Environmental Toxicology

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RESEARCH ARTICLE OPEN ACCESS

Antimicrobial Residues in a Key Cerrado River: Distribution, Persistence, and Effects on Zebrafish Embryo-Larval Development

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Received: 9 September 2025 | **Revised:** 26 March 2026 | **Accepted:** 3 April 2026

Keywords: Cerrado | contaminants of emerging concern | *Danio rerio* | metronidazole | sulfamethoxazole

ABSTRACT

The current study aimed to quantify the antimicrobials amoxicillin (AMX), cefazolin (CFZ), chloramphenicol (CHL), metronidazole (MTZ), and sulfamethoxazole (SX) in effluents and surface water of an important Cerrado river, calculate their half-lives, and analyze their ecotoxicity following single and combined exposures. Four sampling surveys were carried out to identify the antimicrobials employing HPLC-MS/MS. Surface water was collected from the river's spring to the mouth, and effluent samples were taken at a sewage treatment plant (STP). The *pySiRC* tool was used to predict the half-life of antimicrobials in aqueous media under •OH-attack. Ecotoxicity was assessed using a zebrafish embryo-larval toxicity assay at environmentally relevant concentrations of the detected antimicrobials. Just MTZ (0.1 to 45.5 ng L^{-1}) and SX (0.1 to 502.7 ng L^{-1}) were detected in this study. The persistence of MTZ and SX in the aqueous environment was estimated at 14 to 139 days and 9 to 88 days, respectively. The single exposure to MTZ induced cardiotoxicity and changes in the swim bladder and tail curvature. MTZ and SX induced sensory and physiological morphometric changes compared to the control. MTZ, alone or combined with SX, affected the larvae's behavior. Our findings contribute to the understanding of the presence, persistence, and ecotoxicity of antimicrobials in aquatic environments.

1 | Introduction

Contaminants of emerging concern (CECs) are synthetic or naturally occurring chemicals that pose concerns for the environment and human health. The presence and impacts of CECs were only recently recognized; their concentrations are increasing in environmental systems, and they are not yet adequately regulated or monitored [1–3]. CECs cover active pharmaceutical ingredients, including antimicrobials [4]. Estimated antimicrobial consumption in 67 countries studied increased 16.3% from 2016 to 2023, from 29.5 to 34.3 billion defined daily doses (DDDs). The increases were most pronounced in upper-middle-income and lower-middle-income countries. By 2030, without reductions in developing nations,

such as investments to improve infrastructure, especially water and sanitation, along with improved access to vaccination, global antibiotic consumption is projected to increase by 52.3%, reaching 75.1 billion DDDs [5].

Since 2010, antimicrobials have been sold with a medical prescription in Brazil. However, excessive and often inappropriate antimicrobial prescription and self-medication are still common practices in the country [6]. Data from the World Health Organization (WHO) showed that Brazil consumed 22.75 DDD of antimicrobials per 1000 inhabitants per day in 2016, equivalent to 2225.47 metric tons, making it the country with the highest consumption in tons among the 65 countries analyzed [7]. In 2021, consumption increased by 26% in hospital environments [8]. From January 2014 to July 2021, approximately 800 million packages containing antimicrobials were sold in pharmacies and drugstores in Brazil [9].

The contamination of aquatic environment with antimicrobials can occur by (i) sewage treatment plants, since they are not entirely removed in wastewater treatment, after improper medication disposal, human excretion, or pharmaceutical manufacturing; (ii) untreated effluent improperly disposed of directly in rivers; (iii) water runoff, since antimicrobials are used in livestock, aquaculture, and crop production to promote growth or treat diseases [10–12]. The presence of antimicrobials in environmental matrices has been studied worldwide, including in the United States [13], Europe [14], and Asia [15]. However, in Latin America, data on antimicrobials in water remain scarce [4, 16–18]. In Brazil, antimicrobials have already been detected in the aquatic environment in the states of Maranhão [19], Minas Gerais [20, 21], Rio Grande do Sul [22–25], Rio de Janeiro [26], São Paulo [27, 28], and Goiás [29]. These compounds have typically been found in aquatic environments at concentrations ranging from $ng\,L^{-1}$ to $\mu g\,L^{-1}$ [30].

Residues, metabolites, and degradation products of antimicrobials can cause environmental health risks [31], including ecotoxic effects to fish [32, 33], microcrustaceans [34], algae, and plants [35–37], even at low concentrations [38, 39]. Antimicrobials in aqueous environments can alter developmental, cardiovascular, metabolic, antioxidant, and immunological responses across various organisms [33, 40]. Most pharmaceutical products are partially degraded in the environment, and their half-lives remain unclear [41], underscoring the need to predict their persistence. Computational modeling can be a useful tool for understanding the degradation kinetics of antimicrobials in the aquatic environments [42, 43].

An important river in the Cerrado biome has been a target of the discharge of emerging pollutants [44]. The Meia Ponte River Basin is located in the south-central region of the Goiás State, encompassing 39 municipalities. Although it concentrates only 4.2% of the State's territory, approximately 40% of Goiás' population lives on the Meia Ponte river's bank, including Goiânia (the State's capital) and several important municipalities, industrial and agro-industrial hubs (around 3 356 708 inhabitants). As a result, 82% of Goiás municipalities discharge their sewage and wastewater into the Meia Ponte River, thereby increasing the risk of antimicrobial presence [45, 46]. The problem is exacerbated because the same river that collects treated sewage is also used for various anthropogenic activities, including public water supply, industrial use, irrigation, fishing, leisure, and tourism [46].

The presence of antimicrobials, namely sulfamethoxazole (SX; ≥ 1 ng/L), metronidazole (MTZ; < 0.5 ng/L), and chloramphenicol (CHL; < 5 ng/L), has been reported in another Cerrado river [29]. Nevertheless, the ecotoxicity of antibiotics at environmentally relevant concentrations on aquatic organisms is poorly understood. Thus, the present study aimed to quantify five antimicrobials, namely amoxicillin (AMX), cefazolin (CFZ), CHL, MTZ, and SX, in effluents and surface water of the Meia Ponte River in the Brazilian Midwest, calculate their half-lives under •OH-attack, and analyze their ecotoxicity on developing zebrafish after single and combined exposure. Therefore, the hypothesis that the antimicrobials are dispersed and persistent in the Meia Ponte River and that the environmentally relevant concentrations of the antimicrobials are toxic for zebrafish was assessed.

2 | Material and Methods

2.1 | Study Area and Sampling

Sewage and surface water samples were collected at a sewage treatment plant in Goiânia (STP Dr. Hélio Seixo de Britto) and along the Meia Ponte River in Goiás State, Brazil, totalizing 303.7 km (Figure 1). Eight collection points were defined from the spring (P1) to the river's mouth (P8), with sewage samples defined as P4 and P5 (Figure 1 and Table S1). Four collections were carried out in 2022 during the two well-defined seasons in the Brazilian Midwest, namely the rainy and dry seasons, in the following months: February (rainy), May (dry), August (dry), and November (rainy). The methodology of Fagundes et al. [47] and Bolívar-Subirats et al. [48] was applied for sample collection and storage. Two liters of surface water and effluents were collected using a stainless-steel bucket with a capacity of 5 L, attached to a rope approximately eight meters long, at a depth of about 25 to 45 cm. The samples were stored in sterile amber glass bottles and transported under refrigerated conditions at 4°C. The map was created and analyzed in QGIS (version 3.34.3), an open-source geoprocessing software, which included the political and ecosystem limits provided by the Brazilian Institute of Geography and Statistics (IBGE) and the Goiás State Geoinformation System (SIEG).

2.2 | Quantification of Antimicrobials

The sample preparation for HPLC-MS/MS was conducted according to Dos Santos et al. [29] with some modifications. Briefly, samples were filtered through 0.45 μm Nitrocellulose membrane filters with 47 mm diameter (GVS North America, Stanford, USA). Approximately 150 mL of each sample was stored at −80°C for a maximum of 24 h after collection to maintain stability, and then the material was lyophilized. The powder residue was resuspended in methanol three times, each time with 0.5 mL added with homogenization, totaling 1.5 mL. This suspension was transferred to a clean microtube and vacuum-dried

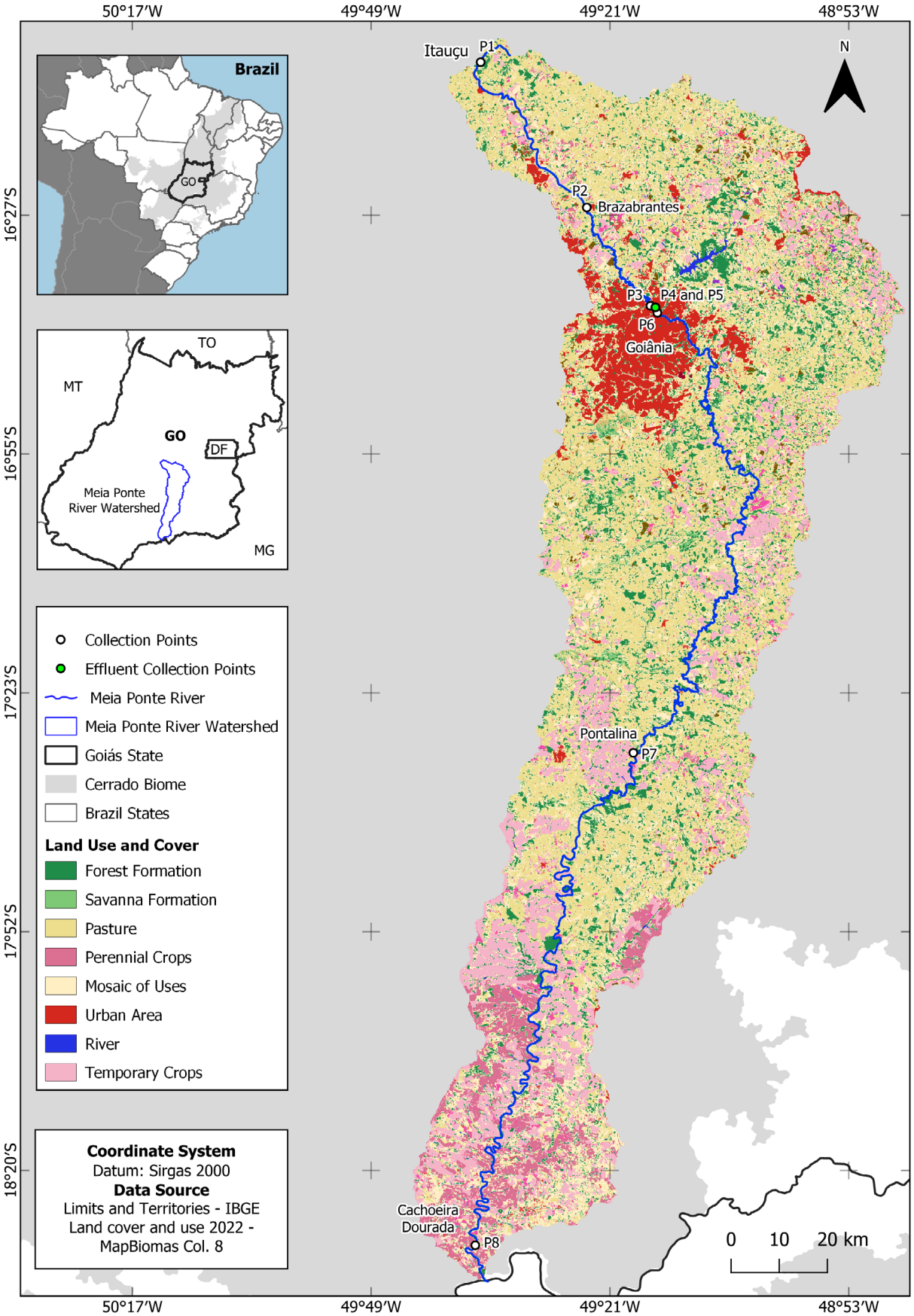

FIGURE 1 | Collection points for effluents and surface water from the Meia Ponte River in Goiás State, Brazilian Midwest. To locate the river globally, inset panels with maps of Brazil, Goiás State, and the Meia Ponte River watershed are shown in the left corner. Land use and cover are also depicted. The area of the original Cerrado biome is represented in gray.

in the SpeedVac. After drying, the sample was resuspended in 100 μL of mobile phase, followed by vortexing for 5 min. It was then centrifuged at 18 800 × g for 10 min at 8°C. Finally, 60 μL of the supernatant was transferred to the insert and injected into the chromatographic system.

The antimicrobials to be identified were selected based on a fast-screening procedure proposed by [49]. The analyses were performed on an ExionLCTM HPLC system (Sciex) coupled to the triple quadrupole mass spectrometer (MS/MS) QTRAP 4500 (Sciex). Chromatographic separation was performed using a Kinetex C8 reversed-phase column (150 × 4.6 mm, 5 μm particle size) equipped with a Phenomenex pre-column (4 × 3 mm) in a column oven at 35°C. The mobile phase used was a mixture of 10 mM ammonium formate with 0.2% formic acid:methanol:acetonitrile (80:15:5, v/v/v), at a flow rate of 0.8 mL min, with a total chromatographic run time of 20 min and an injection volume of 20 μL.

An electrospray ionization (ESI) source was used. The ions were monitored in positive and negative ionization modes depending on the analyte. Two transitions were selected for each antimicrobial, one for quantification and the other for confirmation. Data acquisition parameters, such as ion-pair monitoring, declustering potential (DP), collision energy (CE), entry potential (EP), and collision cell exit potential (CXP), were optimized for each antimicrobial, as presented in the Table S2.

Linearity was determined through calibration curves constructed from chromatographic peak areas versus standard concentrations, with adequate precision, accuracy, and excellent correlation coefficients ($r > 0.99$) for all target antimicrobials, as depicted in Tables S3 and S4. Selectivity was assessed by analyzing a blank sample without antibiotics. There was no interference at the retention times of the target antibiotics (Figure S1). In addition, limits of detection (LOD) and quantification (LOQ) were calculated using the standard deviation and slope of the curve obtained during the linearity test, as outlined in Equations (1 and 2).

$$\mathrm{LOD} = 3.3\,\sigma / S \tag{1}$$

$$\mathrm{LOQ} = 10\,\sigma / S \tag{2}$$

where σ is the standard deviation of intercept and S is the slope average of the curve.

2.3 | Molecular and Kinetic Modeling of Studied Antimicrobials

The electronic structure properties of the antimicrobials were calculated at the M062X/6-31G+(d) level with the solvation model density (SMD). The local minimum stationary points were characterized by analytical harmonic frequency calculations. To evaluate the main sites of reactivity, the Fukui functions (f) of nucleophilic, electrophilic, and oxidative attack were calculated according to the equations $f^{+}_{NBO} \approx \rho^{HOMO}_{NBO} = \sum_i |c_i|^2_{HOMO}$, $f^{-}_{NBO} \approx \rho^{LUMO}_{NBO} = \sum_i |c_i|^2_{LUMO}$, and $f^{0}_{NBO} \approx \left(f^{+}_{NBO} + f^{-}_{NBO}\right)/2$, respectively. The Multiwfn package program [50] was used to study the topological and Fukui functions, where the contributions of the atomic orbitals of the frontier molecular orbital are weighted by the c_i coefficients of HOMO and LUMO orbitals. Quantum chemical calculations were carried out by Gaussian 16 package [51].

Reactivity indices allow for the direction of the calculation of the degradation mechanism of organic compounds. However, the computational cost involved is still Herculean for obtaining the kinetic parameters of this process [52]. To accelerate the process of obtaining these parameters, artificial intelligence procedures have become important allies: the persistence and potential environmental impact of antimicrobials can be approaching estimated computing the half-life from *py*SiRC machine-learning platform with the formula $t_{1/2} = ln2 / \left(k_{total} \times [^{\cdot}\mathrm{OH}]\right)$, where the kinetics parameters k_{total} represents the total reaction rate constant and [OH] denotes the concentration of hydroxyl radicals in aqueous media. The half-life of the reaction was examined at a temperature of 298.15 K, with $[^{\cdot}\mathrm{OH}]$ ranging from 10^{-16} to 10^{-17} mol L^{-1}, a range that typically corresponds to the values observed in surface waters [53, 54]. The hydroxyl radical—an archetypal system of degradation reactions—was selected to mimic the oxidation effect promoted by the oxygenation process in an aqueous environment.

2.4 | Ecotoxicity Assessment

2.4.1 | Zebrafish Embryo-Larval Toxicity Test (ZELT)

Adult *Danio rerio* (zebrafish) reproduction was induced at a 1:1 male-to-female ratio using an AB strain and placed in three separate spawning tanks to obtain the embryos. Five treatments were used for the zebrafish embryo exposure: metronidazole (MTZ, 15 ng L^{-1}), sulfamethoxazole (SX, 15 ng L^{-1}), metronidazole + sulfamethoxazole (MIX, 15 ng L^{-1} each), negative control (NC, reconstituted water), and positive control (PC, 4.0 ng L^{-1} 3,4-dichloroaniline) (Figure 2). The antimicrobial concentrations were defined based on the quantification data obtained from this study with that collected at the Meia Ponte River (P6, Table 1). The embryos were transferred to Petri dishes with reconstituted water [55] and assessed for viability within 4 h post-fertilization (hpf) using a stereomicroscope. Viable embryos were selected and distributed into microplates for each treatment. The tests were conducted in triplicate for 144 h, with 30 embryos per condition. They were incubated in a controlled environment (BOD incubator), with constant temperature (26°C ± 0.5°C), pH (7.2), and photoperiod (14:10 h light/dark cycles). The test was validated when the fertilization and survival rates of the negative control exceeded 90%.

During the exposure period (144 h), mortality rate, hatching rate, and morphological changes were monitored daily using an Axiolab microscope (Zeiss) coupled to an Axiocam 105 color camera and Software ZEN 2.6 blue edition (Figure 2). Spontaneous contractions per minute were recorded after 24 h post-exposure (hpe) to identify neurotoxicity, while heartbeats per minute were analyzed after 48 hpe to assess cardiotoxicity. The experiment followed the OECD 236 guidelines [56] and was performed as described by Pereira et al. [57].

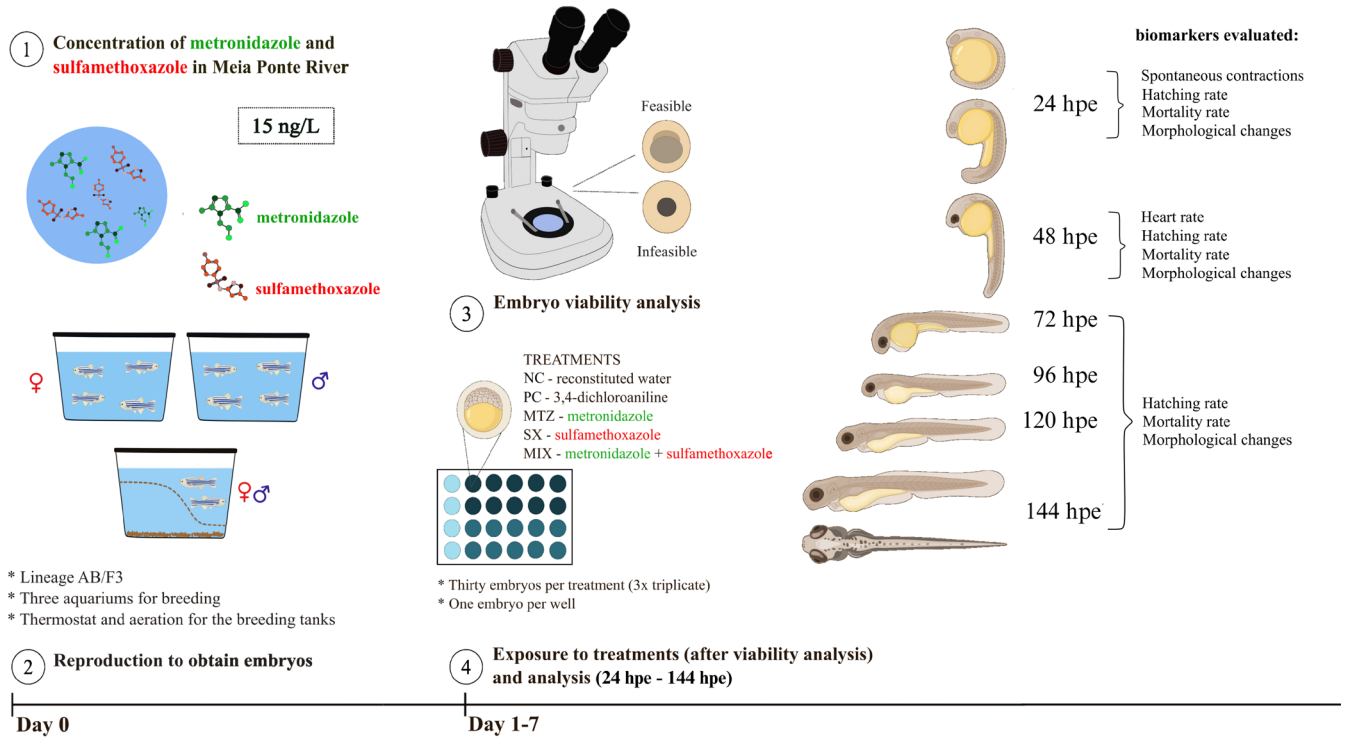

FIGURE 2 | Illustrative scheme of the experimental design of the zebrafish embryo-larval toxicity test (ZELT). The zebrafish embryos were exposed to environmentally relevant concentrations (15 ng L^{-1}) of metronidazole and sulfamethoxazole, isolated or in a mixture, and multiple biomarker responses were analyzed along 144 h post-exposure (hpe). NC, negative control; PC, positive control; MTZ, metronidazole; SX, sulfamethoxazole; MIX, metronidazole and sulfamethoxazole; AB/F3, Indicates that the zebrafish belong to the AB lineage and are in the third generation of laboratory breeding.

2.4.2 | Behavioral Analysis

At the end of the exposure period (144 hpe), zebrafish larvae were placed in a 12-well microplate with 4 mL of reconstituted water to be analyzed for 60 s using a chamber system, as previously reported by Santos et al. [58]. The environment was carefully controlled to ensure reliable results, including maintaining silence, a stable temperature, and optimal lighting. The analysis and capture chamber has high resolution and is positioned inside a box to assist with capturing images. The illumination was manually controlled to ensure a shadow- and reflection-free image, preventing interference with the analysis. The larvae were filmed for 60 s using the MatLab software. The behavior of all surviving larvae of the experiment was filmed, and data from 15 larvae were used in the analyses. Data extraction was carried out using the information generated by MatLab, including total distance traveled (mm/s), time spent in the periphery (mm/s), average speed (mm/s), and maximum speed (mm/s).

2.4.3 | Morphometric Analysis

After the behavior test, the larvae were euthanized by hypothermia, fixed by immersion in 4% paraformaldehyde, washed with 0.2 M phosphate-buffered saline (PBS) solution at pH 7.2 for 4 h, and stored in 70% ethanol at 4°C for morphometric analyses. Subsequently, the larvae were photographed using a Stemi 508 photomicroscope coupled with an image capture system (ZEISS—Axiocam 105 color) and ZEN 2.6 software. The photographs were taken in dorsal and lateral views. Morphometric analyses were conducted with five larvae per replicate ($n = 15$ per experimental group). The larvae were measured using ImageJ software (Figure S2). Individual measurements for each larva were normalized to their total length (from the head tip to the final portion of the notochord). Additionally, morphometric alterations were classified and analyzed according to three categories: (i) sensory measurements—eye area, minimum interocular distance, and maximum interocular distance; (ii) physiological measurements—swim bladder area and yolk sac/liver area; and (iii) skeletal/muscular measurements—mouth-to-anus distance, head length, head width, head height, and total length (Figure S2) [59].

2.5 | Statistical Analysis

Initially, the data were submitted to the Shapiro–Wilk and Levene tests to verify normality and homoscedasticity, respectively. When violating the normality assumption, Kruskal–Wallis was performed with Dunn's post-test. ANOVA was performed with Tukey's post-test for survival and hatching parameters, then compared using the Log Rank test. Descriptive analysis and the Kruskal–Wallis test with Dwass–Steel–Critchlow–Fligner multiple comparisons were used to evaluate the behavior analysis results. Results were considered significant when $p < 0.05$. Graphs were obtained using GraphPad Prism software, while statistical analyses were performed using RStudio software (version 3.5.2) and PCA with significant variables.

3 | Results

3.1 | Antimicrobial Detection

Two (MTZ and SX) of the five evaluated antimicrobials were detected in the STP and along the Meia Ponte River surface water (Table 1). MTZ and SX were detected at all collection points, with values ranging from 0.1 to 45.5 ng L^{-1} and 0.1 to 502.7 ng L^{-1}, respectively. Their highest values were found at P4 (MTZ: 45.5 ng L^{-1}; SX: 502.7 ng L^{-1}) and P5 (MTZ: 43.9 ng L^{-1}; SX: 316.8 ng L^{-1}) at STP Dr. Hélio Seixo de Britto. Quantification of antimicrobials in P4 and P5 was similar, and in P6 (MTZ: 14.5 ng L^{-1}; SX: 36.3 ng L^{-1}) was higher than in P3 (MTZ: 0.3 ng L^{-1}; SX: 0.7 ng L^{-1}), indicating that the removal of antimicrobials in primary treatment is inefficient, and they are dumped into the water bodies of the Meia Ponte River. However, the values detected in P8 are low, suggesting a dilutional effect.

3.2 | Molecular and Kinetic Modeling of Studied Antimicrobials

To assess the degradation process of the antimicrobials under study, Fukui indices were computed. These functions are pivotal in elucidating the reactivity within chemical systems subjected to radical attacks. According to Fukui's formulation, higher values of f^0_{NBO} parameter are indicative of an increased likelihood of radical attacks. Figure 3 shows significant values of f^0_{NBO} indices for the selected atoms across the five antimicrobials investigated in this study.

The f^0_{NBO} values for the atoms S1, C22, C23, C24, and C21 in the AMX molecule, O2, O3, C11, N6, and C9 in the MTZ molecule, S2, O6, C23, C20, and C19 in the CFZ molecule, C13, N7, C14, C10, and C9 in the SX molecule, and O7, O6, C18, C14, and N9 in the CHL molecule are presented (Figure 3). These results suggest that regions with high aromaticity (benzene, carbonyl, and alkenes) and electronegativity (O, N, and S atoms) are the most susceptible to radical attacks, thereby indicating potential sites of reactivity within the molecular structure of the antimicrobials under investigation.

P*ySIRC*, a machine learning computational platform, simulated the half-life times for $^{\cdot}$ • OH-mediated oxidation. This calculation just provides an indicative of environmental persistence under assumed •OH exposure scenarios, thus guiding the accuracy of its quantification. Table 2 presents the half-life values for all the antimicrobials investigated in this study, ranging from 7 to 140 days. For the antimicrobials MTZ and SX, the observed half-life values ranged from 14 to 139 days and from 9 to 88 days, respectively (Table 2).

3.3 | Ecotoxicity Assessment Using Developing Zebrafish

No significant differences in the survival, hatching rate, and spontaneous contraction were found in the exposed groups (MTZ, SX, and MIX) when compared to the NC ($p > 0.05$; Figure 4A–C). However, zebrafish embryos exposed to single MTZ showed an increased heart rate compared with NC ($p < 0.0001$; Figure 4D), whereas a mixture of MTZ and SX attenuated the cardiotoxic effect compared with MTZ alone ($p < 0.0001$). For the PC group, all embryos died within 24 hpe; therefore, these data are not shown in the graphs presented in this section.

3.4 | Morphological Changes

Larvae treated with environmentally relevant concentrations of MTZ presented significant morphological alterations, such as the uninflated swim bladder and deformities in the curvature of the tail, compared to the NC (Figure 5A). These alterations were more pronounced with increasing exposure time to the antimicrobial. The first changes were observed after 72 hpe, intensifying up to 144 hpe (Figure 5B).

3.5 | Morphometric Parameters

Zebrafish larvae exposed to environmentally relevant concentrations of the antimicrobials MTZ or SX for 144 h presented morphometric changes compared to NC, mainly sensory and

TABLE 1 | Quantification (ng L^{-1}) of Metronidazole (MTZ) and Sulfamethoxazole (SX) using HPLC system (Sciex) coupled to the triple quadrupole mass spectrometer (MS/MS) from the collection points of surface water and effluents of the Meia Ponte River collected in 2022, in Goiás State, Brazilian Midwest.

	February (rainy)		May (dry)		August (dry)		November (rainy)	
Collection points	**MTZ**	**SX**	**MTZ**	**SX**	**MTZ**	**SX**	**MTZ**	**SX**
P1	nd	nd	nd	0.2	nd	nd	0.2	nd
P2	0.1	0.2	nd	0.2	0.1	0.2	0.3	0.2
P3	nd	0.2	0.1	0.7	nd	0.2	0.3	0.1
P4	45.5	156.0	18.2	177.0	43.0	502.7	32.5	300.2
P5	43.9	140.9	31.9	142.4	34.4	316.8	20.8	151.9
P6	12.7	23.5	10.9	8.6	14.5	14.7	11.5	36.3
P7	4.6	2.9	3.7	6.2	5.5	2.2	1.3	4.5
P8	1.0	5.9	nd	0.4	nd	0.1	0.4	nd

Abbreviation: nd, not detected.

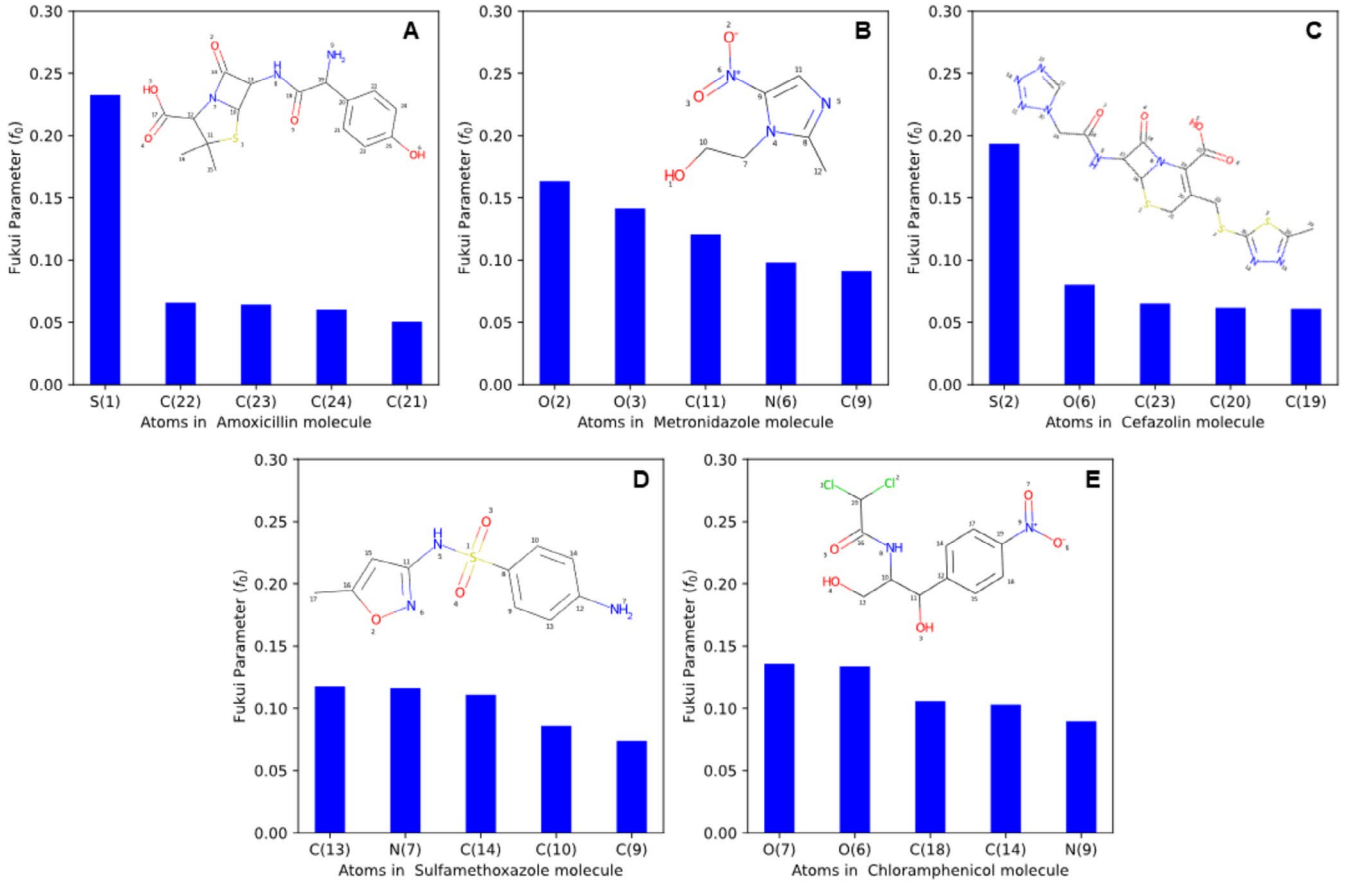

FIGURE 3 | Values of the Fukui function f^0_{NBO}, considering an oxidative attack on selected atoms of antimicrobials amoxicillin (AMX), metronidazole (MTZ), cefazolin (CFZ), sulfamethoxazole (SX), and chloramphenicol (CHL). (A) Atoms in amoxicillin molecule; (B) Atoms in metronidazole molecule; (C) Atoms in cefazolin molecule; (D) Atoms in sulfamethoxazole molecule; and (E) Atoms in chloramphenicol molecule.

TABLE 2 | Half-life time under ·OH oxidation predicted, in days, mimicking the degradation of antimicrobials amoxicillin (AMX), cefazolin (CFZ), chloramphenicol (CHL), metronidazole (MTZ), and sulfamethoxazole (SX) relative to the concentration of the OH radical at a temperature of 298.15 K, with [OH] ranging from 10^{-16} to 10^{-17} mol L^{-1}.

	Half-life (days)				
log([OH])	AMX	CFZ	CHL	MTZ	SX
−17.0	107	68	140	139	88
−16.5	34	21	44	44	28
−16.0	11	7	14	14	9

physiological alterations (Table 3). Larvae exposed to single MTZ or SX showed increased maximum interocular distance compared to those from the NC. Furthermore, larvae exposed to single SX showed an increase in the swim bladder area compared to NC, indicating a possible morphological alteration. Larvae exposed to SX showed an increased minimum interocular distance compared to those treated with MIX. Similarly, larvae exposed to MTZ or SX showed an increase in head width compared to those exposed to MIX ($p < 0.05$; Table 3), indicating that the mixture MTZ + SX can induce differential morphometric changes in zebrafish larvae in comparison with isolated exposure to MTZ or SX.

3.6 | Behavior Test

The exposure of zebrafish larvae to antimicrobials showed no significant difference for average speed (Figure 6A), maximum speed (Figure 6B), and the total distance moved (Figure 6C). On the other hand, the exposure of zebrafish larvae to MTZ, both alone or in a mixture with SX, reduced the time spent in the periphery in comparison with the negative control group ($p < 0.05$; Figure 6D).

4 | Discussion

The widespread use of antimicrobials has led to their increasing release and presence in freshwater ecosystems, posing risks to the Cerrado biome, the most diverse tropical savanna in the world. This study detected MTZ and SX at a Cerrado river spring, as recently observed in a low-order stream spring in the Brazilian Midwest [29]. This situation is concerning since springs are a pivotally important conservation target [60]. The highest values of MTZ and SX were observed in the STP (P4 and P5), with similar values in rainy or dry seasons. The primary treatment (P4) could not remove the antimicrobials (P5) that are dumped into the river (P6), indicating that urbanization contributes to the presence of the antimicrobials in the Meia Ponte River. These antimicrobials could be reintroduced into the population through the public water supply, as this river supplies water to 50% of Goiânia's population [46]. Moreover, the

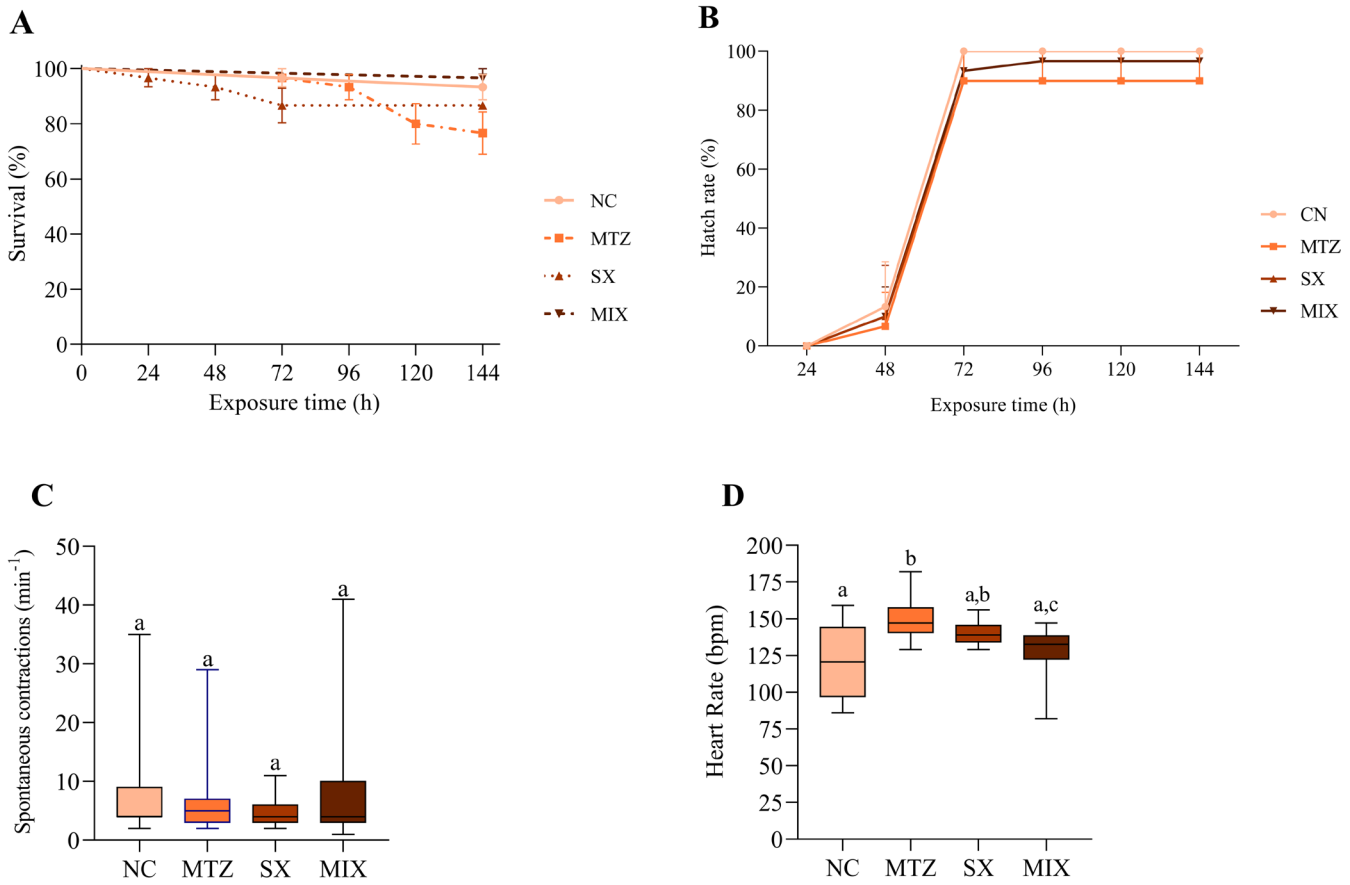

FIGURE 4 | Multiple biomarker analysis of zebrafish embryos and larvae during 144 h of exposure to environmentally relevant concentrations of metronidazole (MTZ) and sulfamethoxazole (SX) alone (15 ng L^{-1}) or in a mixture (MIX, 15 ng L^{-1} each). Survival (A), hatching rate (B), spontaneous contraction frequency (C), and heart rate (D). NC, negative control. Data are presented as mean ± standard deviation (SD). Statistical differences are indicated by different letters ($p < 0.05$).

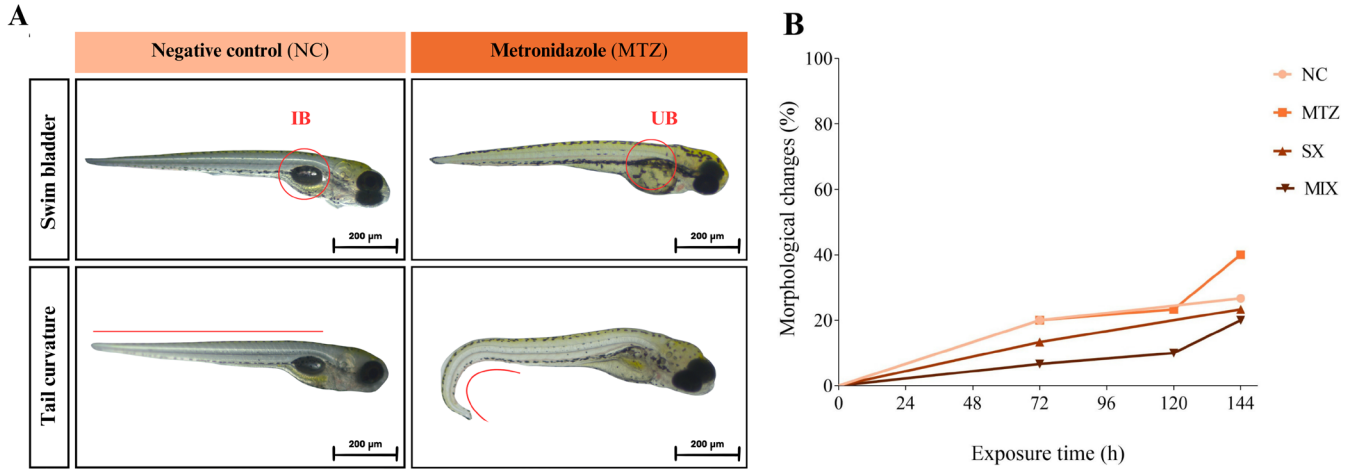

FIGURE 5 | Morphological changes of zebrafish larvae after exposure to environmentally relevant concentrations of the antimicrobials metronidazole (MTZ) and sulfamethoxazole (SX) alone (15 ng L^{-1}) or in a mixture (MIX, 15 ng L^{-1} each). (A) Representation of morphological changes observed in zebrafish larvae treated with MTZ at 144 h post-exposure (hpe). The most commonly observed morphological alterations were the absence of swim bladder inflation and tail curvature deformities. (B) The proportion of morphological changes (%) over the exposure time (24–144 hpe). IB, inflated swim bladder; NC, negative control group; UB, uninflated swim bladder.

presence of antimicrobials in natural environments is a major driver of antimicrobial resistance [61]. Another concern is the ecotoxicological risk of antimicrobials in surface water, which was investigated in this work.

The antimicrobial MTZ, detected in the STP and along the Meia Ponte River surface water (0.1–45.5 ng L^{-1}), belongs to the nitroimidazole group, widely used in human and veterinary medicine to treat anaerobic bacteria and protozoa [62].

TABLE 3 | Morphometric parameters of zebrafish larvae from the negative control group (NC) and after exposure to environmentally relevant concentrations of the antimicrobials metronidazole (MTZ) and sulfamethoxazole (SX), alone or in a mixture (MIX) for 144 h post-exposure.

Ratings	Morphological changes	NC	MTZ	SX	MIX
Sensory measurements	Eye area	0.095 ± 0.564a	1.359 ± 0.252a	1.424 ± 0.099a	1.266 ± 0.232a
	Minimum interocular distance	0.009 ± 0.007a	0.008 ± 0.001a	0.026 ± 0.005a,b	0.006 ± 0.001a,c
	Maximum interocular distance	0.015 ± 0.007a	0.028 ± 0.005b	0.026 ± 0.004b	0.022 ± 0.002a,b
Physiological measurements	Vitelline sac/liver area	3.253 ± 1.124a	4.381 ± 1.011a	4.324 ± 0.401a	4.340 ± 0.811a
	Swimming bladder area	0.791 ± 0.801a	1.312 ± 0.288a,b	1.778 ± 0.242b	1.472 ± 0.811a,b
Skeletal/muscle measurements	Head height	0.018 ± 0.002a	0.021 ± 0.003a	0.021 ± 0.001a	0.020 ± 0.001a
	Head width	0.017 ± 0.007a	0.024 ± 0.002a,b	0.024 ± 0.002a,b	0.021 ± 0.002a,c
	Head length	0.074 ± 0.100a	0.032 ± 0.006a	0.032 ± 0.006a	0.032 ± 0.001a
	Distance from mouth to anus	0.087 ± 0.009a	0.100 ± 0.013a	0.100 ± 0.013a	0.095 ± 0.001a
	Total length	0.146 ± 0.015a	0.168 ± 0.024a	0.168 ± 0.024a	0.162 ± 0.008a

Note: The results are expressed as mean ± standard deviation. Statistical differences are indicated by different letters ($p < 0.05$).

Although MTZ was recently detected in a low-order stream spring (< 0.5 ng L^{-1}) in Brazil [29], this study is the pioneer in detecting MTZ in an important Brazilian river (~15 ng L^{-1}). The highest concentration of MTZ found in a river was in Bangladesh (~40 000 ng L^{-1}) [4].

MTZ is poorly soluble in water but is hydrolytic and photostable [63]. It is considered recalcitrant to biodegradation [64], accumulating in the aquatic environment [65]. In this study, the predicted MTZ half-life in an aquatic environment under $^{\cdot}$ • OH oxidation was 14 to 139 days. This range aligns with values reported in the literature, which range from 14 to 227 days [66]. This high stability enabled detection of MTZ along the Meia Ponte River. Also, it made it listed as one of the seven pharmaceutical pollutants with the highest concentrations detected in the world's rivers, along with SX (also detected in this study), paracetamol, caffeine, metformin, fexofenadine, and gabapentin [4].

The impact of the MTZ on aquatic organisms is poorly known, but it was detected in fish (rainbow trout) muscle tissue (1.5 ng g^{-1}) collected in a northern Polish River [62]. Although the exposure of zebrafish embryos to environmentally relevant concentrations of MTZ and SX, either alone or in a mixture, did not induce changes in the survival, hatching rate, and spontaneous contraction, this study showed that the single exposure to MTZ promoted cardiotoxic effects (increased heart rate) on zebrafish embryos. This cardiotoxicity may be associated with increased reactive oxygen species (ROS), which can cause oxidative damage in cardiac tissue, as demonstrated in albino mice [67]. MTZ metabolites promote DNA fragmentation properties, as the original nitroimidazole ring is retained [68]. Moreover, substantial evidence indicates a hormetic response in the heart rates of fish embryos to contaminants, particularly antibiotics, microplastics, and herbicides. Low doses of these contaminants often promote tachycardia, while high doses induce bradycardia [69].

Regarding morphological changes, this study demonstrated that MTZ impaired swim bladder inflation and promoted tail curvature, which may affect the overall development, behavior, and survival of zebrafish larvae. MTZ can cause effects on the thyroid, increasing hypothyroidism [70, 71]. Thyroid damage has been involved with swim bladder inflation impairment in zebrafish [72–74]. The lack of swim bladder inflation can result in adverse changes in swimming behavior, as its buoyancy and stability will be impaired. Thus, this morphological change can cause irreversible damage, affecting the larvae's ability to swim in their habitat, search for food, and avoid predators [75, 76]. Likewise, the tail is a vital structure for fish, particularly in locomotion and efficient behavioral responses to their environment. Changes in tail curvature interfere with environmental exploration, predator escape, and other vital activities [75]. These findings align with other studies indicating that antimicrobials can impair the normal development of vital organs in fish [77, 78]. Furthermore, the increase in morphological changes with prolonged exposure highlights the risks associated with environmental contamination by MTZ, which was predicted to persist between 14 to 139 days in water. This persistence may lead to increasingly severe and frequent abnormalities in zebrafish development. As a result, these deformities can lead to exclusion and reduced reproductive success in adults, as well as hinder reproduction and long-term adaptation [78, 79].

SX, also detected in this study, is a sulfonamide antimicrobial used to treat bacterial infections, including urinary tract infections, bronchitis, and prostatitis, in humans, and is active against both Gram-negative and Gram-positive bacteria. It is also used in livestock and in the aquaculture industry to cure bacterial infections [80]. SX was detected in the Meia Ponte River and presented higher values in sewage (P4 and P5), exceeding 500 ng L^{-1}. In Brazil, SX was quantified in effluents and rivers of Maranhão (22–120 ng L^{-1}) [19]; São Paulo (0.78–106 ng L^{-1}) [27, 81]; Rio Grande do Sul (< 300 ng L^{-1}) [22, 23, 25]; Paraná,

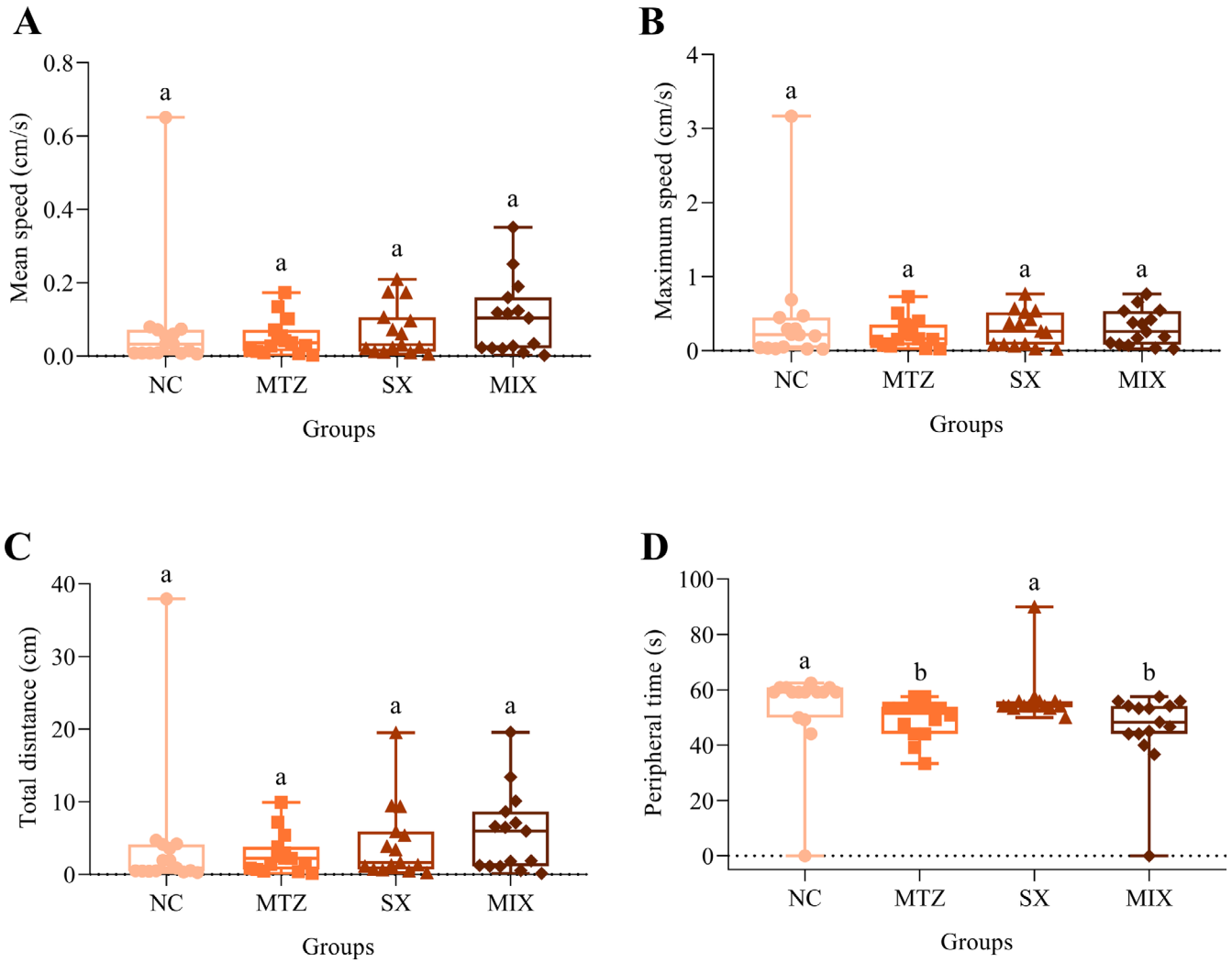

FIGURE 6 | Evaluation of the behavior of zebrafish larvae exposed to environmentally relevant concentrations of the antimicrobials metronidazole (MTZ) and sulfamethoxazole (SX) alone (15 ng L^{-1}) or in a mixture (MIX, 15 ng L^{-1} each). Average speed (A), maximum speed (B), total distance (C), and peripheral time (D) were evaluated. The results are expressed as mean ± standard deviation. Statistical differences are indicated by different letters ($p < 0.05$) and were determined using Kruskal–Wallis followed by Dunn's test. NC, negative control group.

(< 1.80 ng L^{-1}) [82]; Rio de Janeiro (< 2420 ng L^{-1}) [26]; and Goiás (≥ 1 ng L^{-1}) [29]. The SX quantification in the Meia Ponte River is among the highest reported for Brazilian rivers (~36 ng L^{-1}). Globally, SX was highly detected in a Congo river at concentrations of ~17 000 ng L^{-1} [4].

In this study, the predicted SX half-life in an aquatic environment under $^{\cdot}$•OH attack was 9 to 88 days. These values are similar to those reported in a previous study, which determined the SX half-life to be 51.7 days in water [83]. These data confirmed that SX can be classified as a persistent pollutant [84]. This persistence could be toxic to aquatic organisms. In this work, morphometric parameters of zebrafish larvae, such as maximal interocular distance and swim bladder area, increased in the presence of environmentally relevant concentrations of SX, indicating embryo-larval toxicity. Irregular cranial and ocular development can affect fish's visual and behavioral capacities, especially in foraging spaces, making it difficult for them to escape from predators [85–87]. SX at higher concentrations (150 or 200 μg/L) impacted even more the zebrafish development, decreasing the hatching rate and causing neurotoxicity, oxidative stress, and morphological abnormalities [40, 88–89]. SX also inhibited acetylcholinesterase (AChE) activity in the brain (> 200 μg/L), decreased zebrafish spontaneous swimming (≥ 1 μg/L), and increased the heart rate (≥ 10 μg/L) [90].

Notably, the ecological risks to organisms exposed to complex pollutant mixtures in aquatic systems may be greater than those predicted for a single pollutant due to toxicological interactions among pollutants [4]. In this sense, the mixture of antibiotics MTZ and SX was investigated in this study (MIX group). It was observed that the MIX attenuated the cardiotoxic effect of isolated MTZ, indicating an interaction between these two antimicrobials. Moreover, the MIX induced differential morphometric changes in zebrafish larvae compared to isolated exposure to MTZ or SX. The potential interactions between MTZ and SX warrant further investigation.

Regarding behavioral aspects, the results showed that MTZ alone or in combination with SX (MIX) reduced time spent in the periphery, which is a risk for larvae because they are more susceptible

to predation [91]. These results are consistent with the literature, which indicates that MTZ alters nervous system function [92–94], potentially influencing motor behavior and spatial perception in organisms. The mechanisms of metronidazole's neurotoxicity are unknown. However, the suggested mechanisms include oxidative stress to nerve tissue [95, 96], modulation of gamma-aminobutyric acid (GABA) in the cerebellum [97], or inhibition of neuronal protein synthesis [98]. No significant results were observed in other behavioral parameters, such as average speed, maximum speed, and total distance traveled, suggesting that the adverse effects observed are not related to a general decrease in activity or the larvae's inability to move, but rather to a specific change in locomotor behavior. Finally, these results support the hypothesis that MTZ alone and in combination with SX can induce behavioral changes in zebrafish larvae. Early-life behavioral alterations may compromise survival, growth, or later developmental stages of zebrafish [99].

The quantification of SX and MTZ, but not AMX, CFZ, or CHL, in surface water samples can be explained by the instability of certain functional groups and the formation of highly polar transformation products that are not detectable by typical monitoring methods. In particular, β-lactams such as AMX undergo fast hydrolysis β-lactam ring opening in water, yielding penicilloic-acid derivatives that can further evolve into numerous secondary products, which plausibly decreases the measurable parent even when oxidative persistence is inferred from simplified scenarios [100]. In parallel, CHL exhibits pronounced photochemical reactivity, where UV-driven pathways generate para-nitrobenzaldehyde and subsequent oxidized acids (p-nitrobenzoic and p-nitrosobenzoic acids), consistent with relatively rapid depletion of the parent under irradiation and reactive-oxygen conditions [101]. By contrast, although SX is reactive, its transformation under oxidative/photochemical stress frequently proceeds through aromatic hydroxylation, amino-group oxidation, and S—N bond cleavage, generating identifiable intermediates while still allowing some residual parent to persist long enough for detection, depending on exposure time and oxidant regime [102, 103]. MTZ under •OH-mediated oxidation can transform via radical adduct formation at the nitroimidazole ring and oxidation of the hydroxyethyl side chain toward carbonylated/carboxylated products, yet direct photodegradation of nitroimidazoles can exhibit low quantum yields, supporting a scenario in which MTZ may remain detectable in the field despite ongoing transformation [104–106].

Finally, because large-scale river monitoring indicates that pharmaceutical detection patterns are strongly modulated by local emissions, hydrology, and analytical coverage [4], the present interpretation should be viewed as chemically plausible but not definitive, and it motivates future work explicitly tracking key transformation products and degradation kinetics to discriminate true absence from rapid conversion as dominant explanation.

5 | Conclusion

As observed globally, the Meia Ponte River in the Cerrado biome has also been affected by antimicrobial discharge. MTZ and SX, two of the five antimicrobials (AMX, MTZ, CFZ, SX, and CHL) evaluated here, were detected in the STP (even after the primary treatment) and in samples along the Meia Ponte River, including the spring. This study is a pioneer in detecting MTZ in an important Brazilian river, and the SX quantification is one of the highest in Brazil. Molecular and kinetic modeling indicated sites of oxidative reactivity and predicted the half-life values under $^{\cdot}$ • OH oxidation for the antimicrobials investigated here, ranging from 7 to 140 days. The persistence of antimicrobials underscores the importance of monitoring their presence in aquatic environments and sediments. The results of this study provide strong evidence that environmentally relevant concentrations of the antimicrobials SX and MTZ, alone or in combination, induce significant changes in zebrafish embryos and larvae that could interfere with survival and behavior, potentially with ecological implications. Given the predicted persistence of MTZ and SX under •OH-mediated oxidation, longer-term exposures may be environmentally relevant and should be addressed in future studies using chronic and multigenerational endpoints.

Author Contributions

Náthala Maria Simão and **Igor Romeiro dos Santos:** conceptualization, investigation, writing. **Felipe Cirqueira Dias**, **Jéssyca Moreira Morais**, **Lorranny Pereira de Assis Valadares:** investigation, methodology. **Flávio Olimpio Sanches Neto** and **Jerônimo Raimundo de Oliveira Neto:** investigation, methodology, writing. **Naiara Raica Lopes de Oliveira:** methodology. **Mirelle Garcia Silva Bailão** and **Luiz Carlos da Cunha:** methodology, resources. **Valter Henrique Carvalho-Silva:** conceptualization, methodology, supervision, writing – review and editing. **Thiago Lopes Rocha** and **Elisa Flávia Luiz Cardoso Bailão:** conceptualization, funding acquisition, supervision, project administration, writing – review and editing.

Acknowledgments

We thank Companhia de Saneamento de Goiás (Saneago) for providing effluent samples from the Dr. Hélio Seixo de Britto sewage treatment plant. During the preparation of this work, the authors used ChatGPT (OpenAI) to assist with language refinement, literature search, and critical evaluation of the manuscript and related studies, as well as ASKCOS for AI-assisted reaction mechanism analysis and systematization. All content was reviewed and validated by the authors, who take full responsibility for the manuscript. The Article Processing Charge for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614).

Funding

This research was funded by Programa Pesquisa para o SUS: gestão compartilhada em saúde—PPSUS (grant number PPSUS proc. 202110267000295), namely Ministério da Saúde (MS), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG), and Secretaria de Estado da Saúde (SES-GO); by Universidade Estadual de Goiás (UEG; Pró-Projetos, grant number 202100020012836); and by Rede HidroCerrado (PDPG, proc. 202410267001424). N.M.S., I.R.S., and J.M.M. were supported by scholarships from CAPES, UEG, and FAPEG, respectively. VHCS acknowledges for CNPq Research Productivity Grant. TLR and EFLCB are granted a productivity fellowship from CNPq (grant number 314413/2023-5) and FAPEG (proc. 202510267001605), respectively. M.G.S.B. acknowledges Programa de Auxílio à Pesquisa Científica e Tecnológica (FAPEG, grant number 202310267000213) and CNPq (grant number 314289/2023-2).

Ethics Statement

This work has received approval for research ethics from the Council of Animal Experimentation from the Federal University of Goiás (No. 073/22), where the experiments were conducted, and a proof/certificate of approval is available upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. M. Čelić, M. Farré, M. L. de Alda, et al., "Chapter 16 - Environmental Analysis: Emerging Pollutants," in *Liquid Chromatography*, vol. 2, Third ed., ed. S. Fanali, B. Chankvetadze, P. R. Haddad, C. F. Poole, and M.-L. Riekkola (Elsevier, 2023), 549–578.

2. N. A. Khan, S. U. Khan, S. Ahmed, et al., "Recent Trends in Disposal and Treatment Technologies of Emerging-Pollutants-A Critical Review," *TrAC Trends in Analytical Chemistry* 122 (2020): 115744, https://doi.org/10.1016/j.trac.2019.115744.

3. A. Kumar, A. Nighojkar, P. Varma, et al., "Algal Mediated Intervention for the Retrieval of Emerging Pollutants From Aqueous Media," *Journal of Hazardous Materials* 455 (2023): 131568, https://doi.org/10.1016/j.jhazmat.2023.131568.

4. J. L. Wilkinson, A. B. A. Boxall, D. W. Kolpin, et al., "Pharmaceutical Pollution of the World's Rivers," *Proceedings of the National Academy of Sciences* 119, no. 8 (2022): e2113947119, https://doi.org/10.1073/pnas.2113947119.

5. E. Y. Klein, I. Impalli, S. Poleon, et al., "Global Trends in Antibiotic Consumption During 2016–2023 and Future Projections Through 2030," *National Academy of Sciences of the United States of America* 121, no. 49 (2024): e2411919121.

6. F. Pellegrino, F. Aguiar-Alves, D. Assumpção, M. Alves, and R. Dias, "Antibiotic Prescription and Consumption in Brazil: Impact in the Global Health," *Annals of Clinical and Medical Case Reports* 12, no. 15 (2024): 1–4.

7. WHO, *Report on Surveillance of Antibiotic Consumption 2016–2018 Early Implementation* (world Health organization, 2018), https://apps.who.int/iris/bitstream/handle/10665/277359/9789241514880-eng.pdf?ua=1.

8. N. C. M. Massarine, G. H. A. de Souza, I. B. Nunes, et al., "How Did COVID-19 Impact the Antimicrobial Consumption and Bacterial Resistance Profiles in Brazil?," *Antibiotics* 12, no. 9 (2023): 1374, https://doi.org/10.3390/antibiotics12091374.

9. F. S. Del Fiol, C. C. Bergamaschi, I. P. De Andrade, et al., "Consumption Trends of Antibiotics in Brazil During the COVID-19 Pandemic," *Frontiers in Pharmacology* 13 (2022): 844818.

10. S. Coutu, L. Rossi, D. A. Barry, S. Rudaz, and N. Vernaz, "Temporal Variability of Antibiotics Fluxes in Wastewater and Contribution From Hospitals," *PLoS One* 8, no. 1 (2013): e53592, https://doi.org/10.1371/journal.pone.0053592.

11. L. H. M. L. M. Santos, M. Gros, S. Rodriguez-Mozaz, et al., "Contribution of Hospital Effluents to the Load of Pharmaceuticals in Urban Wastewaters: Identification of Ecologically Relevant Pharmaceuticals," *Science of the Total Environment* 461–462 (2013): 302–316, https://doi.org/10.1016/j.scitotenv.2013.04.077.

12. United Nations Environment Programme (UNEP), Environmental Dimensions of Antimicrobial Resistance: Summary for Policymakers, 2022, https://wedocs.unep.org/bitstream/handle/20.500.11822/38373/antimicrobial_R.pdf.

13. K. He, E. Hain, A. Timm, M. Tarnowski, and L. Blaney, "Occurrence of Antibiotics, Estrogenic Hormones, and UV-Filters in Water, Sediment, and Oyster Tissue From the Chesapeake Bay," *Science of the Total Environment* 650 (2019): 3101–3109.

14. U. Szymańska, M. Wiergowski, I. Sołtyszewski, et al., "Presence of Antibiotics in the Aquatic Environment in Europe and Their Analytical Monitoring: Recent Trends and Perspectives," *Microchemical Journal* 147 (2019): 729–740.

15. H. Q. Anh, T. P. Q. Le, N. Da Le, et al., "Antibiotics in Surface Water of East and Southeast Asian Countries: A Focused Review on Contamination Status, Pollution Sources, Potential Risks, and Future Perspectives," *Science of the Total Environment* 764 (2021): 142865.

16. M. Llorca, M. Farré, E. Eljarrat, et al., "Review of Emerging Contaminants in Aquatic Biota From Latin America: 2002–2016," *Environmental Toxicology and Chemistry* 36, no. 7 (2016): 1716–1727.

17. C. Peña-Guzmán, S. Ulloa-Sánchez, K. Mora, et al., "Emerging Pollutants in the Urban Water Cycle in Latin America: A Review of the Current Literature," *Journal of Environmental Management* 237 (2019): 408–423, https://doi.org/10.1016/j.jenvman.2019.02.100.

18. J. L. D. Pizzol, T. L. Lubschinski, E. T. B. Mohr, et al., "Pharmaceuticals, Personal Care Products, and Illicit Drugs in the Aquatic Environment and Occurrence of the Antimicrobial Resistance in Brazil: A Systematic Review," *Environmental Science and Pollution Research* 32 (2025): 20799–20824, https://doi.org/10.1007/s11356-025-36795-5.

19. M. J. S. Chaves, S. C. Barbosa, M. M. Malinowski, et al., "Pharmaceuticals and Personal Care Products in a Brazilian Wetland of International Importance: Occurrence and Environmental Risk Assessment," *Science of the Total Environment* 734 (2020): 139374.

20. A. L. C. de Barros, F. F. Schmidt, S. F. de Aquino, and R. J. C. F. Afonso, "Determination of Nine Pharmaceutical Active Compounds in Surface Waters From Paraopeba River Basin in Brazil by LTPE-HPLC-ESI-MS/MS," *Environmental Science and Pollution Research* 25, no. 20 (2018): 19962–19974.

21. E. O. Reis, A. F. S. Foureaux, J. S. Rodrigues, et al., "Occurrence, Removal and Seasonal Variation of Pharmaceuticals in Brasilian Drinking Water Treatment Plants," *Environmental Pollution* 250 (2019): 773–781.

22. J. B. Arsand, R. B. Hoff, L. Jank, et al., "Wide-Scope Determination of Pharmaceuticals and Pesticides in Water Samples: Qualitative and Confirmatory Screening Method Using LC-qTOF-MS," *Water, Air, & Soil Pollution* 229 (2018): 399, https://doi.org/10.1007/s11270-018-4036-2.

23. J. B. Arsand, R. B. Hoff, L. Jank, et al., "Presence of Antibiotic Resistance Genes and Its Association With Antibiotic Occurrence in Dilúvio River in Southern Brazil," *Science of the Total Environment* 738 (2020): 139781, https://doi.org/10.1016/j.scitotenv.2020.139781.

24. S. S. Caldas, C. Rombaldi, J. L. de Oliveira Arias, L. C. Marube, and E. G. Primel, "Multi-Residue Method for Determination of 58 Pesticides, Pharmaceuticals and Personal Care Products in Water Using Solvent Demulsification Dispersive Liquid–Liquid Microextraction Combined With Liquid Chromatography-Tandem Mass Spectrometry," *Talanta* 146 (2016): 676–688, https://doi.org/10.1016/j.talanta.2015.06.047.

25. M. Perin, A. Dallegrave, L. S. Barnet, L. Z. Meneghini, A. A. Gomes, and T. M. Pizzolato, "Pharmaceuticals, Pesticides and Metals/Metalloids in Lake Guaíba in Southern Brazil: Spatial and Temporal Evaluation and a Chemometrics Approach," *Science of the Total Environment* 793 (2021): 148561, https://doi.org/10.1016/j.scitotenv.2021.148561.

26. J. A. Sabino, A. L. de Sá Salomão, P. M. de Oliveira Muniz Cunha, R. Coutinho, and M. Marques, "Occurrence of Organic Micropollutants in an Urbanized Sub-Basin and Ecological Risk Assessment,"

Ecotoxicology 30 (2021): 130–141, https://doi.org/10.1007/s10646-020-02304-2.

27. C. C. Montagner, F. F. Sodré, R. D. Acayaba, et al., "Ten Years-Snapshot of the Occurrence of Emerging Contaminants in Drinking, Surface and Ground Waters and Wastewaters From São Paulo State, Brazil," *Journal of the Brazilian Chemical Society* 30, no. 3 (2019): 614–632.

28. R. C. Pivetta, C. Rodrigues-Silva, A. R. Ribeiro, and S. Rath, "Tracking the Occurrence of Psychotropic Pharmaceuticals in Brazilian Wastewater Treatment Plants and Surface Water, With Assessment of Environmental Risks," *Science of the Total Environment* 727 (2020): 138661, https://doi.org/10.1016/j.scitotenv.2020.138661.

29. I. R. dos Santos, I. N. M. da Silva, J. R. de Oliveira Neto, et al., "The Presence of Antibiotics and Multidrug-Resistant *Staphylococcus Aureus* Reservoir in a Low-Order Stream Spring in Central Brazil," *Brazilian Journal of Microbiology* 54, no. 2 (2023): 997–1007.

30. P. Kovalakova, L. Cizmas, T. J. McDonald, B. Marsalek, M. Feng, and V. K. Sharma, "Occurrence and Toxicity of Antibiotics in the Aquatic Environment: A Review," *Chemosphere* 251 (2020): 126351, https://doi.org/10.1016/j.chemosphere.2020.126351.

31. C. Bouki, D. Venieri, and E. Diamadopoulos, "Detection and Fate of Antibiotic Resistant Bacteria in Wastewater Treatment Plants: A Review," *Ecotoxicology and Environmental Safety* 91 (2013): 1–9, https://doi.org/10.1016/j.ecoenv.2013.01.016.

32. L. Gao, Y. Shi, W. Li, J. Liu, and Y. Cai, "Occurrence, Distribution and Bioaccumulation of Antibiotics in the Haihe River in China," *Journal of Environmental Monitoring* 14, no. 4 (2012): 1247–1254.

33. C. Yang, G. Song, and W. Lim, "A Review of the Toxicity in Fish Exposed to Antibiotics," *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 237 (2020): 108840, https://doi.org/10.1016/j.cbpc.2020.108840.

34. W. Deng, N. Li, H. Zheng, and H. Lin, "Occurrence and Risk Assessment of Antibiotics in River Water in Hong Kong," *Ecotoxicology and Environmental Safety* 125 (2016): 121–127, https://doi.org/10.1016/j.ecoenv.2015.12.002.

35. M. P. Gomes, D. S. Tavares, V. S. Richardi, et al., "Enrofloxacin and Roundup Interactive Effects on the Aquatic Macrophyte *Elodea canadensis* Physiology," *Environmental Pollution* 249 (2019): 453–462, https://doi.org/10.1016/j.envpol.2019.03.026.

36. Q. Li, J. Gao, Q. Zhang, L. Liang, and H. Tao, "Distribution and Risk Assessment of Antibiotics in a Typical River in North China Plain," *Bulletin of Environmental Contamination and Toxicology* 98 (2017): 478–483, https://doi.org/10.1007/s00128-016-2023-0.

37. Q. Zhou, G. Liu, M. Arif, X. Shi, and S. Wang, "Occurrence and Risk Assessment of Antibiotics in the Surface Water of Chaohu Lake and Its Tributaries in China," *Science of the Total Environment* 807 (2022): 151040, https://doi.org/10.1016/j.scitotenv.2021.151040.

38. K. Kümmerer, "Antibiotics in the Aquatic Environment: A Review–Part I," *Chemosphere* 75, no. 4 (2009): 417–434, https://doi.org/10.1016/j.chemosphere.2008.11.086.

39. D. A. Palacio, F. L. Aranda, and B. É. L. Rivas, "Removal of Antibiotic Emerging Pollutants: An Overview," *Journal of the Chilean Chemical Society* 67, no. 3 (2022): 5547–5561, https://doi.org/10.4067/S0717-97072022000305547.

40. B. S. Diogo, S. Rodrigues, and S. C. Antunes, "Mixture Matters: Exploring the Overlooked Toxicity of Sulfamethoxazole and Trimethoprim in Aquatic Environments," *Environmental Toxicology* 40 (2025): 1277–1293, https://doi.org/10.1002/tox.24528.

41. J. Wang and R. Zhuan, "Degradation of Antibiotics by Advanced Oxidation Processes: An Overview," *Science of the Total Environment* 701 (2020): 135023, https://doi.org/10.1016/j.scitotenv.2019.135023.

42. Y.-Y. Cai, Q. Q. Zhang, X. T. Yan, et al., "Antibiotic Pollution in Lakes in China: Emission Estimation and Fate Modeling Using a Temperature-Dependent Multimedia Model," *Science of the Total Environment* 842 (2022): 156633, https://doi.org/10.1016/j.scitotenv.2022.156633.

43. Z. Cheng, Q. Dong, Z. Yuan, X. Huang, and Y. Liu, "Fate Characteristics, Exposure Risk, and Control Strategy of Typical Antibiotics in Chinese Sewerage System: A Review," *Environment International* 167 (2022): 107396, https://doi.org/10.1016/j.envint.2022.107396.

44. P. T. Santos and A. P. Martins, "Socioeconomic and Spatial Analysis of the Meia Ponte River Basin (GO)," *Geographies Journal* 18, no. 2 (2022): 1–20, https://doi.org/10.35699/2237-549X.2022.37885.

45. R. P. Gomes, T. Oliveira, A. Rodrigues, L. Ferreira, J. Vieira, and L. Carneiro, "Occurrence of Antibiotic Resistance Genes, Antibiotics-Resistant and Multi-Resistant Bacteria and Their Correlations in One River in Central-Western Brazil," *Water* 15, no. 4 (2023): 747, https://doi.org/10.3390/w15040747.

46. SEMAD—State Secretariat for Environment and Sustainable Development, Action Plan for the UPGRH of the Meia Ponte River, 2020, http://pbapgo.meioambiente.go.gov.br/wp-content/uploads/2020/12/RT-04-Plano-de-Acoes-UPGRH-Meia-Ponte-V01.pdf.

47. A. K. B. Fagundes, T. A. Mendes, and T. S. R. Pereira, "Classificação Preliminar de Corpos D'água Com Base Na Resolução Conama nº 357/2005: Caso Do Rio Meia Ponte - GO," *Ciência e Natura* 38, no. 3 (2016): 1382.

48. G. Bolívar-Subirats, C. Rivetti, M. Cortina-Puig, C. Barata, and S. Lacorte, "Occurrence, Toxicity and Risk Assessment of Plastic Additives in Besos River, Spain," *Chemosphere* 263 (2021): 128022, https://doi.org/10.1016/j.chemosphere.2020.128022.

49. S. Teixeira, C. Delerue-Matos, A. Alves, and L. Santos, "Fast Screening Procedure for Antibiotics in Wastewaters by Direct HPLC-DAD Analysis," *Journal of Separation Science* 31, no. 16-17 (2008): 2924–2931.

50. T. Lu and F. Chen, "Multiwfn: A Multifunctional Wavefunction Analyzer," *Journal of Computational Chemistry* 33, no. 5 (2012): 580–592, https://doi.org/10.1002/jcc.22885.

51. M. J. Frisch, G. W. Trucks, H. B. Schlegel, et al., Gaussian 16 Rev. C.01 [computer program]. Wallingford, CT2016.

52. F. O. Sanches-Neto, J. R. Dias-Silva, L. H. Keng Queiroz Junior, and V. H. Carvalho-Silva, ""pySiRC": Machine Learning Combined With Molecular Fingerprints to Predict the Reaction Rate Constant of the Radical-Based Oxidation Processes of Aqueous Organic Contaminants," *Environmental Science & Technology* 55, no. 18 (2021): 12437–12448.

53. F. O. Sanches-Neto, B. Ramos, A. M. Lastre-Acosta, A. C. S. C. Teixeira, and V. H. Carvalho-Silva, "Aqueous Picloram Degradation by Hydroxyl Radicals: Unveiling Mechanism, Kinetics, and Ecotoxicity Through Experimental and Theoretical Approaches," *Chemosphere* 278 (2021): 130401, https://doi.org/10.1016/j.chemosphere.2021.130401.

54. D. Vione, G. Falletti, V. Maurino, et al., "Sources and Sinks of Hydroxyl Radicals Upon Irradiation of Natural Water Samples," *Environmental Science & Technology* 40, no. 12 (2006): 3775–3781, https://doi.org/10.1021/es052206b.

55. ISO, "Water Quality – Determination of the Acute Lethal Toxicity of Substances to a Freshwater Fish [Brachydanio Rerio Hamilton-Buchanan (Teleostei, Cyprinidae)]. In. 2 Ed: ISO - International Organization for Standardization," (1996).

56. OECD, *Test No. 236: Fish Embryo Acute Toxicity (FET) Test. OECD Guidelines for Testing Chemicals, Section 2* (OECD Publishing, 2013).

57. A. C. Pereira, B. B. Gonçalves, R. S. Brito, L. G. Vieira, E. C. O. Lima, and T. L. Rocha, "Comparative Developmental Toxicity of Iron Oxide Nanoparticles and Ferric Chloride to Zebrafish (*Danio rerio*) After Static and Semi-Static Exposure," *Chemosphere* 254 (2020): 126792.

58. A. L. Santos, L. C. Rodrigues, C. C. Rodrigues, et al., "Polystyrene Nanoplastics Induce Developmental Impairments and Vasotoxicity in Zebrafish (*Danio Rerio*)," *Journal of Hazardous Materials* 464 (2024): 132880.

59. J. Mamboungou, A. Canedo, G. Qualhato, T. L. Rocha, and L. G. Vieira, "Environmental Risk of Titanium Dioxide Nanoparticle and Cadmium Mixture: Developmental Toxicity Assessment in Zebrafish (*Danio Rerio*)," *Journal of Nanoparticle Research* 24, no. 9 (2022): 186.

60. M. Cantonati, R. J. Fensham, L. E. Stevens, et al., "Urgent Plea for Global Protection of Springs," *Conservation Biology* 35, no. 1 (2021): 378–382.

61. Samreen, I. Ahmad, H. A. Malak, and H. H. Abulreesh, "Environmental Antimicrobial Resistance and Its Drivers: A Potential Threat to Public Health," *Journal of Global Antimicrobial Resistance* 27 (2021): 101–111, https://doi.org/10.1016/j.jgar.2021.08.001.

62. M. Wagil, J. Maszkowska, A. Białk-Bielińska, M. Caban, P. Stepnowski, and J. Kumirska, "Determination of Metronidazole Residues in Water, Sediment and Fish Tissue Samples," *Chemosphere* 119 (2015): S28–S34, https://doi.org/10.1016/j.chemosphere.2013.12.061.

63. M. Sánchez-Polo, J. Rivera-Utrilla, G. Prados-Joya, and R. Ocampo-Pérez, "Metronidazole Photodegradation in Aqueous Solution by Using Photosensitizers and Hydrogen Peroxide," *Journal of Chemical Technology & Biotechnology* 87, no. 8 (2012): 1202–1208, https://doi.org/10.1002/jctb.3750.

64. N. Aarab, M. Laabd, H. Eljazouli, R. Lakhmiri, H. Kabli, and A. Albourine, "Experimental and DFT Studies of the Removal of Pharmaceutical Metronidazolefrom Water Using Polypyrrole," *International Journal of Industrial Chemistry* 10, no. 3 (2019): 269–279, https://doi.org/10.1007/s40090-019-0190-7.

65. C. F. Rediguieri, V. Porta, D. S. G. Nunes, et al., "Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Metronidazole," *Journal of Pharmaceutical Sciences* 100, no. 5 (2011): 1618–1627, https://doi.org/10.1002/jps.22409.

66. L. Lian, B. Yao, S. Hou, J. Fang, S. Yan, and W. Song, "Kinetic Study of Hydroxyl and Sulfate Radical-Mediated Oxidation of Pharmaceuticals in Wastewater Effluents," *Environmental Science & Technology* 51, no. 5 (2017): 2954–2962, https://doi.org/10.1021/acs.est.6b05536.

67. F. Coşkun, E. Yalçın, and K. Çavuşoğlu, "Metronidazole Promotes Oxidative Stress and DNA Fragmentation-Mediated Myocardial Injury in Albino Mice," *Chemosphere* 352 (2024): 141382.

68. M. W. Bariweni, V. B. Patel, G. M. Zariwala, and R. I. Ozolua, "Biomarkers of Antibiotic Toxicity: A Focus on Metronidazole," in *Biomarkers in Toxicology, Biomarkers in Disease: Methods, Discoveries and Applications*, ed. V. B. Patel (Springer Nature, 2023), 139–154, https://doi.org/10.1007/978-3-031-07392-2_75.

69. E. Agathokleous, "The Hormetic Response of Fish Embryo Heart Rate to Contaminants: Implications for Research and Policy," *Science of the Total Environment* 806 (2022): 152911, https://doi.org/10.1016/j.scitotenv.2021.152911.

70. M. Autumn, J. Zeng, I. Ranieri, and S. K. McMenamin, "Experimentally Manipulating the Thyroid Hormone Axis in Zebrafish," *Methods in Molecular Biology* 2876 (2025): C1, https://doi.org/10.1007/978-1-0716-4252-8_17.

71. J. L. Bakke, N. Lawrence, and B. Campbell, "The Effect of Metronidazole on the Synthesis of Thyroid Stimulating Hormone in the Hypothyroid Rat," *Metabolism - Clinical and Experimental* 14, no. 5 (1965): 647–651.

72. A. Godfrey, B. Hooser, A. Abdelmoneim, et al., "Thyroid Disrupting Effects of Halogenated and Next Generation Chemicals on the Swim Bladder Development of Zebrafish," *Aquatic Toxicology* 193 (2017): 228–235.

73. E. Stinckens, L. Vergauwen, A. L. Schroeder, et al., "Impaired Anterior Swim Bladder Inflation Following Exposure to the Thyroid Peroxidase Inhibitor 2-Mercaptobenzothiazole Part II: Zebrafish," *Aquatic Toxicology* 173 (2016): 204–217, https://doi.org/10.1016/j.aquatox.2015.12.023.

74. E. Stinckens, L. Vergauwen, B. R. Blackwell, et al., "Effect of Thyroperoxidase and Deiodinase Inhibition on Anterior Swim Bladder Inflation in the Zebrafish," *Environmental Science & Technology* 54, no. 10 (2020): 6213–6223.

75. B. Baldisserotto, E. C. Urbinati, and J. E. P. Cyrino, *Biology and Physiology of Neotropical Freshwater Fishes* (Academic Press, 2014).

76. L. Prestinicola, C. Boglione, and S. Cataudella, "Relationship Between Uninflated Swim Bladder and Skeletal Anomalies in Reared Gilthead Seabream (*Sparus aurata*)," *Aquaculture* 432 (2014): 462–469.

77. W. Qiu, X. Liu, F. Yang, et al., "Single and Joint Toxic Effects of Four Antibiotics on Some Metabolic Pathways of Zebrafish (*Danio Rerio*) Larvae," *Science of the Total Environment* 716 (2020): 137062.

78. Z. Yan, G. Lu, Q. Ye, and J. Liu, "Long-Term Effects of Antibiotics, Norfloxacin, and Sulfamethoxazole, in a Partial Life-Cycle Study With Zebrafish (*Danio rerio*): Effects on Growth, Development, and Reproduction," *Environmental Science and Pollution Research* 23 (2016): 18222–18228, https://doi.org/10.1007/s11356-016-7018-1.

79. L. Zhou, S. M. Limbu, M. Shen, et al., "Environmental Concentrations of Antibiotics Impair Zebrafish Gut Health," *Environmental Pollution* 235 (2018): 245–254, https://doi.org/10.1016/j.envpol.2017.12.073.

80. J. Wang and S. Wang, "Microbial Degradation of Sulfamethoxazole in the Environment," *Applied Microbiology and Biotechnology* 102 (2018): 3573–3582, https://doi.org/10.1007/s00253-018-8845-4.

81. M. A. F. Locatelli, F. F. Sodré, and W. F. Jardim, "Determination of Antibiotics in Brazilian Surface Waters Using Liquid Chromatography–Electrospray Tandem Mass Spectrometry," *Archives of Environmental Contamination and Toxicology* 60 (2011): 385–393, https://doi.org/10.1007/s00244-010-9550-1.

82. B. Böger, M. Surek, R. O. Vilhena, et al., "Occurrence of Antibiotics and Antibiotic Resistant Bacteria in Subtropical Urban Rivers in Brazil," *Journal of Hazardous Materials* 402 (2021): 123448, https://doi.org/10.1016/j.jhazmat.2020.123448.

83. M. F. Murrieta, E. Brillas, J. L. Nava, and I. Sirés, "Photo-Assisted Electrochemical Production of HClO and Fe^{2+} as Fenton-Like Reagents in Chloride Media for Sulfamethoxazole Degradation," *Separation and Purification Technology* 250 (2020): 117236, https://doi.org/10.1016/j.seppur.2020.117236.

84. B. Xu, D. Mao, Y. Luo, and L. Xu, "Sulfamethoxazole Biodegradation and Biotransformation in the Water–Sediment System of a Natural River," *Bioresource Technology* 102, no. 14 (2011): 7069–7076, https://doi.org/10.1016/j.biortech.2011.04.086.

85. L. Baumann, A. Ros, K. Rehberger, S. C. F. Neuhauss, and H. Segner, "Thyroid Disruption in Zebrafish (*Danio rerio*) Larvae: Different Molecular Response Patterns Lead to Impairment of Ocular Development and Visual Functions," *Aquatic Toxicology* 172 (2016): 44–55, https://doi.org/10.1016/j.aquatox.2015.12.015.

86. L. Dill, "The Escape Response of the Zebra Danio (*Brachydanio rerio*) II. The Effect of Experience," *Animal Behaviour* 22 (1974): 723–730, https://doi.org/10.1016/S0003-3472(74)80023-0.

87. Y. R. Yanagitsuru, O. Akanyeti, and J. C. Liao, "Head Width Influences Flow Sensing by the Lateral Line Canal System in Fishes," *Journal of Experimental Biology* 221, no. 21 (2018): jeb180877.

88. B. S. Diogo, S. Rodrigues, O. Golovko, and S. C. Antunes, "From Bacteria to Fish: Ecotoxicological Insights Into Sulfamethoxazole and Trimethoprim," *Environmental Science and Pollution Research* 31 (2024): 52233–52252, https://doi.org/10.1007/s11356-024-34659-y.

89. J. Liu, T. Wei, X. Wu, H. Zhong, W. Qiu, and Y. Zheng, "Early Exposure to Environmental Levels of Sulfamethoxazole Triggers Immune and Inflammatory Response of Healthy Zebrafish Larvae," *Science of the Total Environment* 703 (2020): 134724, https://doi.org/10.1016/j.scitotenv.2019.134724.

90. T. Lin, S. Yu, Y. Chen, and W. Chen, "Integrated Biomarker Responses in Zebrafish Exposed to Sulfonamides," *Environmental Toxicology and Pharmacology* 38, no. 2 (2014): 444–452, https://doi.org/10.1016/j.etap.2014.07.020.

91. S. J. Schnörr, P. J. Steenbergen, M. K. Richardson, and D. L. Champagne, "Measuring Thigmotaxis in Larval Zebrafish," *Behavioural Brain Research* 228, no. 2 (2012): 367–374.

92. A. M. El-Moslemany, M. H. Abd-Elfatah, N. A. Tahoon, et al., "Mechanistic Assessment of Anise Seeds and Clove Buds Against the Neurotoxicity Caused by Metronidazole in Rats: Possible Role of Antioxidants, Neurotransmitters, and Cytokines," *Toxics* 11, no. 9 (2023): 724.

93. K. I. Park, J. M. Chung, and J. Y. Kim, "Metronidazole Neurotoxicity: Sequential Neuroaxis Involvement," *Neurology India* 59, no. 1 (2011): 104–107.

94. C. G. Sørensen, W. K. Karlsson, F. M. Amin, and M. Lindelof, "Metronidazole-Induced Encephalopathy: A Systematic Review," *Journal of Neurology* 267 (2020): 1–13, https://doi.org/10.1007/s00415-018-9147-6.

95. N. Tokanová, R. Dobšíková, V. Doubková, J. Blahová, Z. Svobodová, and P. Maršálek, "The Effect of Sulfamethoxazole on Oxidative Stress Indices in Zebrafish (*Danio rerio*)," *Drug and Chemical Toxicology* 44 (2021): 58–63, https://doi.org/10.1080/01480545.2018.1560465.

96. S. Chaturvedi, M. Y. Malik, M. Rashid, et al., "Mechanistic Exploration of Quercetin Against Metronidazole Induced Neurotoxicity in Rats: Possible Role of Nitric Oxide Isoforms and Inflammatory Cytokines," *Neurotoxicology* 79 (2020): 1–10.

97. J. Evans, D. Levesque, K. Knowles, R. Longshore, and S. Plummer, "Diazepam as a Treatment for Metronidazole Toxicosis in Dogs: A Retrospective Study of 21 Cases," *Journal of Veterinary Internal Medicine* 17, no. 3 (2003): 304–310.

98. W. G. Bradley, "Metronidazole Neuropathy," *BMJ* 2, no. 6087 (1977): 610, https://doi.org/10.1136/bmj.2.6087.610.

99. D. G. Valcarce, A. Sellés-Egea, M. F. Riesco, et al., "Early Stress Exposure on Zebrafish Development: Effects on Survival, Malformations and Molecular Alterations," *Fish Physiology and Biochemistry* 50, no. 4 (2024): 1545–1562, https://doi.org/10.1007/s10695-024-01355-0.

100. K. Hirte, B. Seiwert, G. Schüürmann, and T. Reemtsma, "New Hydrolysis Products of the Beta-Lactam Antibiotic Amoxicillin, Their pH-Dependent Formation and Search in Municipal Wastewater," *Water Research* 88 (2016): 880–888.

101. Y. A. Belikov, O. A. Snytnikova, D. G. Sheven, R. G. Fedunov, V. P. Grivin, and I. P. Pozdnyakov, "Laser Flash Photolysis and Quantum Chemical Studies of UV Degradation of Pharmaceutical Drug Chloramphenicol: Short-Lived Intermediates, Quantum Yields and Mechanism of Photolysis," *Chemosphere* 351 (2024): 141211.

102. M. Gómez-Ramos Mdel, M. Mezcua, A. Agüera, et al., "Chemical and Toxicological Evolution of the Antibiotic Sulfamethoxazole Under Ozone Treatment in Water Solution," *Journal of Hazardous Materials* 192, no. 1 (2011): 18–25.

103. B. T. Kengne, Y. Sun, S. Wang, et al., "Kinetic Analysis and Transformation Pathways of Sulfamethoxazole Degradation in Water and Wastewater Under Electron Beam Irradiation," *Water* 17 (2025): 1596, https://doi.org/10.3390/w17111596.

104. S. M. Maldonado Domínguez, C. E. Barrera-Díaz, P. Balderas Hernández, D. Amado-Piña, T. Torres-Blancas, and G. Roa-Morales, "Metronidazole Electro-Oxidation Degradation on a Pilot Scale," *Catalysts* 15 (2025): 29, https://doi.org/10.3390/catal15010029.

105. G. Prados-Joya, M. Sánchez-Polo, J. Rivera-Utrilla, and M. Ferro-garcía, "Photodegradation of the Antibiotics Nitroimidazoles in Aqueous Solution by Ultraviolet Radiation," *Water Research* 45, no. 1 (2011): 393–403, https://doi.org/10.1016/j.watres.2010.08.015.

106. J. Sun, R. Chu, and Z. U. H. Khan, "A Theoretical Study on the Degradation Mechanism, Kinetics, and Ecotoxicity of Metronidazole (MNZ) in •OH- and SO4•− Assisted Advanced Oxidation Processes," *Toxics* 11 (2023): 796, https://doi.org/10.3390/toxics11090796.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Description and georeferencing of collection points for surface water and effluents from the Meia Ponte River, in the State of Goiás, Brazilian Midwest. **Table S2:** Triple quadrupole mass spectrometer (MS/MS) parameters for antimicrobial analysis. **Table S3:** Linear ranges, correlation coefficient values, and quantification (LOQ) and detection (LOD) limits obtained during method validation. **Table S4:** Precision and accuracy **Figure S1:** Representative chromatograms of the antibiotic standards (black) and the blank sample (gray). **Figure S2:** Morphometric parameters evaluated in zebrafish larvae after 144 h of exposure. (A) sensory measurements: EA: eye area; IDmin: minimum interocular distance; IDmax: maximum interocular distance. The TL measurement, total length, serves as a parameter for the others. (B) physiological measurements: SBA: swim bladder area; SVA: yolk sac area. (C) skeletal/muscular measurements: TL: total length; HW: head width; HH: head height; DMA: distance from mouth to anus; and HL: head length.