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Anthropogenic impacts on water quality in southern Brazil: a multidimensional analysis of tributaries and the Chavantes reservoir

Impactos antrópicos na qualidade da água no sul do Brasil: uma análise multidimensional dos tributários e do reservatório de Chavantes

Laiana Domingues Queiroz[1], *Emanuely Cristina de Araúijo Batista*[1], *Eliezer de Oliveira da Conceição*[2], *Tatiane Mantovano*[1], *Anna Carolina Leonelli Pires de Campos*[1]

ABSTRACT

Freshwater is a vital resource, and its quality is critical for both human and environmental health. This study evaluated physicochemical and microbiological parameters of water from the Chavantes Reservoir, upstream tributaries to the reservoir, and downstream sites in the Paranapanema River and in its downstream tributaries, as well as the antimicrobial susceptibility of *Escherichia coli*. The results showed that variables such as temperature, turbidity, and total dissolved solids remained within the limits established by Brazil's Environment Council (CONAMA) Resolution 357/2005 and Minister's Office/ Ministry of Health (GM/MS) Ordinance 888/2021. In contrast, thermotolerant coliforms and *E. coli* exceeded the permitted values in several tributaries upstream. A strong correlation was observed between turbidity and coliforms, indicating fecal pollution sources related to the lack of sanitation and inadequate waste management. Antimicrobial resistance analysis revealed the presence of tetracycline- and gentamicin-resistant genes in *E. coli* strains, highlighting the impacts of agricultural activities and the indiscriminate use of antibiotics. Principal component analysis indicated greater dispersion among the upstream tributaries, reflecting high variability, while reservoir samples exhibited lower microbiological contamination, suggesting a dilution effect. These findings reinforce the need for continuous monitoring, riparian zone restoration, and the implementation of integrated sanitation and environmental management strategies. This study contributes to understanding anthropogenic impacts on water resources and supports public policies aimed at conserving water quality in the Chavantes Reservoir region.

Keywords: water quality assessment; bioindicators; fecal contamination; antimicrobial resistance; Paranapanema Basin.

RESUMO

A água doce é um recurso vital, e sua qualidade é determinante para a saúde humana e ambiental. Este estudo avaliou parâmetros físico-químicos e microbiológicos das águas do reservatório de Chavantes, de tributários a montante do reservatório e de trechos a jusante no rio Paranapanema e em seus tributários a jusante, além da suscetibilidade antimicrobiana de *Escherichia coli.* Os resultados mostraram que variáveis como temperatura, turbidez e sólidos dissolvidos totais permaneceram dentro dos limites estabelecidos pela Resolução do Conselho Nacional do Meio Ambiente (CONAMA) 357/2005 e pela Portaria do Gabinete do Ministro/Ministério da Saúde (GM/MS) 888/2021. Em contrapartida, coliformes termotolerantes e *E. coli* ultrapassaram os valores permitidos em diversos tributários a montante. Observou-se alta correlação entre turbidez e coliformes, indicando fontes de poluição fecal relacionadas à ausência de saneamento e ao manejo inadequado de resíduos. A análise de resistência antimicrobiana revelou genes de resistência à tetraciclina e à gentamicina em cepas de *E. coli*, evidenciando os impactos das atividades agropecuárias e do uso indiscriminado de antibióticos. A análise de componentes principais indicou maior dispersão entre os tributários a montante, refletindo elevada variabilidade, enquanto as amostras do reservatório apresentaram menor contaminação microbiológica, sugerindo efeito de diluição. Esses achados reforçam a necessidade de monitoramento contínuo, recuperação de áreas ripárias e implementação de estratégias integradas de saneamento e gestão ambiental. O estudo contribui para a compreensão dos impactos antrópicos sobre os recursos hídricos e subsidia políticas públicas voltadas à conservação da qualidade da água na região do reservatório de Chavantes.

Palavras-chave: avaliação da qualidade da água; bioindicadores; contaminação fecal; resistência antimicrobiana; Bacia do Rio Paranapanema.

[1]Universidade Estadual do Norte do Paraná – Jacarezinho (PR), Brazil.
[2]Universidade Estadual do Paraná – Paranaguá (PR), Brazil.
Corresponding author: Anna Carolina Leonelli Pires de Campos – Grupo de Estudos e Pesquisa em Recursos Hídricos e Ecologia Aplicada, Universidade do Norte do Paraná – Av. Manoel Ribas, 215 – Centro – CEP: 86400-000 – Jacarezinho (PR), Brazil. E-mail: aclpcampos@gmail.com
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Introduction

Water is an essential element for the existence of life on Earth, and Brazil possesses around 15% of the volume of freshwater available in the world. Distributed in underground aquifers and surface water bodies, it is used for population supply, recreation, navigation, industrial activity, agribusiness, energy generation, mineral extraction, tourism and leisure (Montoya and Finamore, 2020; NWA, 2020).

In Paraná, there are 16 hydrographic basins, such as the Itararé basin and the Paranapanema basins 1, 2, 3 and 4 (SEMA, 2010). The Chavantes reservoir, located in the Paranapanema 1 and Itararé basins, has an area of 419 km^2 and a water storage capacity of 9.4 billion m^3, with a 1,085 km shoreline borders that bathes 15 municipalities in Paraná and São Paulo, including Carlópolis, Jacarezinho, Ribeirão Claro, Chavantes and Piraju (CTG Brasil, 2023).

Beyond the energy generation function provided by the reservoir's turbines, the reservoir has stimulated tourism in the "Angra Doce" region, promoting activities such as boat trips, fishing, camping and lodging by the water (NWA, 2019). The area is also heavily occupied by agriculture, livestock and industries, such as dairies and meatpacking plants (SEMA, 2010).

Freshwater conservation is essential not only to ensure a good quality of life, but also to minimize conflicts related to water resources. However, the preservation of water sources faces significant challenges due to the introduction of microbiological, chemical and emerging contaminants, such as antibiotics, from untreated sewage, industrial activities and agricultural practices (Abdulmumin Muhammad et al., 2024; Sikhakhane Nwokediegwu et al., 2024).

Globally, some microbiological indicators are used to examine the contamination of water bodies and among the most expressive microorganisms are enterobacteria, of the thermotolerant coliform group, *Escherichia coli*. These pathogens are mostly transmitted by the fecal-oral route and are directly associated with the contamination of water sources (APHA, 2017; Vasconcellos et al., 2022). Antimicrobial resistance, previously focused on clinical settings, is also emerging as a critical environmental issue. Recent studies indicate that the environment acts as a reservoir for resistant bacteria and their genes, highlighting the need for integrated monitoring and mitigation strategies to reduce the impacts of this contamination (Morin-Crini et al., 2022; Singh et al., 2022; Parry, 2025). Brazilian legislation, such as the Minister's Office/Ministry of Health (GM/MS) Ordinance No. 888, which amends Annex XX of Consolidation Ordinance No. 5 of the MS, establishes strict quality control standards for water sources intended for human consumption, with an emphasis on the analysis of *E. coli* as an indicator of fecal contamination (Brasil, 2021). Brazil's National Environment Council (CONAMA) Resolution No. 357 provides for the classification of hydric bodies and the environmental guidelines for their classification, and establishes the parameters for the discharge of effluents.

Given the relevance of water uses in this region, this study aimed to evaluate water quality along the Chavantes Reservoir, upstream tributaries to the reservoir, and downstream sites in the Paranapanema River and its downstream tributaries in Paraná, focusing on physicochemical and microbiological parameters, as well as *E. coli* antimicrobial resistance.

Despite the growing use of water for recreation, there are few studies and monitoring programs that assess bathing conditions, especially in freshwater bodies of water in Paraná, including the Paranapanema and Itararé rivers, since both the Chavantes reservoir and tributaries stand out for their multiple uses (SEMA, 2010; NWA, 2018; Branco et al., 2019).

Material and Methods

Study area

The Chavantes reservoir, upstream tributaries to the reservoir, and downstream sites in the Paranapanema River and in its downstream tributaries are in the Paranapanema 1 and Itararé river basins. According to data provided by the Instituto Água e Terra (IAT, 2020), the area of the Paranapanema 1 Basin is 1,238.91 km^2, with soils with a varied texture, from clayey to sandy, predominantly Nitisol, Red Latosol and Argillosols in undulating to gently undulating reliefs. The Itararé basin covers 5,007.28 km^2 and has several aquifers. In this basin, there is also a greater occurrence of Red-Yellow Argillosols. In its southern portion, Humic Cambisols and undulating relief predominate (IAT, 2020). The Chavantes reservoir borders 15 municipalities, among which two are highlighted in this study: Carlópolis and Ribeirão Claro (Figure 1).

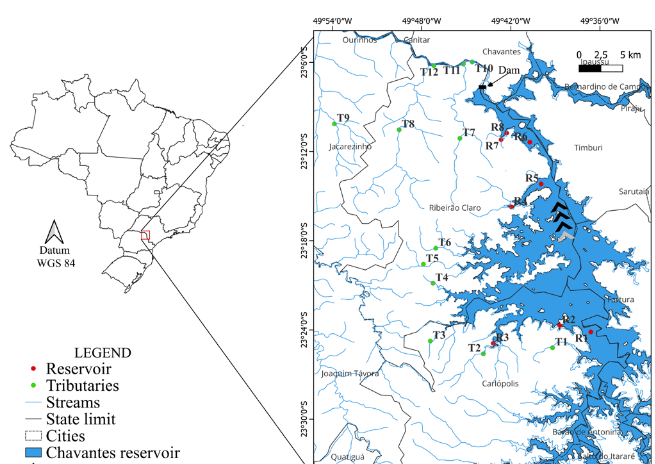


Figure 1 – Sampling locations in upstream tributaries to the reservoir (T1–T6), in-reservoir sites (R1–R8), and downstream sites in the Paranapanema River (T10–T12) and its downstream tributaries (T7–T9), in the municipalities of Carlópolis, Ribeirão Claro and Jacarezinho, northern Paraná, Brazil.

Strategic areas of the Chavantes Reservoir were selected by sampling of 20 points (Table 1). Eight samples were collected within the Chavantes Reservoir, located in the municipalities of Carlópolis and Ribeirão Claro. Twelve samples were collected outside the reservoir, comprising six in upstream tributaries to the reservoir (Espírito Santo Stream, Jaboticabal Stream, Novo River, Água da Mula Stream, Taquaruçu Stream, and Da Cruz Stream) and six at downstream sites: three in tributaries of the Paranapanema River (Claro Stream, Anhumas Stream, and Ouro Grande River) and three in the Paranapanema River below the dam (Paranapanema River 1, 2, and 3). Downstream sites are below the dam, and do not contribute to the reservoir's water mass. This sampling design covers various zones of the reservoir under different anthropogenic impacts, including areas with pastures, proximity to highways, as well as industrial, fishing, and tourism activities (see Supplementary Material).

Three samples were collected between September and October 2023, a period of variable temperatures with mild maxima (18 to 23°C) and above-average precipitation, particularly in October. In September, rainfall was below the average of 164.0 mm, but this was offset by a record volume in October, which exceeded the monthly average by more than 90 mm (IDR-Paraná, 2023).

Microbiological analysis

For this study, triplicate water samples were collected at each site in sterile 100 mL bottles for microbiological analysis of TC, thermotolerant coliforms (TTC), and *Escherichia coli*. Total and thermotolerant coliform counts were carried out using Fluorocult LMX Broth modified (Merck KGaA, Darmstadt, Germany) and the Most Probable Number (MPN) technique. Three sets of tubes with inverted Durham tubes were inoculated with 1, 0.1 and 0.01 mL of each water sample. The tubes were incubated at 37 °C for 48 hours in a bacteriological oven, after which the results were read.

In addition, Lauryl Sulfate Tryptose Broth with 4-methylumbelliferyl-β-D-glucuronide — MUG was used as a fluorogenic medium to allow rapid detection of E. coli, by observing fluorescence under ultraviolet (UV) light at 366 nm. The presence of TC was indicated by gas formation and turbidity of the medium, without fluorescence, while the presence of *E. coli* was confirmed by fluorescence due to MUG hydrolysis by β-glucuronidase. Tubes that showed turbidity and gas production but were negative for fluorescence and indole were considered positive for TC. Tubes with gas production, turbidity of the medium, light blue fluorescence, and a positive indole test after addition of 0.3

Table 1 – Location of collection points in-reservoir sites (R1–R8), upstream tributaries to the reservoir (T1–T6); downstream sites in the Paranapanema River (T10–T12) and in its downstream tributaries (T7–T9).

Locations collected (reservoir and tributaries)	Location (latitude, longitude)	Position
Reservoir collection point 1 (R1)	-23.402226, -49.610217	In the reservoir
Reservoir collection point 2 R2)	-23.394961, -49.645185	In the reservoir
Reservoir collection point 3 (R3)	-23.414983, -49.719768	In the reservoir
Reservoir collection point 4 (R4)	-23.262020, -49.699409	In the reservoir
Reservoir collection point 5 (R5)	-23.236369, -49.666020	In the reservoir
Reservoir collection point 6 (R6)	-23.189532, -49.678611	In the reservoir
Reservoir collection point 7 (R7)	-23.187348, -49.711145	In the reservoir
Reservoir collection point 8 (R8)	-23.186600, -49.710872	In the reservoir
Espírito Santo stream (T1)	-23.419957, -49.653167	Upstream
Jaboticabal stream (T2)	-23.426812, -49.730817	Upstream
Novo river (T3)	-23.412438, -49.790351	Upstream
Água da Mula stream (T4)	-23.347615, -49.787313	Upstream
Taquaruçu stream (T5)	-23.326361, -49.798079	Upstream
da Cruz stream (T6)	-23.308406, -49.784177	Upstream
Claro stream (T7)	-23.185187, -49.757102	Downstream
Anhumas stream (T8)	-23.175591, -49.825303	Downstream
Ouro Grande river (T9)	-23.168962, -49.897949	Downstream
Paranapanema river (T10)	-23.099551, -49.743303	Downstream
Paranapanema river (T11)	-23.101880, -49.753268	Downstream
Paranapanema river (T12)	-23.103823, -49.786290	Downstream

T: tributaries; R: reservoir.

mL of Kovacs reagent were considered TTC (Merck, 2005; Chantarasiri et al., 2015).

The results were expressed as MPN/100 mL, according to the Environmental Sanitation Technology Company of the State of São Paulo — CETESB (2018) and CONAMA (2005). After confirming TTC, methylene blue eosin (EMB) agar plates (Merck) were used to detect the presence of *Escherichia coli*. A sterile loop was dipped into the broth of each positive tube and streaked onto the plates, which were then incubated and inspected for characteristic bacterial growth (Anwar et al., 2010; Ali et al., 2011; Lal and Cheeptham, 2007).

Antibiogram

From the growth of *E. coli* on the plates, five colonies were isolated from each positive sample and the antimicrobial susceptibility test was carried out using the disk diffusion method on Muller - Hinton agar (Merck KGaA, Darmstadt, Germany), according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2021). A total of 16 different antimicrobial disks from six different classes were used to assess the susceptibility of the *Escherichia coli* isolates. The antimicrobial agents under examination included amoxicillin+clavulanate (AMC), ampicillin (AMP), aztreonam (ATM), cefoxitin (CFO), ciprofloxacin (CIP), cefepime (CPM), chloramphenicol (CLO), enrofloxacin (ENR), gentamicin (GEN), imipenem (IMP), meropenem (MPM), nalidixic acid (NAL), piperacillin+tazobactam (PIT), streptomycin (STM), sulfamethazole+trimethoprim (SUT), tetracycline (TET). The reference values previously tabulated by the CLSI criteria (2021) were used to classify susceptibility.

Physicochemical analysis

A Horiba model U-52G multi-parameter probe was used *in loco* to measure dissolved oxygen, hydrogen potential (pH), biochemical oxygen demand (BOD), water temperature, turbidity and total dissolved solids (TDS). The measured values were displayed in bar plots using the 'ggplot2' (Wickham, 2016), 'tidyverse' (Wickham et al., 2019), and 'patchwork' (Pedersen, 2024) packages in the R platform version 4.3.1 (R Core Team, 2024).

Statistical analysis

A basic descriptive statistical analysis was carried out to interpret the maximum and minimum values for the physicochemical and microbiological analyses, with arithmetic averages and percentages, and the results are shown in the tables. To assess the relationship between the physicochemical and microbiological variables of the water samples collected in the reservoir and both the upstream and downstream tributaries, a Pearson correlation analysis was carried out using the Microsoft 365 Excel program, where correlation values ranged from -1 (perfect negative correlation) to 1 (perfect positive correlation). The correlation values were presented directly on the graph, and the variables were organized on both axes to facilitate interpretation. Principal component analysis (PCA) has been widely used for water quality monitoring and management, due to the lack of defined water quality assessment criteria and the complex range of contamination sources (Sharif et al., 2015; Singh et al., 2017; Eid et al., 2024). PCA was applied in this study to reduce data dimensionality and identify patterns of variation between samples. Data were normalized to ensure that all variables had the same scale, minimizing the impact of differences in magnitude between them. The 'ggplot2' package was used to obtain graphical visualizations of the results. All analyses were performed using the R platform version 4.3.1 (R Core Team, 2024).

Results and Discussion

Figure 2 shows the results obtained for the physical-chemical parameters assessed at the different points in the Chavantes Reservoir (R1–R8), upstream tributaries to the reservoir (T1–T6), and downstream sites in the Paranapanema River (T10–T12) and in its downstream tributaries (T7–T9) in Paraná. The values obtained for each parameter analyzed were compared to those described in CONAMA Resolution 357 of 2005, which determines the classification of Class 2 raw water bodies and regulates water quality based on its designated uses, establishing limits for the discharge of harmful substances into rivers, lakes, and other aquatic systems, and to Ordinance 888 of 2021, which determines the quality of surface water.

Temperature values ranged from 17.56 to 28.60°C, with an average of 25.6°C, all within the limit set by CONAMA Resolution 357/2005 for Class 2 freshwaters (≤40C). The variation observed reflects the environmental conditions of the collection season (spring), characterized by higher temperatures, which can influence the increase in biological activity and, consequently, in BOD.

Coates et al. (2022) highlight that periods of elevated temperatures are associated with an increased incidence of anoxia, resulting from intensified microbial metabolism, which consumes more oxygen, and the reduced solubility of gases in water. As water temperature rises, the solubility of gases, including oxygen, decreases significantly, thereby compromising the levels of dissolved oxygen available in aquatic ecosystems. The literature demonstrates that this decline in solubility becomes particularly pronounced above 35°C, as observed with noble gases, and this trend can be extrapolated to understand similar behavior in oxygen (Schwenk et al., 2022). At the same time, higher temperatures accelerate microbial metabolism, especially in eutrophic environments, intensifying oxygen consumption and worsening anoxic conditions (Coates et al., 2022).

The pH values ranged from 7.09 to 10.70, with an average of 8.23. Although most points were within the limits established by CONAMA Resolution 357/2005 (6.0 to 9.0), two exceeded the upper limit: R3 (9.64) and T9 (10.70). These deviations can be attributed to high organic loads resulting from anthropogenic activities, such as the discharge of domestic sewage, agricultural runoff, and industrial processes.

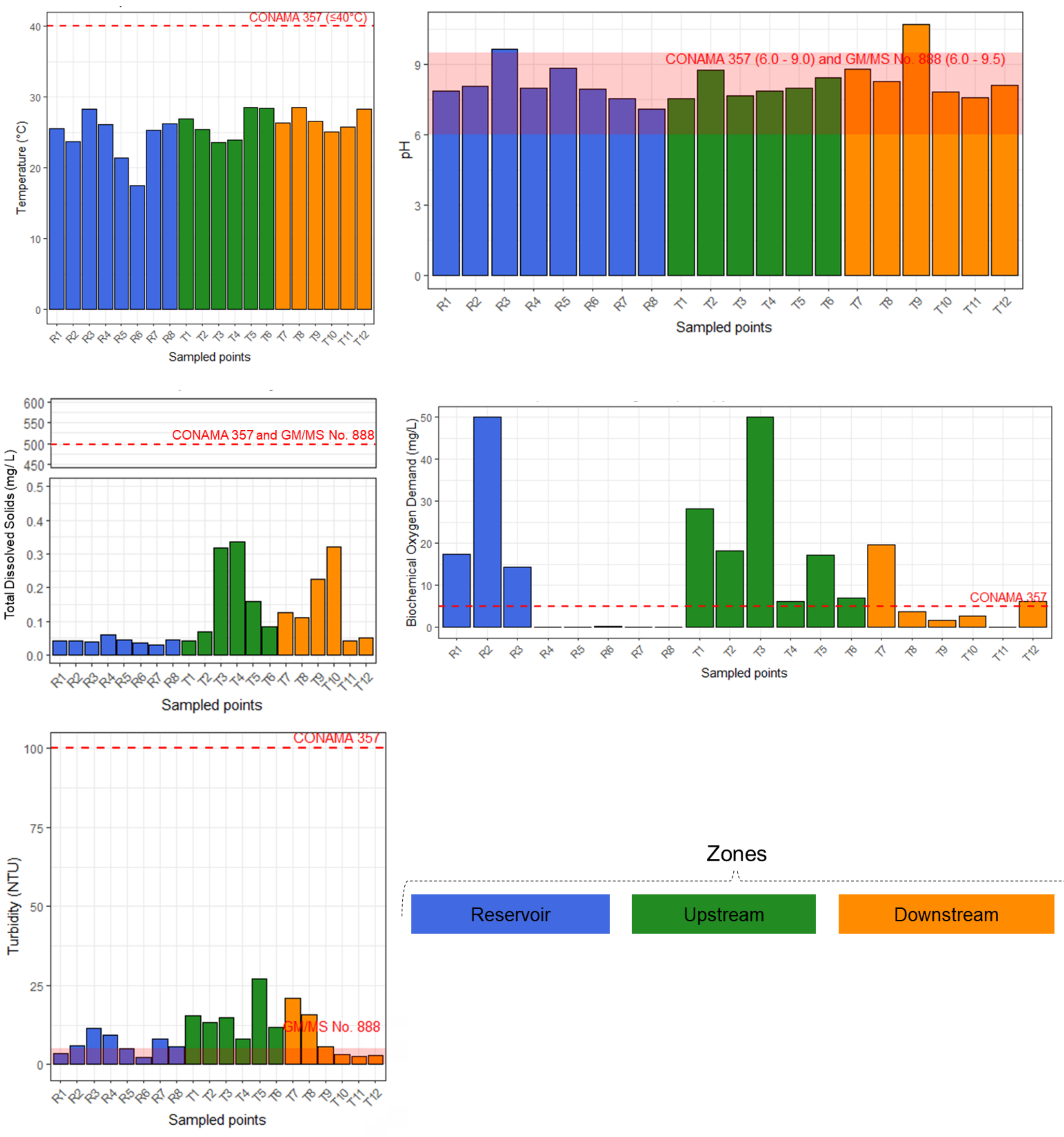


Figure 2 – Mean physical and chemical parameters of water samples from Chavantes Reservoir (R1–R8), upstream tributaries to the reservoir (T1–T6), and downstream sites in the Paranapanema River (T10–T12) and in its downstream tributaries (T7–T9) in Paraná, and the maximum values allowed according to legislation of the Ministry of Health — MS (2017), amended by Minister's Office — GM/MS Ordinance No. 888 (2021)* and 357 Resolution, National Environment Council — CONAMA (2005) — river class 2. *recommended value to pH.

Additionally, geological formations and climate change can alter water alkalinity, especially when minerals present in the soil contribute to natural increases in pH (Akhtar et al., 2021). Moreover, pH and temperature influence the distribution of dissolved substances in rivers and lakes. In lakes, pH tends to be higher at the surface due to photosynthesis, which consumes carbon dioxide, and lower at the bottom due to respiration, which releases the gas. This pH gradient contributes to high concentrations of dissolved substances in the hypolimnion, a region with low oxygen levels (Brasil, 2006). Elevated pH levels impact water quality by influencing nutrient availability and microbial activity, potentially altering aquatic species composition and promoting algal blooms (Dewangan et al., 2023).

The turbidity of the samples ranged from 2.34 to 27.20 NTU, with an average of 9.64 NTU, all below the maximum limit established by CONAMA Resolution 357/2005 for Class 2 waters and by Ordinance 888/2021 (≤100 NTU). This low turbidity indicates a high level of water quality, suggesting minimal suspended solids and organic matter, which are generally associated with domestic sewage. Thus, low turbidity serves as a positive indicator of overall water quality in this context (Halicki and Halicki, 2022).

The increase in BOD in water bodies can be attributed to both point sources, such as domestic and industrial effluent discharges (Mondal et al., 2022), and diffuse sources, such as nutrient-laden agricultural runoff (Shim et al., 2023; Navratilova et al., 2025). Hydrological factors, such as water temperature and river flow, also directly influence microbial activity, intensifying BOD levels (Marcheva et al., 2024). Accelerated urbanization and intensive agriculture further aggravate this scenario, especially during periods of high temperatures and prolonged droughts (Shim et al., 2023). In comparison with other studies, such as that of Ribeiro et al. (2022) in the Paraná River, where 57% of the samples exceeded the BOD limit, the results of this work reinforce the need for integrated and continuous water quality monitoring actions, especially in regions with multiple uses of water resources.

The TDS results ranged from 0.031/L to 0.320 mg/L, with an average of 0.111 mg/L, all within the limit of 500 mg/L established by legislation. This is an excellent indicator of water quality, considering that high concentrations of dissolved solids can compromise the use of water for human consumption and other purposes. Furthermore, TDS concentrations are strongly influenced by river flow and seasonal variations. High flows promote dilution of dissolved solids, while low flows increase their concentration. Dry seasons tend to have higher TDS levels compared to rainy seasons, due to lower dilution and increased evaporation (Chui et al., 2023; Putman et al., 2024).

The quantification of TC and TTC revealed high contamination at various points in the water bodies analyzed, indicating a significant environmental impact (Table 2). In the TC analysis, 55% of the points (R2, R4, R7, R8, T1, T2, T4, T5, T7, T8 and T10) showed values higher than 16,000 NMP/100 mL. This result reflects the influence of anthropogenic activities, especially in areas close to communities, highways and pastures, where there is inadequate sewage disposal and poor soil management (see Supplementary Material). Points with lower counts (≤3,500 NMP/100 mL) are in areas with less anthropogenic impact, corroborating studies such as Fuentes et al. (2024), who also associated moderate levels of TC with recreational areas with less human interference. Additionally, data from a survey in rural and urban areas indicate that 77.54% of water samples collected in rural areas were contaminated by coliforms, in contrast to only 10.95% in urban areas, reinforcing the association between environmental vulnerability and inadequate sanitation standards. The study also points to a correlation between rainfall patterns and increased bacterial contamination, indicating potential risks in various water sources, even if not exclusively focused on rivers and dams (Santos et al., 2023).

As for TTC, CONAMA Resolution 357 (2005) establishes a limit of 1,000 NMP/100 mL for class 2 fresh waters. Of the 20 points sampled, 35% (T1, T2, T4, T5, T7, T8 and T12) exceeded this limit, with values between 1,700 and >16,000 NMP/100 mL. Significant contamination in tributaries is directly related to reduced flow and proximity to urbanized areas (see Supplementary Material), which converges with the pattern observed in other regions of the world (Davis et al., 2022; Díaz-Gavidia et al., 2022).

In contrast, in Brazil, Santos et al. (2023) reported alarming levels of contamination in rural areas, where 77.54% of the samples were contaminated with coliform bacteria and 44.50% with *Escherichia coli*. Urban areas, on the other hand, showed significantly lower contamination rates, with 10.95% of samples containing coliforms and only 2.53% containing *E. coli*. Another relevant factor is the impact of rainfall: in rural areas, 20.95% of the contaminated samples were associated with precipitation events, while in urban areas this association was observed in 9.33% of the samples. This suggests that rainfall exacerbates water contamination, possibly through surface runoff or inadequate infiltration (Santos et al., 2023).

However, this overlap of factors — disorderly urban expansion, poor infrastructure, intensive agricultural practices, and climate variations — creates a challenging scenario for water quality management (Singh et al., 2022).

As for *Escherichia coli*, current legislation (Ordinance GM/MS No. 888/2021) requires the absence of this bacterium in water intended for human consumption. However, 100% (7/7) of the points that exceeded the permitted limit for TTC showed the presence of *E. coli*. This association has already been observed in other contexts: in Cascavel (PR), for example, all six rivers analyzed presented high levels of TTC and confirmed presence of *E. coli*, evidencing the link between exceeding legal limits and the presence of this pathogenic bacterium (Malagi et al., 2020). In the La Paz River basin in Bolivia, 100% of samples from impacted sites revealed contamination by enteropathogens, including *E. coli*, precisely when the levels of TTC exceeded health guidelines

(Poma et al., 2016). In addition, this same study revealed that half of the enteropathogens identified were resistant to multiple antibiotics, which increases the risk to public health. In African countries such as South Africa and Nigeria, studies have found the presence of pathogenic strains of *E. coli* in drinking and recreational water sources, reiterating the threat that these contaminated waters pose to vulnerable communities (Potgieter et al., 2020; Dick and Okparanta, 2022; Delair et al., 2024). These data reinforce the importance of constant monitoring of water quality and the rigorous implementation of public policies.

Varied antimicrobial resistance profiles were observed at the sampling sites where *E. coli* was detected. In upstream tributaries T2, T4, and T5, resistance to tetracycline (TET) was predominant, being detected in 100% of the samples in T2, in 60% of the samples in T4, and in 20% of the samples in T5. Additionally, T5 also showed resistance to gentamicin (GEN) in 60% of the samples. Site T2 corresponds to the surface water intake used for part of the municipal supply of Carlópolis (Sanepar, 2021), and is characterized by a highway crossing above the river, nearby rural properties with grazing areas, and preserved riparian vegetation. Site T4 is similarly influenced by a highway and rural pastures, but also exhibits the presence of domestic waste along the river margins, although the riparian forest remains preserved. Site T5 is also intersected by a highway and surrounded by rural land use, with maintained riparian vegetation.

Previous studies in several regions of Brazil and Latin America also observed antibiotic resistance in *E. coli* isolates: in the Tietê Ecological Park (SP), high resistance to erythromycin (82%) and amoxicillin was observed, with multidrug-resistant strains more common in the hot and rainy seasons (Storto et al., 2021). *E. coli* strains isolated from the Meia Ponte River, in the Central-West region of Brazil, showed high resistance to antibiotics of the lincosamide and β-lactam classes.

Table 2 – Mean bacterial count of water samples, by most probable number, from the Chavantes Reservoir (R1–R8), upstream tributaries to the reservoir (T1–T6), and downstream sites in the Paranapanema River (T10–T12) and in its downstream tributaries (T7–T9) in Paraná, with the maximum value allowed according to legislation and antimicrobial resistance profile of isolated *E. coli*.

	Collection points	TC *(MPN/ 100 mL)	TTC** (MPN/100 mL)	*E. coli* resistance profile [number (%)]
Reservoir	R1	3,500	<18	NA****
	R2	>16,000	<18	NA****
	R3	1,300	<18	NA****
	R4	>16,000	<18	NA****
	R5	45	<18	NA****
	R6	<18	<18	NA****
	R7	>16,000	<18	NA****
	R8	>16,000	<18	NA****
Upstream	T1	>16,000	>16,000	Sensible [5/5 (100%)]
	T2	>16,000	>16,000	TET [5/5 (100%)]; GEN [1/5 (20%)]
	T3	210	210	NA****
	T4	>16,000	>16,000	TET [3/5 (60%)]
	T5	>16,000	>16,000	TET [1/5 (20%)]; GEN [3/5 (60%)]
	T6	410	20	NA****
Downstream	T7	>16,000	>16,000	Sensible [5/5 (100%)]
	T8	>16,000	>16,000	Sensible [5/5 (100%)]
	T9	110	20	NA****
	T10	>16,000	20	NA****
	T11	3,500	130	NA****
	T12	3,500	1,700	Sensible [5/5 (100%)]
	Attachment XX of Ordinance nº 5 of the Ministry of Health (2017) amended by Ordinance GM/MS No. 888 (2021)	NR***	NR***	NR***
	357 Resolution, CONAMA (2005) — river class 2	NR***	≤1000	NR***

*TC: total coliforms; **TTC: thermotolerant coliforms; ***NR: not regulated; ****NA: not applicable.

A significant percentage, 59.37%, was classified as multidrug-resistant, that is, resistant to multiple classes of antibiotics. In all samples tested, the *sul*2 and *erm*C resistance genes were detected, evidencing widespread dissemination of these genetic elements in the aquatic environment. The statistical analysis also showed a strong anthropogenic impact on the river basin, raising doubts about the effectiveness of current Brazilian legislation to contain contamination (Gomes et al., 2023).

In Rio Grande do Sul, in Lajeado Pardo, there was total resistance to ampicillin and high rates for other antibiotics such as ciprofloxacin (50%) (Flach et al., 2024). Finally, in Bolivia, in the La Paz River basin, all samples from impacted areas presented E. coli and other enteropathogens, and half of these bacteria showed resistance to multiple antibiotics, aggravating health risks (Poma et al., 2016).

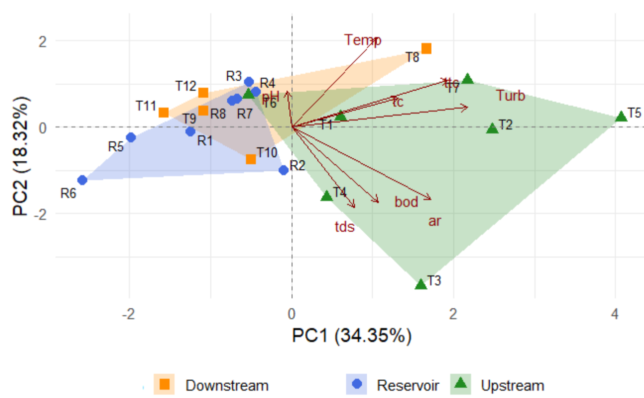
The detection of antimicrobial resistance is worrying, as it represents a risk not only to the environment, but also to public health, due to the possibility of transmitting resistance genes to other pathogens. This reinforces the need for continuous monitoring and stricter regulations on the use of antimicrobials in rural and urban areas.

Also, from the correlation matrix shown in Table 3, it can be seen that the parameters of turbidity and TTC showed a strong positive correlation (r=0.71), indicating that higher levels of turbidity are related to higher concentrations of TTC and pH and TC showed a moderate negative correlation (r=-0.39), suggesting that water with a lower pH may have a higher amount of TC.

TC (NMP/100mL) showed a moderate correlation with turbidity (r=0.38) and with TTC (r=0.58). On the other hand, pH and TDS showed weak correlations with TC and TTC. The weak correlation between pH, TDS and TC and TTC can be attributed to the complexity of the processes that affect water quality. Although pH and the concentration of dissolved solids influence water characteristics, the presence of coliforms is more related to fecal contamination and factors such as organic matter and sanitation management. Therefore, it is possible that the variation in pH and TDS is not a determining factor for the presence of these microorganisms, which depend more directly on specific sources of pollution and sanitary conditions than on physical-chemical parameters.

The Principal Component Analysis (PCA) graph with clusters was generated to explore the separation between the groups of samples collected from the Reservoir (R1–R6), the upstream tributaries to the reservoir (T1–T6), and the downstream sites in the Paranapanema River and its downstream tributaries (T7–T12). The analysis was based on physical, chemical, and microbiological variables, and the first two principal components jointly explained 52.67% of the total variance of the data (34.35% for the first component and 18.32% for the second) (Figure 3).

The points related to the tributaries (T) show greater dispersion in the PCA space, reflecting higher variability in the measured characteristics. Samples T2, T3, T4, and T5 (upstream tributaries) stand out as being more distant from the cluster containing the reservoir samples, indicating significantly different characteristics, particularly associated with turbidity, antibiotic resistance, TTC, and TDS.



*Temp: temperature; Turb: turbidity; tds: total dissolved solids, bod: biochemical oxygen demand, pH; ar: antibiotic resistance, ttc: termotolerant coliforms, tc: total coliforms.

Figure 3 – Principal component analysis (PCA) graph PC1 × PC2 with the collection points from the Chavantes Reservoir (R1–R8), upstream tributaries to the reservoir (T1–T6) and the downstream sites in the Paranapanema River and its downstream tributaries (T7–T12) on the physical, chemical and microbiological analyses. PC1 explains 34.57% of the variation in the data, while PC2 explains 18.32%.

Table 3 – Correlation matrix between the water parameters analyzed.

	Temp. (°C)	pH	Turbidity (NTU)	BOD (mg/L)	TDS (mg/L)	TC (MNP/100 mL)	TTC (MNP/100 mL)
Temperature (°C)	-						
pH	0.16	-					
Turbidity (NTU)	**0.44**	0.07	-				
BOD (mg/L)	-0.02	-0.10	**0.36**	-			
TSD (mg/L)	-0.03	0.08	0.15	0.15	-		
TC (MNP/100 mL)	0.24	**-0.39**	**0.38**	0.07	0.08	-	
TTC (MNP/100 mL)	0.27	-0.02	**0.71**	0.15	0.19	**0.58**	-

Temp. (°C): temperature; BOD: biochemical oxygen demand; TDS: total dissolved solids; TC: total coliforms; TTC: thermotolerant coliforms. Numbers in bold indicate moderate or strong correlation.

These variables are more strongly related to the tributaries, suggesting greater vulnerability to pollution in these sites, possibly due to surface runoff, lack of sanitation, and agricultural activities.

In contrast, downstream sites, T7–T9 in tributaries of the Paranapanema River and T10–T12 in the Paranapanema River, are positioned closer to the reservoir group, indicating more similar characteristics, even though they are located below the dam and do not contribute to the reservoir's water mass. Accordingly, values observed at these downstream sites are interpreted as a downstream impact gradient, not as drivers of the reservoir's intrinsic water quality. The smaller dispersion of the reservoir samples (R) indicates more homogeneous water quality, suggesting that the dam may act as a dilution and regulation zone, reducing the microbiological and physicochemical variability observed in the tributaries. Thus, the graph highlights the influence of local environmental conditions on tributaries (particularly upstream), underscoring the importance of management strategies to reduce pollutant inputs into the reservoir.

The results of this study are in line with other reports in the literature, such as Poma et al. (2016), who observed a high microbial load in areas of greater anthropogenic interference. In addition, Malagi et al. (2020) reported massive contamination in urbanized areas, highlighting that the lack of basic sanitation and inadequate sewage disposal are the main contributing factors to the degradation of water quality.

The absence of riparian forests, essential for the protection of water bodies, compromises the microbiological quality of the water, alters soil permeability and intensifies the leaching of substances such as antibiotics (Filoso et al., 2017; Bortoloti et al., 2018; Frak and Jankiewicz, 2018). In livestock farming, the intensive use of antibiotics to ensure food quality and safety results in the excretion of more than 90% of these drugs into the environment, causing bioaccumulation and leaching, in addition to environmental impact (Amarasiri et al., 2020; Seyoum et al., 2024).

On the other hand, in regions with a larger water course and volume, such as the Chavantes reservoir, the results are more promising, with an absence of TTC and *E. coli*, indicating that the pollution of the tributaries, the reservoir or the Paranapanema River, is in fact of anthropogenic and agricultural origin. This scenario highlights the importance of conservation and recovery measures for riparian areas, as well as investments in basic sanitation and environmental education.

Conclusion

The results obtained in this study highlight the complexity of the water quality of the Chavantes reservoir, upstream tributaries to the reservoir, downstream sites in the Paranapanema River and in its downstream tributaries, emphasizing both natural and anthropogenic factors as determinants of this variability. Although physical-chemical parameters such as temperature, turbidity and TDS were mostly within the limits set by national regulations, microbiological indicators such as TTC and *Escherichia coli* showed worrying levels, especially at points with greater anthropogenic influence.

High microbiological contamination, associated with the presence of antimicrobial resistance genes, highlights the influence of intensive agricultural and livestock practices, as well as the lack of adequate basic sanitation in adjacent urban and rural areas. These results are in consonance with previous studies, confirming the central role of inadequate waste management and the absence of riparian forests in the degradation of water quality.

The statistical analysis reinforced the influence of point and diffuse sources of pollution, with the upstream tributaries showing greater dispersion in the data and variability in the measured characteristics, while the reservoir showed better overall quality, highlighting its role as a dilution and buffer zone. However, the high BOD loads and the presence of *E. coli* in tributaries the reservoir and the Paranapanema River warn of the risk of compromising the aquatic ecosystem and human health, mainly due to the possible spread of pathogens and antimicrobial resistance genes.

This study therefore emphasizes the urgency of integrated measures for the conservation and sustainable management of the region's water resources. Investments in basic sanitation, restoration of riparian forests and continuous monitoring are crucial to mitigate the impacts of anthropogenic activities and guarantee water quality. It should also be noted that further studies should focus on the genetic characterization of resistant bacteria, and effective control actions, such as environmental education initiatives and regulating the use of antibiotics in agriculture, are fundamental to minimizing the risks associated with antimicrobial resistance. These efforts not only contribute to environmental preservation but are also essential for public health and sustainable development in the region.

Authors' contributions

Queiroz, L.D.: investigation, methodology, resources, writing – original draft. **Batista**, E.C.A.: investigation, methodology, resources, writing – original draft. **Conceição**, E.O.: formal analysis, software, resources, writing – review & editing. **Mantovano,** T.: formal analysis, software, resources, writing – review & editing. **Campos**, A.C.L.P.: conceptualization, data curation, formal analysis, methodology, resources, project administration, supervision, writing – original draft, writing – review & editing.

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