sub-Saharan Africa and a major driver of *Plasmodium falciparum* transmission (WHO, 2016). Although *Anopheles gambiae* s.l. has traditionally been associated with small, temporary and relatively clean freshwater habitats, increasing evidence indicates that members of this species complex can also exploit polluted aquatic habitats, particularly in urban environments (Sattler et al., 2005; Owusu-Asenso et al., 2025). Such adaptation of a major malaria vector to polluted breeding sites poses a significant threat to strategies for malaria control in urban and polluted environments. Importantly, higher larval densities have been demonstrated in moderately contaminated waters, likely due to reduced predation and increased microbial food resources (Awolola et al., 2007; Bakonyi et al., 2022; Avramov et al., 2024). However, high concentrations of waterborne pesticides, heavy metals, and hydrocarbons can impair larval survival and development, indicating a complex, non-linear relationship between pollutant levels and mosquito life history and fecundity (Abbas et al., 2025).

Microplastics (MPs) are now globally widespread contaminants in freshwater systems and are increasingly detected in mosquito breeding habitats. MPs may be ingested by larvae or adhere to the exoskeleton, altering feeding behaviour, developmental timing, and survival depending on MP concentration and polymer type (Aryaprema et al., 2025). The surfaces of MPs can also adsorb pesticides and other pollutants, acting as a sink for a range of chemical stressors (Martín et al., 2022; Chen et al., 2024). A recent global meta-analysis has shown strong negative effects of MPs on freshwater invertebrate larvae, yet the ecological and evolutionary consequences of MP exposure for malaria vectors remain largely unexplored (Abbas et al., 2025; Jones et al., 2024). However, exposure to environmental pollutants, including MPs, may contribute to stress tolerance and pre-adaptation in mosquito populations (Prud'homme et al., 2017; Shilla et al., 2024). Aquatic insects respond to chronic stress through detoxification enzyme upregulation, cuticular modification, and metabolic adjustments - mechanisms that overlap with pathways underlying insecticide resistance, which is a growing challenge for malaria vector control (Mack and Attardo, 2024). Although multigenerational exposure to larvae from a laboratory colony of insecticide-susceptible *An. gambiae* can increase insecticide tolerance in adult mosquitoes (Shilla et al., 2024), empirical evidence linking environmental pollution gradients to changes in insecticide susceptibility in *An. gambiae* is still lacking.

Current knowledge on the effects of MPs on mosquitoes has largely been derived from laboratory colonies exposed to simplified exposure conditions that are not linked to real-world pollutant mixtures (Al-Jaibachi et al., 2019; Edwards et al., 2023; Shilla et al., 2024). Therefore, there is a critical need for studies that integrate environmental chemical profiles with multigenerational biological responses. This study addresses this gap by combining field measurements of micropollutants in mosquito breeding grounds, with subsequent controlled multigenerational exposure experiments using *An. gambiae* larvae collected from a waterborne pollution gradient in the Msimbazi River. Specifically, we test whether MPs (either alone or in combination with insecticides) interact with environmental stress histories and influence subsequent larval development, survival, and adult insecticide tolerance. Our findings provide new insights into how pollution may inadvertently enhance the environmental resilience of malaria vectors.

2. Materials and methods

2.1. Study area

The Msimbazi River (Fig. 1) in Dar es Salaam, Tanzania, originates in the Kisarawe highlands and flows ~35 km through a 300 km^2 catchment, receiving inputs from the Luhanga, Ubungo, and Sinza tributaries, before discharging into the Indian Ocean (Chanzi, 2017). The river is heavily impacted by effluents from the textile, food-processing, metal, and plastic industries (Ngassapa et al., 2018). Two sites with confirmed occurrence of *Anopheles gambiae* larvae were sampled: an upstream Pugu Forest site representing baseline freshwater conditions (site MS1), and a downstream densely populated urban section heavily influenced by industrial discharge, domestic sewage, and urban waste (site MS2). Water samples and mosquito larvae were collected from both sites in December 2024, at the onset of the light rains, using standardised protocols.

2.2. Sampling and analysis of micropollutants

A total of 24 water samples (12 from each site) were collected from known larval breeding habitats within the Msimbazi River, and the concentrations of heavy metals (Pb, Cd, Hg, and As), pesticides (organochlorine and organophosphate), nutrients (e.g., NO_3^-, PO_4^{3-}, NH_4^+), and microplastics (size >20 to <500 μm) were quantified.

For heavy metal analysis, water samples were immediately filtered (0.45 μm), acidified with nitric acid (pH < 2), before subsequent analyses (Masoud et al., 2023). Quantification of heavy metals was performed by atomic absorption spectrophotometry (AAS), with calibration against internal standards, blanks, and certified reference materials (Jin et al., 2020), and the mean for each sample was calculated from six technical replicates.

Analytical procedures for quantifying pesticide concentrations followed Chidewe et al. (2025), with minor adaptations. Briefly, water samples were processed using liquid–liquid extraction (with analytical-grade solvents) and concentrated and cleaned prior to GC–MS analysis. Certified standards were added to ensure accurate calibration and correction for matrix effects. GC–MS conditions were optimised to accommodate both stable organochlorines (OCPs) and thermally sensitive organophosphates (OPs). Quantification was performed using the internal-standard method, and quality control included procedural blanks, spike recoveries, and replicate analyses.

Concentrations of inorganic nutrients (nitrite, nitrate, ammonium, and phosphate) were quantified using standard spectrophotometric procedures following APHA (2017), and methodologies previously applied in East African river systems (Shilla, 2020). Briefly, NO_2^- was determined using the Griess–Ilosvay colorimetric method, while NO_3^- was quantified after cadmium reduction to NO_2^-, followed by the Griess–Ilosvay colorimetric reaction. The concentration of NH_4^+ was quantified using the indophenol blue (phenate) method, and orthophosphate quantified using the ascorbic acid molybdenum blue method (APHA, 2017). Absorbance was recorded at the respective wavelengths using a UV–Vis spectrophotometer, with calibration curves prepared

from analytical-grade standards. Quality assurance included blanks, duplicate samples, and standard spike recoveries.

For MP analysis, surface-water samples were collected in triplicate (5 L each) from the Msimbazi River using a clean metallic bucket, and sieved through a 5 mm stainless-steel mesh. Replicate samples of filtered water were added to glass bottles, stored on ice, and transported back to the lab. In the laboratory, particles >63 μm were fractionated by wet-sieving through stacked stainless-steel sieves (63, 125, 250, 500 μm), while particles ranging from 20 to <63 μm were collected by vacuum filtration onto 20 μm nylon membrane filters (Merck Millipore). All retained particles were examined under a stereomicroscope, manually picked with metal forceps, and classified by size and morphology. Quality assurance and quality control (QA/QC) measures included exclusive use of metallic/glass equipment, pre-filtered reagents, covering samples during processing, cotton laboratory attire, and routine field and laboratory blanks to detect and correct for airborne or procedural contamination (Qiu et al., 2016).

Procedural blanks (distilled water, reagents, and solvents) analysed in parallel to Msimbazi River samples showed no detectable heavy metals (Pb, Cd, Hg, and As) or pesticide residues, confirming the absence of laboratory contamination. Method performance was evaluated using spike recovery experiments and method detection limits, with recoveries ranging from 75 to 121% for heavy metals, 87–119% for organophosphate pesticides, and 88–122% for organochlorine pesticides (Table S1). Detection limits were sufficiently low to allow quantification of environmentally relevant concentrations.

2.3. Collection and rearing of mosquito larvae

Mosquito larvae were collected from the two sites in the Msimbazi River using a 350 mL dipper and transferred into aerated 10 L buckets for transport to the Dar es Salaam Institute of Technology (DIT). Larvae were placed in labelled 2 L ceramic bowls (Shenzhen Tao Hui Industrial Co., Ltd) and reared under standard insectary conditions (26–30 °C; 65–80% RH) and fed daily with finely ground Tetramine® fish food (Tetra, UK) until pupation. Pupae were collected with pipettes, transferred into 500 mL ceramic cups, and placed inside emergence cages. Adult mosquitoes were maintained on 10% glucose solution, and female adults were blood-fed with cow blood using the membrane-feeding system to stimulate egg development (Costa-da-Silva, 2023).

2.4. Preparation of microplastics for exposure experiments

Polyethylene terephthalate (PET) particles (1.0–7.5 μm; density 1.38 g/cm^3) were sourced from Cospheric LLC (Santa Barbara, USA). PET particles were prepared following Shilla et al. (2024): a stock suspension (100 mg/20 mL) was stored at 4 °C in the dark, and prior to use, the stock was agitated to disperse particles and subsequently diluted to the two required exposure concentrations (1 g/L and 10 g/L).

2.5. Assessment of larval survival and development time

Larval survival and development assays were conducted at the Dar es Salaam Institute of Technology following procedures described in Shilla et al. (2024). Briefly, six treatments (Table 1), including a control (distilled water), microplastic (1 or 10 g/L PET MPs), or the insecticide deltamethrin, either alone or in a mixture with MP, were used for exposure assays for larvae originating from the two river sites (MS1 and MS2), with six independent replicates for each treatment from each site. This approach followed WHO guidelines, which require at least four replicates, each comprising 25 larvae (WHO, 2005). An initial pre-test identified a concentration of 0.140×10^{-10} g/L deltamethrin (Decis®; AGRO SHOPY–Syngenta India Ltd.) as the LD_{15}, i.e., the dose producing ~15% mortality. In the control and MP-only treatments, 900 larvae were used per generation across all six generations (G1–G6) to assess multigenerational responses to repeated MP exposure. In contrast, 900

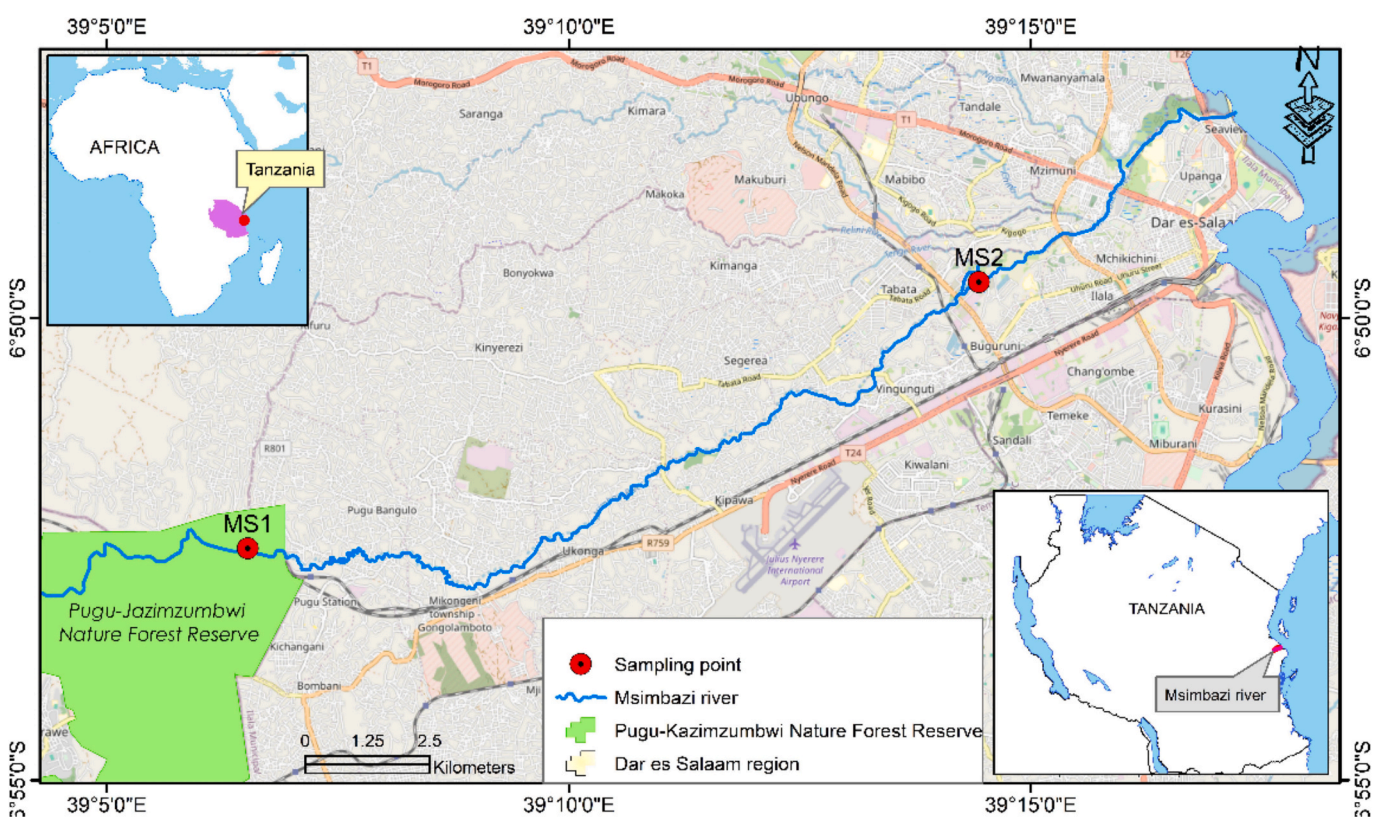

Fig. 1. Map of the Msimbazi River catchment showing the upstream (MS1) and downstream (MS2) sampling sites in relation to major urban and industrial land-use features.

Table 1
Treatments and exposure design. Each treatment included six replicates with 60 larvae each. Control and MP-only treatments were evaluated across G1–G6, whereas insecticide-only and MP–insecticide mixture treatments were evaluated at G1, G3 and G6.

Treatment	Microplastics	Insecticide*
1	–	–
2	1 g/L	–
3	10 g/L	–
4	–	+
5	1 g/L	+
6	10 g/L	+

* 0.140×10^{-10} g/L of deltamethrin

larvae per generation were used for the insecticide-only and MP–insecticide mixture treatments at selected generations (G1, G3, and G6) to assess whether the presence of MPs modified the effects of deltamethrin on larval survival and development. For each treatment, six replicate cohorts were maintained separately in 500 mL ceramic bowls (Shenzhen Tao Hui Industrial Co., Ltd.) throughout successive generations and monitored daily. Offspring from each replicate were used to establish the corresponding replicate in the subsequent generation, with no pooling among replicates between generations. Pupae were collected using a dropper and transferred to labelled net cages for adult emergence. Emerged adults were provided with a 10% sucrose solution via filter papers, and females were fed cow blood using a membrane-feeding system to support egg development. Larvae were reared through six successive generations (G1–G6) under consistent treatment conditions, while the parental generation (G0) remained untreated and served as the baseline control.

2.6. WHO resistance bioassay tests

Insecticide susceptibility was assessed across three mosquito generations G0 (unexposed), G3, and G6 for larvae originating from both sites, in accordance with WHO guidelines (WHO, 2022). These generations were selected to evaluate changes in tolerance over time. For the G0 generation, 100 adult female mosquitoes originating from each site were divided into four replicate groups of 25 mosquitoes, together with a control group of 25 mosquitoes. This assay was repeated twice, resulting in 200 test mosquitoes and 50 controls per site. For G3, mosquitoes from each study area were evaluated after larval exposure to the six treatment conditions (Table 1). As above, 100 adult female mosquitoes originating from each treatment were divided into four replicate groups of 25 mosquitoes, together with a control group of 25 mosquitoes, and the assay was repeated twice (yielding 1200 test mosquitoes and 300 control mosquitoes per site). The same experimental design was applied for mosquitoes from G6.

Adult female mosquitoes aged 3–5 days were aspirated and allowed to acclimatize for 1 h before being transferred to WHO bioassay tubes lined with 0.05% deltamethrin-impregnated paper. At the same time, control groups were placed in insecticide-free tubes. Knockdown was recorded at 5, 10, 15, 20, 30, 40, 50, and 60 min, and mortality was assessed 24 h after exposure. All assays were conducted under controlled conditions of 24–28 °C and 68–74% RH. According to WHO criteria (WHO, 2022), mortality rates of 98–100% indicate susceptibility, 90–97% suggest possible resistance, and values <90% confirm resistance.

2.7. Statistical analysis

Data normality was evaluated using Q–Q plots of residuals (Ghasemi and Zahediasl, 2012; Law and Jackson, 2017). Following confirmation of normality, mean environmental variables between the two sites (MS1 and MS2) were compared using independent-samples *t*-tests. Principal component analysis (PCA) was used to summarise the multivariate structure of the measured pollutants and visualise differences in overall pollutant profiles between MS1 and MS2. Given the strong covariance among pollutants and the inclusion of only two sampling sites, the PCA was interpreted descriptively rather than as evidence of independent effects of individual pollutants on insecticide tolerance. Differences in larval survival and development time across treatments and generations were analysed using a univariate general linear model (GLM) with Bonferroni-adjusted pairwise comparisons, and Kaplan–Meier survival curves were used to illustrate survival patterns. All analyses were conducted in IBM SPSS Statistics version 22.

Insecticide susceptibility was evaluated using probit regression to estimate knockdown times for 50% (KDT50) and 95% (KDT95) and mortality within 24 h post-exposure of adult female mosquitoes in the control, G0, G3, and G6 groups. Resistance was quantified as the ratio of KDT50 in treated groups (G3 or G6) to that of the untreated baseline (G0). Mortality recorded 24 h post-exposure was also analysed using a GLM with Bonferroni-adjusted multiple comparisons.

3. Results

3.1. Waterborne micropollutants and subsequent deltamethrin tolerance in adult An. gambiae s.l. reared from field-collected larvae

Concentrations of waterborne micropollutants differed between the two sites in the Msimbazi River, with very low or non-detectable levels of most pollutants at site MS1, and significantly higher concentrations of heavy metals, pesticide residues, nutrients, and microplastics at site MS2 ($p < 0.05$), demonstrating a clear upstream–downstream gradient in pollutant levels (Table 2).

Principal Component Analysis (PCA) reduced the pollutant dataset into four components, with eigenvalues >1 explaining 79% of the total variance (Fig. 2 and Fig. S1). PC1 explained 55% of the variance and was driven by NO_3^-, p,p'-DDT, Malathion, NH_4^+, Pb, Parathion, As, NO_2^-, Parathion-methyl and PO_4^{3-}. PC2 (10%) was associated with Chlorpyrifos, Hg and p,p'-DDE; PC3 (8%) with Lindane/γ-HCH, α-HCH and MPs; and PC4 (6%) with Dieldrin. The PCA showed a pronounced separation between samples from MS1 and MS2, consistent with the substantially higher concentrations of most measured pollutants at MS2. Given the strong covariance among pollutants and the two-site design, these components primarily characterise differences in the overall pollutant profiles of MS1 and MS2 and were therefore not interpreted as independent effects of specific pollutants.

3.2. Impact of MPs and MP–insecticide mixture on larval survival

The survival of larvae exposed to MPs significantly decreased compared to the control (with significantly lower survival in larvae exposed to the higher concentration of MPs), irrespective of which site the larvae had been collected from (site MS1: $F_{(2,108)} = 670.9$, $p < 0.0001$; site MS2: $F_{(2,108)} = 319.5$, $p < 0.0001$) (Fig. 3; Table S2). The mean survival of larvae exposed to 1 g/L decreased between 68 and 80% (site MS1) and 86–94% (site MS2), while exposure to 10 g/L decreased survival between 67 and 74% (MS1) and 79–81% (MS2). Larval survival was markedly higher ($p < 0.0001$) in the corresponding controls (MS1: 99%; MS2: 98–99%; Table S2). Despite the initial decline, survival rates increased progressively over generations. For larvae from site MS1, the rate of survival rose from 67 to 68% in G1 to 74–80% in G6 ($F_{(5, 72)} = 17.9$, $p < 0.0001$), while the survival rate of larvae from site MS2 increased from 79 to 86% to 81–94% ($F_{(5, 72)} = 3.1$, $p = 0.014$). Pairwise comparisons showed that for larvae from site MS1, survival during G2–G6 was significantly higher than in G1 ($p < 0.05$), although with no significant differences for larvae between G3–G6 (Table S3). For larvae from site MS2, significant increases in the rate of survival occurred only in G5 and G6 relative to G1 and G2 ($p < 0.05$), with no differences detected for larvae between G3–G6.

Larval survival in the MP–insecticide mixture treatments, also varied

Table 2
Comparison of the mean concentration of micropollutants between sites MS1 and MS2.

Pollutant (mg/L)*	Site MS1	Site MS2	Statistics
Pb	0.023 ± 0.01	0.450 ± 0.55	$t_{(23)} = -2.662$, $p = 0.012$
Cd	0.005 ± 0.00	0.103 ± 0.23	$t_{(23)} = -2.08$, $p = 0.049$
Hg	0.007 ± 0.00	0.068 ± 0.09	$t_{(23)} = -3.19$, $p = 0.004$
As	0.021 ± 0.02	0.791 ± 0.66	$t_{(23)} = -5.666$, $p < 0.0001$
Parathion	0.005 ± 0.00	0.433 ± 0.32	$t_{(23)} = -6.579$, $p < 0.0001$
Parathion-methyl	0.009 ± 0.01	1.379 ± 0.77	$t_{(23)} = -8.761$, $p < 0.0001$
Malathion	0.010 ± 0.01	0.531 ± 0.48	$t_{(23)} = -5.308$, $p < 0.0001$
Diazinon	0.007 ± 0.00	0.723 ± 0.84	$t_{(23)} = -4.183$, $p < 0.0001$
Chlorpyrifos	0.005 ± 0.00	0.099 ± 0.07	$t_{(23)} = -6.848$, $p < 0.0001$
p,p'-DDT	0.005 ± 0.00	0.219 ± 0.21	$t_{(23)} = -4.983$, $p < 0.0001$
p,p'-DDE	0.005 ± 0.00	0.093 ± 0.04	$t_{(23)} = -10.349$, $p < 0.0001$
Dieldrin	0.005 ± 0.00	0.086 ± 0.07	$t_{(23)} = -5.42$, $p < 0.0001$
Lindane (γ-HCH)	0.005 ± 0.00	0.094 ± 0.06	$t_{(23)} = -7.092$, $p < 0.0001$
α-HCH	0.005 ± 0.00	0.077 ± 0.06	$t_{(23)} = -6.119$, $p < 0.0001$
NO_2^-	0.114 ± 0.09	3.783 ± 1.95	$t_{(23)} = -9.218$, $p < 0.0001$
NO_3^-	2.080 ± 0.95	58.110 ± 29.32	$t_{(23)} = -9.353$, $p < 0.0001$
NH_4^+	0.135 ± 0.08	10.619 ± 6.59	$t_{(23)} = -7.793$, $p < 0.0001$
PO_4^{3-}	0.054 ± 0.09	1.517 ± 1.17	$t_{(23)} = -6.099$, $p < 0.0001$
MPs (particles/L)	57.00 ± 28.03	1957.00 ± 138.64	$t_{(23)} = -64.549$, $p < 0.0001$

* Concentrations of all pollutants are expressed as mg/L, except for MPs which are reported as particles/L. Values represent the mean of twelve replicates ± standard deviation.

significantly with concentration of the MPs, irrespective of site (site MS1: $F_{(2, 54)} = 26.1$, $p < 0.0001$; site MS2: $F_{(2, 54)} = 316.5$, $p < 0.0001$) (Fig. 4; Table S2). Similarly, survival was significantly affected over the generations (site MS1 ($F_{(2, 36)} = 8.6$, $p = 0.001$; site MS2 ($F_{(2, 36)} = 4.7$, $p = 0.017$), with increased survival from 58 to 70% to 69–77% in larvae at G1 to G6 from site MS1, and from 80 to 84% to 83–88% from larvae from site MS2. Pairwise comparisons indicated that in larvae from site MS1, G6 survival was significantly higher than in G1 and G3 ($p < 0.01$), whereas for larvae from site MS2, a significant increase was observed only between G1 and G6 ($p < 0.05$) (Table S3). Overall, survival of larvae exposed to the MP–insecticide mixture treatments was significantly higher than for larvae exposed to just the insecticide (site MS1: $p < 0.0001$; and site MS2: $p < 0.001$) (Table S2).

3.3. Impact of MPs and MP–insecticide mixture on larval development time

Exposure to the higher concentration of MPs (10 g/L) resulted in significant variation, with a trend toward increased larval development time (site MS1: $F_{(2, 2080)} = 11.35$, $p < 0.0001$; site MS2: $F_{(2, 2165)} = 9.72$, $p < 0.0001$, Table S4). For larvae originating from site MS1, development time rose from between 8.9 and 9.7 days with exposure to 1 g/L, to between 9.1 and 10.0 days with exposure to 10 g/L, and for larvae from site MS2 from between 8.7 and 9.3 days to between 9.0 and 9.4 days (Table 3); overall, exposure to MPs significantly increased larval developmental times compared to the control ($p < 0.0001$). Importantly, development time decreased markedly between larvae of different generations. Development time of larvae from site MS1 decreased from between 9.7 and 10.0 days in G1 to between 8.9 and 9.1 days in G6 ($F_{(5, 3147)} = 52.09$, $p < 0.0001$), while development time of larvae from site MS2 decreased from between 9.3 and 9.4 in G1 to between 8.7 and 9.0 days in G6 ($F_{(5, 3534)} = 21.31$, $p < 0.0001$), with the greatest reduction observed in the later generations (G5 to G6).

In the MP–insecticide mixture treatments, development time also decreased significantly with the higher concentration of MPs. In larvae from site MS1 development time decreased from between 8.9 and 9.5

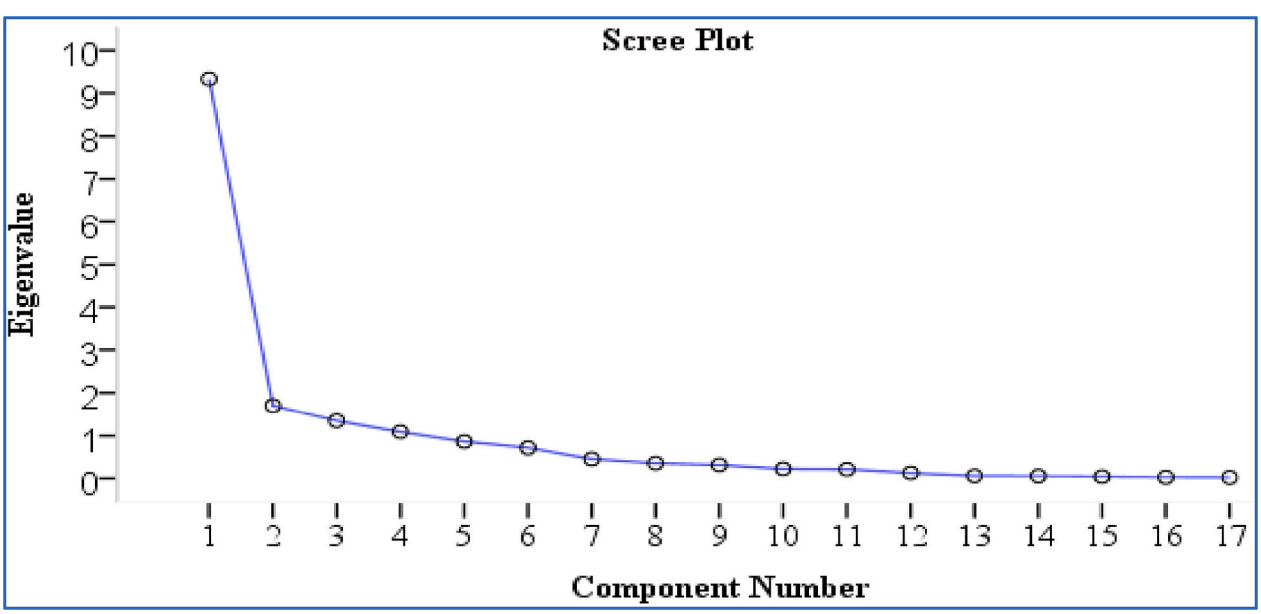

Fig. 2. Scree plot indicating the principal components.

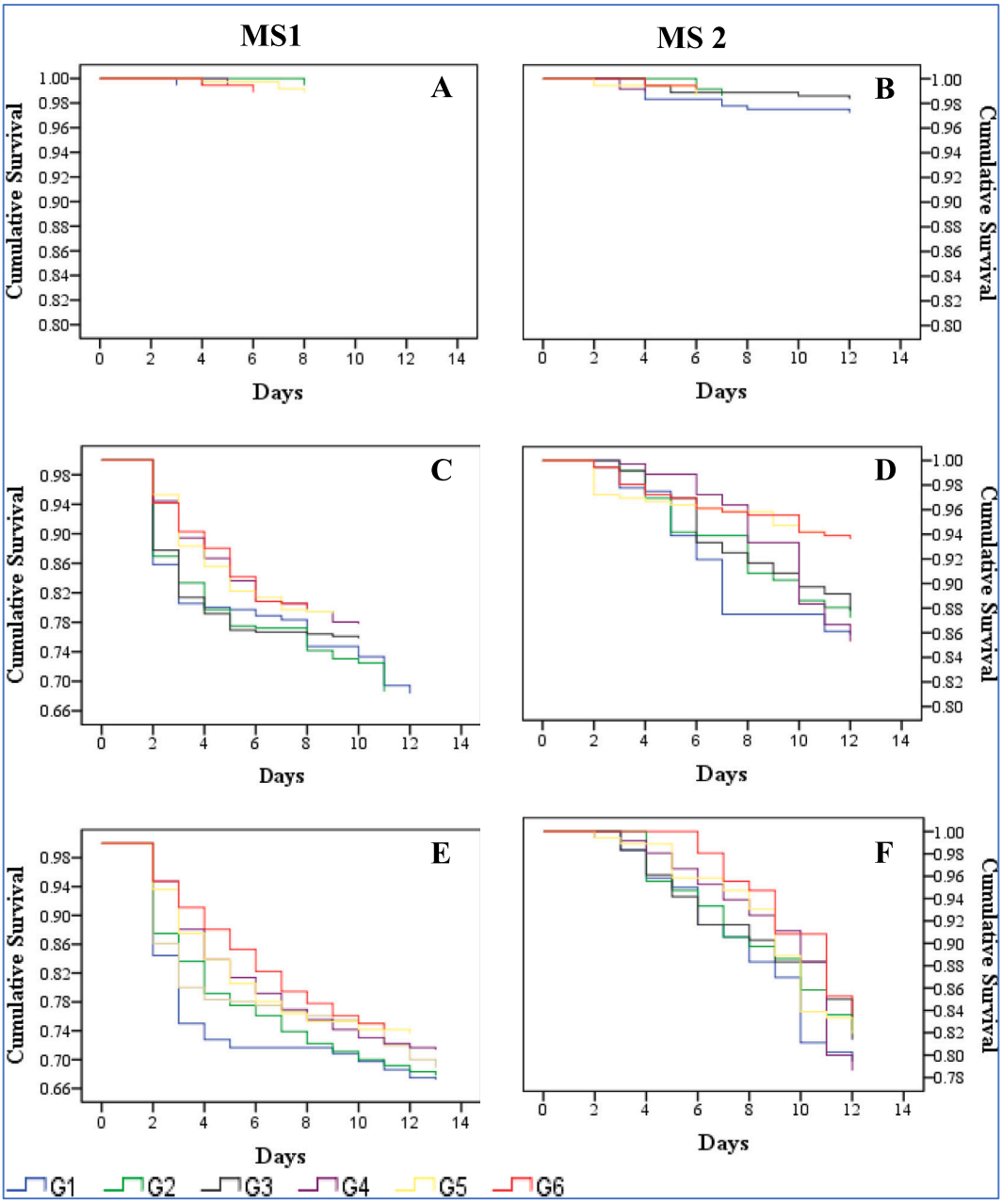

Fig. 3. Survival curves of larvae (originating from sites MS1 and MS2) exposed to control (A and B), or microplastics at either 1 g/L (C and D), or 10 g/L (E and F).

days to between 8.8 and 9.3 days ($F_{(2,\ 2080)} = 11.35$, $p < 0.0001$), while development time decreased in larvae from site MS2 from between 9.2 and 9.8 days to between 9.1 and 9.6 days ($F_{(2,\ 2165)} = 9.72$, $p < 0.0001$). Generational declines in larval development time were also observed: from between 9.3 and 9.5 days in G1 to 8.8–8.9 days in G6 for larvae from site MS1 ($p < 0.0001$), with the development time in larvae at G6 significantly shorter than for G1 and G3 ($p < 0.01$). For larvae from site MS2, development time decreased from between 9.6 and 9.8 to 9.1–9.2 days for larvae at G1 compared to G6 ($p = 0.014$).

In general, the larval development time after exposure to just MPs was longer for larvae from site MS1 (9.4 days) than MS2 (9.2 days) ($t_{(6464.6)} = 8.62$, $p < 0.0001$). The same pattern was observed in MP–insecticide mixture treatments (site MS1: 9.5 days compared to 9.3 days for larvae from site MS2; $t_{(2874.3)} = 2.63$, $p < 0.01$). Overall, development time was slightly shorter for larvae exposed to just MPs (9.3 days) compared to larvae exposed to the MP–insecticide mixture treatments (9.4 days) ($t_{(4885.2)} = -3.78$, $p < 0.0001$, Table S4).

3.4. WHO bioassay insecticide susceptibility of adult An. gambiae s.l

3.4.1. Knockdown time (KDT_{50} and KDT_{95})

Following larval exposure to MP-only treatments, the deltamethrin knockdown time for the subsequent adult mosquitoes was increased (Table 4). Mosquitoes derived from larvae from site MS1 had a KDT_{50} of between 18.6 and 28.7 min and a KDT_{95} of between 68.4 and 106.0 min (to give a resistance ratio (RR) of between 1.0 and 1.5), while mosquitoes from site MS2 had a greater delay, with a KDT_{50} of between 26.7 and 48.6 min and a KDT_{95} of between 98.4 and 179.2 min (RR: 1.0–1.8). Adult mosquitoes derived from larvae exposed to the higher MP concentration, and from later larval generations, consistently exhibited longer knockdown times.

For larvae previously exposed to the MP–insecticide mixture treatments, knockdown times for adult mosquitoes also increased. Mosquitoes derived from larvae from site MS1 had a KDT_{50} of between 20.4 and 32.9 min and a KDT_{95} of between 75.3 and 121.3 min (to give a RR of

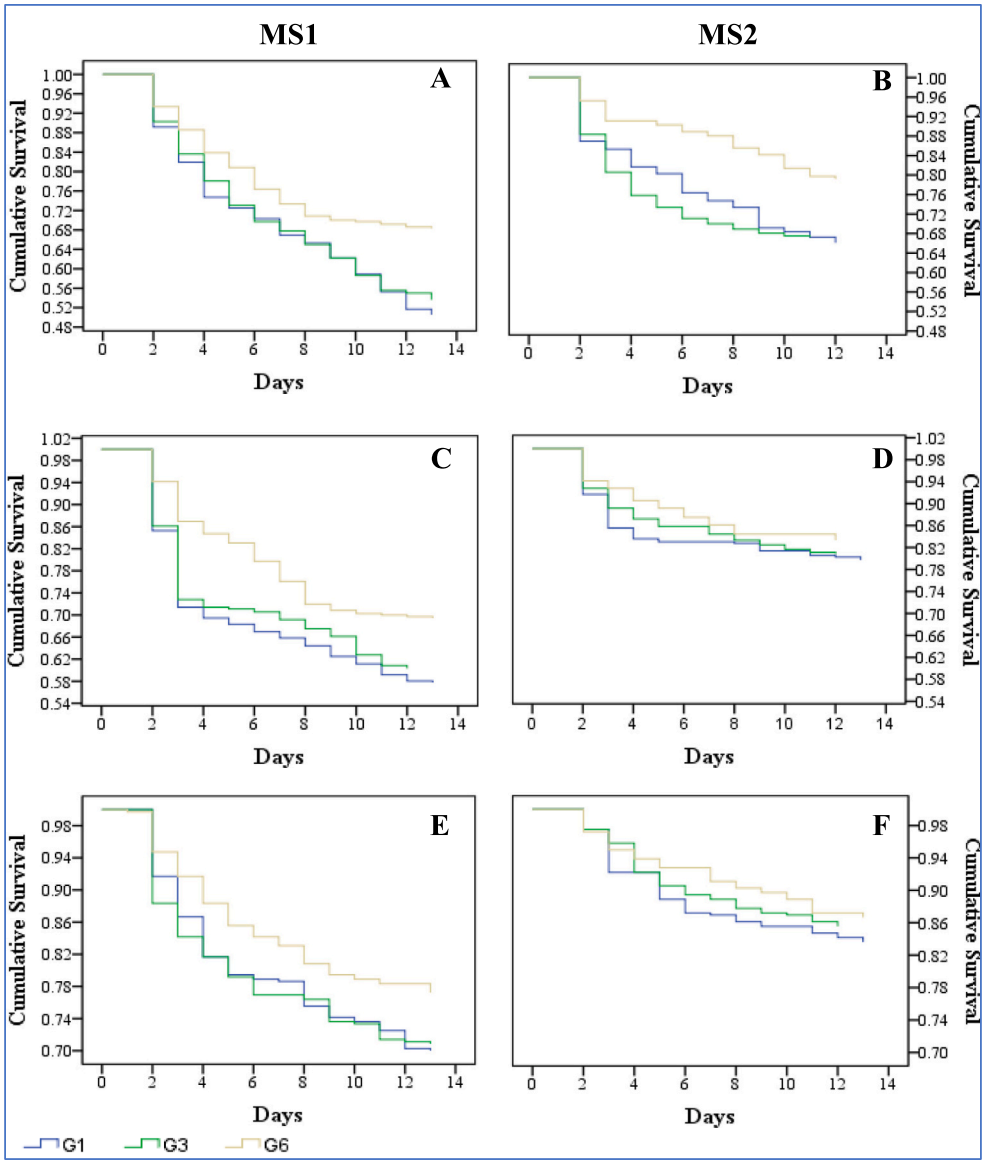

Fig. 4. Survival curves of larvae (originating from sites MS1 and MS2) exposed to insecticide (A and B), or an insecticide mixture containing MPs at 1 g/L mixture (C and D), or 10 g/L (E and F).

Table 3
The mean (± SD) development time (in days) of larvae exposed to MPs and an MP–insecticide mixture.

	MPs treatments	G1	G2	G3	G4	G5	G6
Site MS1	Control	9.0 ± 0.9	8.9 ± 0.9	8.9 ± 0.9	8.9 ± 0.9	8.9 ± 0.8	9.0 ± 0.9
	1 g/L MPs	9.7 ± 1.1	9.5 ± 1.1	9.4 ± 1.1	9.3 ± 1.0	9.0 ± 1.2	8.9 ± 1.1
	10 g/L MPs	10.0 ± 1.3	9.6 ± 1.3	9.5 ± 1.3	9.4 ± 1.3	9.3 ± 1.3	9.1 ± 1.4
Site MS2	Control	8.7 ± 1.0	8.5 ± 1.0	8.7 ± 1.1	8.5 ± 1.1	8.7 ± 1.1	8.6 ± 1.1
	1 g/L MPs	9.3 ± 1.1	9.3 ± 1.2	9.2 ± 1.1	9.1 ± 1.0	8.9 ± 1.4	8.7 ± 1.3
	10 g/L MPs	9.4 ± 1.2	9.4 ± 1.0	9.3 ± 1.2	9.3 ± 1.1	9.0 ± 1.0	9.0 ± 1.1

	Mixture treatments	G1	G3	G6
Site MS1	Insecticide	9.9 ± 1.2	9.6 ± 1.1	8.9 ± 1.3
	1 g/L MP-insecticide	9.5 ± 1.3	9.4 ± 1.4	8.9 ± 1.4
	10 g/L MP-insecticide	9.3 ± 1.1	9.2 ± 1.4	8.8 ± 1.3
Site MS2	Insecticide	10.0 ± 1.3	9.8 ± 1.6	9.3 ± 1.6
	1 g/L MP-insecticide	9.8 ± 1.3	9.8 ± 1.2	9.2 ± 1.4
	10 g/L MP-insecticide	9.6 ± 1.2	9.4 ± 1.5	9.1 ± 1.6

Table 4
Knockdown time (KDT_{50} and KDT_{95}) of adult female *An. gambiae* s.l. after 60 min exposure to insecticide.

	Adult mosquito knockdown time				
Site	Generation	Larval treatment	KDT_{50} (95% CI)	KDT_{95} (95% CI)	Resistance ratio
MS1	G0	Baseline	18.6 (15.3–22.4)	68.4 (56.2–84.0)	1.0
	G3	Control	20.4 (16.9–24.6)	75.3 (61.8–92.4)	1.1
		1 g/L MPs	26.2 (21.8–31.5)	96.5 (79.3–118.7)	1.4
		10 g/L MPs	27.6 (22.9–33.2)	101.9 (83.7–125.2)	1.5
	G6	Control	18.9 (15.6–22.9)	69.6 (57.1–85.7)	1.0
		1 g/L MPs	27.8 (23.2–33.4)	102.6 (84.4–126.0)	1.5
		10 g/L MPs	28.7 (23.9–34.5)	106.0 (87.1–130.3)	1.5
MS2	G0	Baseline	26.7 (22.2–32.0)	98.4 (81.0–120.7)	1.0
	G3	Control	28.5 (23.7–34.1)	104.9 (86.4–128.8)	1.1
		1 g/L MPs	40.1 (33.9–47.1)	147.6 (132.2–171.5)	1.5
		10 g/L MPs	42.7 (35.4–52.4)	157.0 (126.8–195.9)	1.6
	G6	Control	27.8 (23.1–33.3)	102.4 (84.3–125.6)	1.0
		1 g/L MPs	48.6 (40.2–58.9)	179.2 (145.2–224.1)	1.8
		10 g/L MPs	48.9 (40.5–59.2)	180.2 (146.1–225.3)	1.8
MS1	G3	Control	20.4 (16.9–24.6)	75.3 (61.8–92.4)	1.1
		Insecticides	24.1 (20.0–29.1)	89.0 (73.2–109.4)	1.3
		1 g/L Mixture	24.6 (20.4–29.6)	90.7 (74.6–111.3)	1.3
		10 g/L Mixture	28.8 (23.9–34.6)	106.1 (87.2–130.4)	1.5
	G6	Insecticide	29.7 (24.7–35.7)	109.5 (90.0–134.6)	1.6
		1 g/L Mixture	30.3 (25.2–36.3)	111.6 (91.7–137.2)	1.6
		10 g/L Mixture	32.9 (27.4–39.6)	121.3 (99.4–149.5)	1.8
MS2	G3	Control	28.5 (23.7–34.2)	105.0 (86.4–129.0)	1.1
		Insecticide	45.1 (37.4–54.6)	166.3 (135.1–207.4)	1.7
		1 g/L Mixture	44.8 (37.2–54.1)	165.2 (134.4–205.7)	1.7
		10 g/L Mixture	51.3 (42.3–62.4)	189.1 (152.7–237.5)	1.9
	G6	Insecticide	50.3 (41.5–61.2)	185.6 (150.0–192.8)	1.9
		1 g/L Mix	51.6 (42.5–62.9)	190.3 (153.5–239.1)	1.9
		10 g/L Mix	56.8 (46.5–69.4)	209.3 (167.8–264.6)	2.1

Resistance ratio = KDT_{50} for each treatment divided by the KDT_{50} of the baseline.

between 1.1 and 1.8); while mosquitoes derived from larvae from MS2 showed a greater response, with a KDT_{50} of between 28.5 and 56.8 min and a KDT_{95} of between 105.0 and 209.3 min (giving a RR of between 1.1 and 2.1). Larval exposure to the higher MP concentration, and adult mosquitoes derived from later larval generations, showed the greatest increases in knockdown times, particularly in mosquitoes originating from site MS2.

3.4.2. Adult mosquito mortality rate after 24 h of insecticide exposure

No significant differences in 24-h mortality were detected for adult mosquitoes derived from larvae at G3 exposed to MP-only treatments from either site (Fig. 5). However, mosquitoes derived from G6 larvae demonstrated reduced mortality (site MS1: $F_{(3,\ 28)} = 34.2$, $p < 0.0001$; site MS2: $F_{(3,28)} = 4.5$, $p < 0.05$). For larvae derived from site MS1, post hoc tests showed reduced mortality in adult mosquitoes following exposure to both the 1 g/L and 10 g/L MP treatments compared to the control ($p < 0.0001$), but only for the 10 g/L treatment for larvae from site MS2 ($p < 0.05$).

For larvae exposed to MP–insecticide mixture treatments, no significant differences in mortality of adult mosquitoes derived from G3 were observed. However, for adult mosquitoes derived from larvae at G6, significant effects appeared at both sites (MS1: $F_{(3,28)} = 41.3$, $p < 0.0001$; MS2: $F_{(3,28)} = 4.5$, $p < 0.05$). Mortality of adult mosquitoes from larvae at G6, which had been exposed to the two MP–insecticide mixture treatments and the insecticide only treatment was significantly lower than the control treatment (site MS1 $p < 0.0001$; site MS2 $p < 0.001$). Overall, 24-h mortality declined with increasing MP concentration and across generations, with MS2 showing the strongest indications of increased tolerance.

4. Discussion

This study provides novel evidence that multigenerational responses of mosquito larvae to MP exposure are influenced by their previous environmental pollution history. While similar responses have previously been demonstrated under controlled laboratory conditions (Shilla et al., 2024), the stronger responses observed in larvae originating from the more contaminated site suggest that real-world pollution exposure may precondition mosquito populations to subsequent MP stress. These findings advance current understanding of how multigenerational MP exposure interacts with environmental pollution gradients to shape phenotypic stress tolerance in an important disease vectors.

4.1. Spatial gradients of micropollutants and subsequent association with insecticide tolerance

The upstream–downstream gradient along the Msimbazi River reflects increasing urban and industrial inputs, consistent with previous assessments of this catchment and, more generally, urban African rivers (Chanzi, 2017; Lema, 2025). Higher concentrations of metals, pesticides, nutrients and microplastics at MS2 indicate substantially greater chemical contamination of the downstream mosquito breeding environment. PCA further illustrated this strong multivariate separation between MS1 and MS2. However, because many pollutants covaried strongly with site, their individual contributions cannot be disentangled from the overall MS1–MS2 contrast. The greater insecticide tolerance observed in mosquitoes originating from MS2 is therefore consistent with, but does not establish, environmental pre-conditioning by pollution. Chronic exposure to complex pollutant mixtures may influence physiological stress-response and detoxification pathways that overlap with mechanisms of insecticide tolerance (Matiya et al., 2019; Owusu-Asenso et al., 2025). Thus, these findings indicate an association between contrasting environmental histories and mosquito tolerance rather than independent effects of particular pollutants.

4.2. Effects of exposure to MPs on survival and development time of mosquito larvae

In this study, MPs initially reduced larval survival and extended development time, consistent with previous evidence that MP

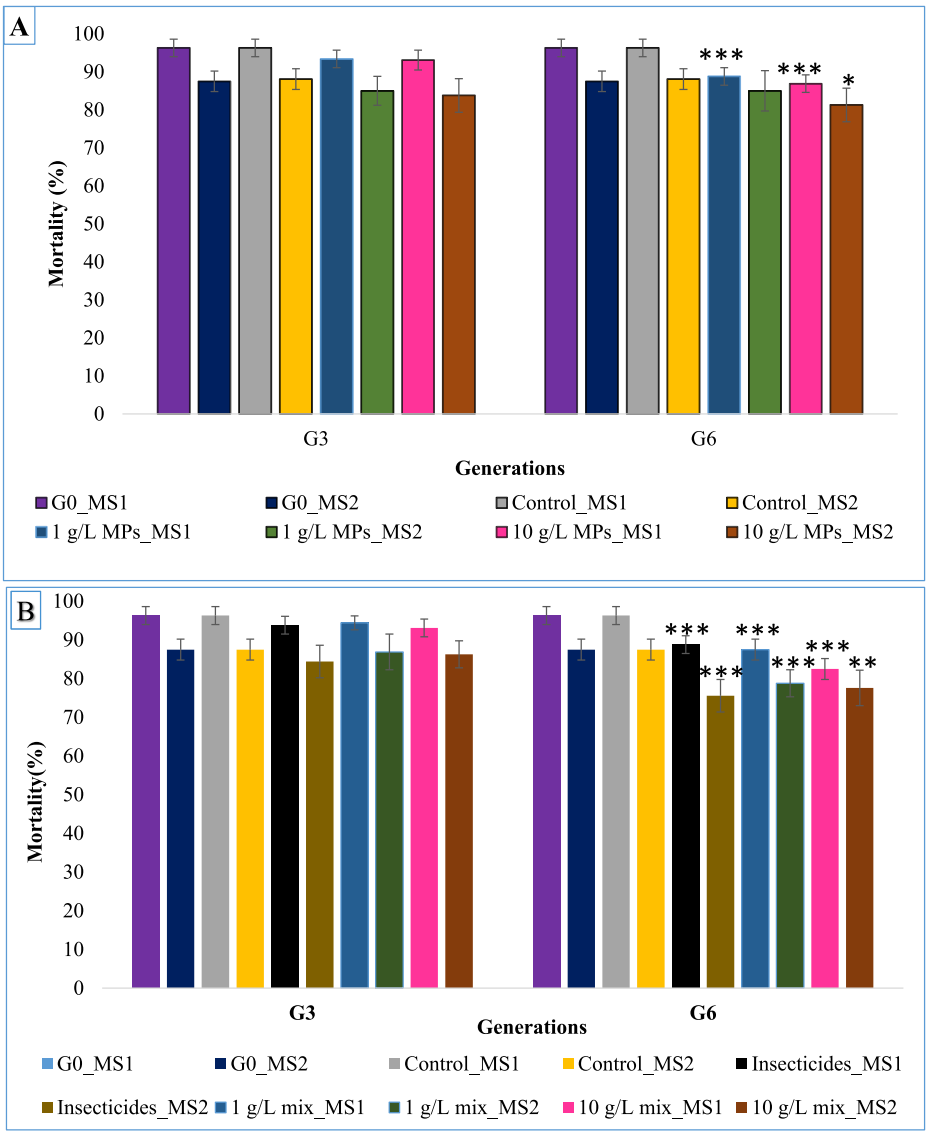

Fig. 5. Mean mortality rates of female *An. gambiae* s.l. reared from larvae (at either G3 or G6) exposed to MPs (A) or an MP–insecticide mixture (B). Larvae were originally collected from an upstream sampling site (MS1) or a downstream site located within a densely populated urban area (MS2). Error bars represent standard deviation. Asterisks denote significant differences between each treatment and the corresponding control (*$p < 0.01$, **$p < 0.001$, ***$p < 0.0001$), general linear model tests.

accumulation can obstruct the larval intestinal tract and reduce energy intake (Li et al., 2024). Yet, with continued exposure across successive generations, larvae exhibited progressively higher survival and shorter development times, indicating progressive multigenerational changes in phenotypic responses to MP exposure. Notably, this adaptive response was most pronounced in larval populations originating from the highly polluted MS2 site, underscoring the role of environmental pre-conditioning by complex pollutant mixtures in enhancing resilience to emerging stressors such as MPs. Morphological adjustments may have contributed to this tolerance, with continual MP exposure potentially selecting for larvae with larger or more efficient guts, thereby improving nutrient assimilation despite MP obstruction and facilitating more effective egestion (Griffin et al., 2023). Evidence from other invertebrates supports this pattern, showing that multigenerational exposure to MPs can activate robust biochemical and genetic stress pathways, including upregulation of oxidative-stress enzymes (Soares et al., 2023), and overexpression of heat shock proteins and superoxide dismutase (Caballero-Carretero et al., 2024; Abbas et al., 2025). Such responses likely underpin the enhanced physiological performance that was observed in subsequent larval generations.

4.3. *Interactive effects of microplastics and insecticides on larval survival and development time*

Larval survival was higher following exposure to MP–insecticide mixture than in insecticide-only treatments, particularly at the higher MP concentration and in larvae at later generations. Importantly, this pattern supports the hypothesis that MPs may reduce effective insecticide bioavailability (e.g., through sorption to plastic surfaces), while simultaneously inducing stress-response pathways that enhance larval tolerance (Li et al., 2024). Microplastics possess large and reactive surfaces that readily sorb both polar and non-polar larvicides (Liu et al., 2022; Rushdi et al., 2023), likely lowering the freely dissolved insecticide fraction available for uptake. Across the selected generations evaluated, exposure to MP–insecticide mixture was associated with increased survival and shortened development time, suggesting progressive changes in phenotypic tolerance to insecticide exposure (Zoh et al., 2021; Shilla et al., 2024). Therefore, our study extends the

emerging evidence that MPs may influence insecticide efficacy and alter survival and developmental trajectories across generations in a major malaria vector, further underscoring the growing relevance of microplastics in insecticide tolerance dynamics within polluted urban environments. However, molecular identification of sibling species within the *An. gambiae* complex was not performed; therefore, differences in species composition between MS1 and MS2 cannot be excluded as a potential contributor to the observed inter-site variation in deltamethrin tolerance.

4.4. Susceptibility of adult An. gambiae sl to insecticide treatments

Deltamethrin assays showed a clear, larval generation-dependent rise in tolerance among adult mosquitoes derived from larvae exposed to MPs or MP–insecticide mixture; with the strongest effect coming from adult mosquitoes derived from larvae from the highly polluted site (MS2). Mortality was markedly reduced in adults derived from G6 larvae, especially those larvae exposed to the higher concentration of MP (10 g/L), indicating that repeated larval exposure to MPs was associated with progressively reduced susceptibility to deltamethrin (Shilla et al., 2024). Gradients of waterborne pollutants and greater exposure to downstream environmental pollution may precondition larval populations to subsequent chemical stress, with MPs acting as an additional environmental stressor. Although physiological mechanisms involved in coping with MPs may overlap with pathways associated with insecticide resistance, including detoxification, oxidative-stress responses and metabolic adjustments (Al Naggar et al., 2025), these mechanisms were not directly measured in the present study. Our findings therefore indicate increased phenotypic tolerance rather than genetically based insecticide resistance. Moreover, because we did not conduct a de-selection experiment, it remains unclear whether the increased tolerance would persist or reverse after cessation of MP exposure. Nevertheless, repeated MP exposure may induce physiological responses that also enhance tolerance to deltamethrin, suggesting a potential cross-tolerance mechanism that warrants further molecular and genetic investigation.

4.5. Implications for environmental management and urban vector control

The findings of this study have significant implications for malaria vector control in rapidly urbanising environments, where aquatic pollution and disease transmission increasingly converge. Larval habitats, especially polluted urban waters, are frequently overlooked in relation to insecticide resistance development in malaria vectors. Our findings suggest that polluted mosquito breeding environments may precondition mosquito populations for increased insecticide tolerance prior to adult-stage exposure. This has important implications for urban vector control, as it suggests that environmental stressors in larval habitats may contribute to reduced insecticide susceptibility before adult mosquitoes encounter insecticide-based interventions, potentially influencing the effectiveness of indoor residual spraying and insecticidal bed nets (Otolorin et al., 2025; Oxborough et al., 2024). Therefore, persistent chemical stress in polluted larval habitats may contribute to elevated phenotypic insecticide tolerance, even without direct insecticide exposure during adult control interventions (Liu et al., 2026).

Microplastics represent a growing but largely unrecognised challenge for urban malaria control (Jones et al., 2024; Li et al., 2024). Unlike regulated chemical pollutants, microplastics are persistent, poorly governed, and continue to accumulate in urban freshwater systems that serve as mosquito breeding habitats. Importantly, microplastics represent a new class of environmental stressors that can indirectly shape insecticide susceptibility, even in the absence of direct insecticide exposure. Reduced insecticide effectiveness in the presence of microplastics suggests that they can lower the effective concentrations of larvicides used to control larvae in known mosquito breeding sites. The findings of this study indicate that microplastic-contaminated environments can weaken larvicidal performance and elevate baseline tolerance, potentially reducing the long-term effectiveness of interventions focused on adult mosquitoes (Otolorin et al., 2025). Despite these implications, current insecticide-resistance management frameworks do not explicitly consider microplastics as contributors to resistance risk, highlighting a critical gap in urban vector-control planning (WHO, 2022).

5. Conclusion

Micropollutant gradients in the Msimbazi River, particularly elevated concentrations of pesticides, metals, nutrients, and microplastics are strongly associated with increased deltamethrin tolerance in *Anopheles gambiae* s.l. Multigenerational exposure experiments demonstrated that microplastics initially reduced larval performance, followed by higher survival, shorter development times and increased adult insecticide tolerance in later generations. These effects were most pronounced in mosquitoes collected from the more polluted site, suggesting potential environmental preconditioning for enhanced phenotypic tolerance, although differences in sibling-species composition between sites cannot be excluded as a contributing factor. These findings provide evidence that chronic, multigenerational microplastic exposure, in combination with real-world pollution histories, can influence both larval traits and adult insecticide susceptibility. Enhancing wastewater treatment, reducing industrial and domestic effluent discharge, and limiting plastic inputs into urban waterways may therefore help reduce environmental conditions associated with elevated insecticide tolerance. Therefore, incorporating pollution metrics into insecticide-susceptibility surveillance may also help clarify spatial variation in mosquito responses to insecticides across urban landscapes. In summary, effective malaria control in urban environments requires a broader environmental perspective. Addressing aquatic pollution alongside conventional vector-control interventions will be essential to maintain insecticide efficacy and mitigate unintended effects of polluted urban breeding habitats on insecticide tolerance.

CRediT authorship contribution statement

Dativa J. Shilla: Writing – original draft, Supervision, Conceptualization. **Deokary Joseph Matiya:** Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization. **Nyanda Laini Nyamandito:** Writing – review & editing, Investigation. **Richard S. Quilliam:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2026.182325.

Data availability

Data will be made available on request.

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