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# Assessment of Water Quality and Presence of Enterobacteria in the Billings-Tamanduateí Watershed and Its Relationship with Social Indicators

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**Abstract:** Water quality in urban streams is closely linked to socioeconomic conditions, particularly in densely populated and poorly sanitized areas. This study evaluates the physicochemical and microbiological quality of water in the Billings-Tamanduateí sub-basin and investigates its correlation with social indicators such as population density, informal settlements, and lack of sewage treatment. Water samples were collected from 14 sub-basins, analyzing parameters, including dissolved organic and inorganic carbon, total dissolved nitrogen, pH, dissolved oxygen, oxidation-reduction potential, conductivity, and the presence of enterobacteria (*Escherichia coli*, *Enterococcus*, and *Pseudomonas aeruginosa*). Statistical analyses revealed strong associations between water quality degradation and increased population density, lack of sanitation, and lower per capita income. The findings reinforce that socially vulnerable areas are the most affected, with higher levels of organic and microbiological contamination. Despite improvements in sewage collection over time, gaps in wastewater management persist, even in major metropolitan areas such as São Paulo. Future studies could expand the analysis to include less vulnerable regions for comparison and explore the impacts of climate change on urban stream water quality. The results highlight the urgent need for improved sanitation infrastructure and policies to mitigate contamination and protect public health.

**Keywords:** urban water quality; socioeconomic factors; sewage treatment; fecal indicators; population density; environmental pollution; climate change impacts; São Paulo metropolitan area

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## 1. Introduction

The Metropolitan Region of São Paulo (MRSP), home to approximately 21 million inhabitants [1], is the largest urban agglomeration in South America and the sixth largest worldwide. Despite being a hub for essential services such as healthcare, education, and employment, the region still faces significant challenges in urban infrastructure, particularly in basic sanitation. In 2022, about 7.6% of the MRSP population [settled in regular areas] lacked access to sewage collection, leading to the discharge of 511,199 $m^3$ of untreated sewage into the environment [2].

The MRSP is situated within the Upper Tietê Basin, which entirely corresponds to the Water Resources Management Unit n. 06 (Unidade de Gerenciamento de Recursos Hídricos n. 06—UGRHI06) of the state of São Paulo. This region features multiple rivers naturally obstructed by the Serra do Mar Mountain range. Historically, the area has undergone significant hydrological interventions, such as the Serra Project in the 1920s, aimed at water storage for hydroelectric power generation. In the 1940s, to enhance the capacity of the Henry Borden Power Plant, the Tietê River was partially diverted, and the course of the Pinheiros River was reversed [3].

The Billings Reservoir, covering an area of 150 $km^2$, is one of the region's primary water systems, supplying municipalities in the Greater ABC region (which comprises the municipalities of Santo André, São Bernardo do Campo, São Caetano do Sul, Diadema, Mauá, Ribeirão Pires, and Rio Grande da Serra) and parts of São Paulo. Located within the Billings-Tamanduateí watershed, this area includes the Billings Watershed Protection and Recovery Area, forming a crucial component of the MRSP's water supply system [4].

The unregulated expansion of the MRSP directly affects the water quality of urban streams, which frequently receive untreated sewage due to the irregular occupation of floodplain areas and the absence of sanitation infrastructure [5]. This discharge of effluents increases nutrient loads, such as nitrogen (N), carbon (C), and phosphorus (P), triggering eutrophication—a process that depletes dissolved oxygen levels and compromises aquatic biodiversity [6].

Sewage contamination also facilitates the spread of pathogenic microorganisms, including *Escherichia coli*, *Salmonella*, and *Enterococcus*, which can cause gastrointestinal diseases and severe hospital-acquired infections [7,8]. Furthermore, the presence of *Pseudomonas aeruginosa*, an antibiotic-resistant bacterium, poses a significant public health risk [9].

Recent studies have highlighted the relationship between urban stream degradation and socioeconomic factors. A recent study analyzed 42 urban streams and found that 93% of sampling sites exhibited contamination by antidepressants [10]. This study also revealed correlations between water quality, population density, per capita income, and lack of basic sanitation. These findings underscore the urgent need for effective public policies to rehabilitate water bodies and improve living conditions in the MRSP. To address these challenges, Brazil has committed to the United Nations (UN) 2030 Agenda, particularly Sustainable Development Goals (SDGs) 6—Clean Water and Sanitation and 3—Good Health and Well-being, aiming to enhance sanitation infrastructure and ensure universal access to treated water [11].

Given this context, the present study aimed to assess water quality and identify the presence of enterobacteria in the Billings-Tamanduateí watershed, correlating these factors with social indicators such as population density, informal settlements, and households lacking sewage treatment, which has never been studied in the region. To this end, we propose the following hypotheses: (i) Will areas with greater social vulnerability exhibit poorer water quality in urban streams? and (ii) Will these same areas show a higher prevalence of enterobacteria? Both hypotheses are grounded in studies demonstrating the link between social vulnerability and limited access to basic sanitation [10,12].

## 2. Materials and Methods

### *2.1. Study Area*

With a drainage area of 824.08 $km^2$, the Billings–Tamanduateí watershed is located within the Alto Tietê basin, in the Metropolitan Region of São Paulo (MRSP) (Figure 1). According to a technical report by the São Paulo State Public Prosecutor's Office (2003), the region experiences two predominant climatic periods: a rainy season from October to April and a dry season from May to September, corresponding, respectively, to summer and

winter in the Southern Hemisphere. Based on Köppen's climate classification, the MRSP climate is categorized as subtropical (Cwa).

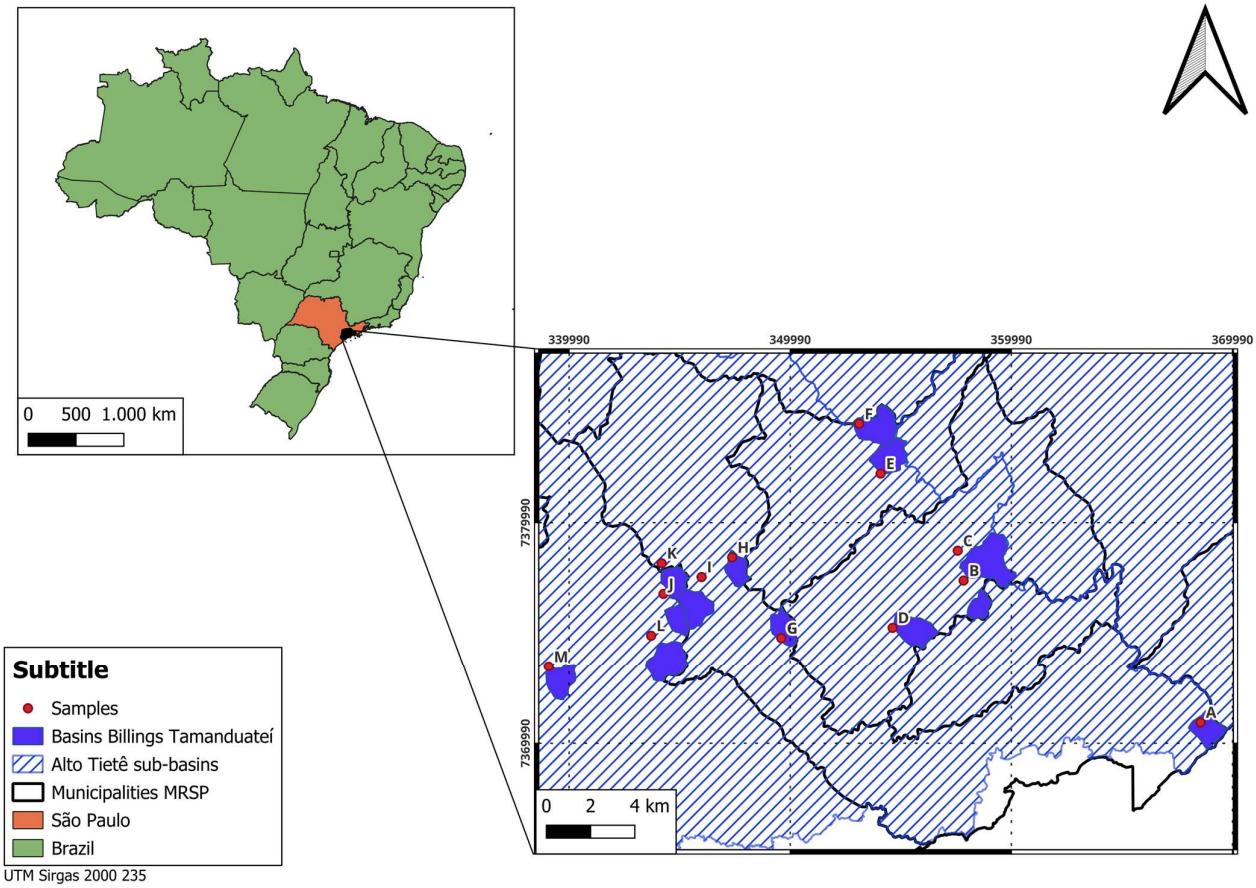


**Figure 1.** Location of the Billings Tamanduateí watershed, in the Alto Tietê River Basin, and the sampling stations of the study (São Paulo State, Brazil). The coordinates of each sampling station (UTM, X,Y) are: A = (−23,708316151703244, −46,39453095615553), B = (−23,69610496453302, −46,39700078254179), C = (−23,72735956729754, −46,426235741322635), D = (−23,65959258433309, −46,43138288262682), E = −23,643845566827636, −46,440161291159164), F = (−23,724407883109244, −46,475096604823506); G = (−23,69787714678419, −46,497079031407345), H = (−23,705806437760955, −46,510734777779625), I = (−23,712515461552805, −46,52772120051972), J = (−23,700012003204588, −46,528387334658625), K = (−23,72959141958858, −46,53338334070041), L = (−23,741787163069624, −46,57901352701734), and M = (−23,754896313941313, −46,585008734267475).

Regarding vegetation cover, the MRSP has a forest cover ranging between 20% and 50%, as reported in the São Paulo State Forest Inventory [13]. This watershed is particularly significant due to the presence of the Billings Reservoir, one of São Paulo State's main reservoirs, which supplies water to the ABC region and parts of the capital while also transferring water to the Guarapiranga system [14].

### *2.2. Sampling Design*

The sampling process involved the use of 500 mL plastic bottles, with three water samples collected using a plastic bucket tied to a rope. At each sampling station, a multiparameter probe (HI9829 Hanna Instruments, Barueri, Brazil) was used to measure dissolved oxygen, pH, temperature, oxidation-reduction potential (ORP), turbidity, and water conductivity. After field collection, samples were refrigerated for enterobacteria analysis and frozen for further assessment of total dissolved carbon, dissolved organic carbon, dissolved inorganic carbon, total dissolved nitrogen, and ions (Figure 2).

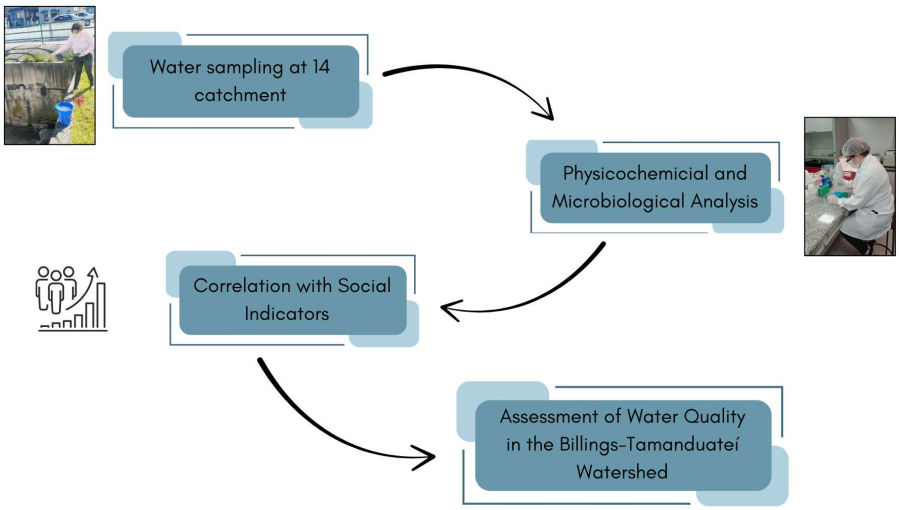


**Figure 2.** Flowchart summarizing the methodological workflow adopted in the study, including water sampling at 14 catchments, physicochemical and microbiological analyses, correlation with social indicators, and assessment of water quality in the Billings–Tamanduateí watershed.

According to the National Institute of Meteorology [15], summer in the Southern Hemisphere begins on 22 December and ends on 20 March. Therefore, all collections were conducted during the rainy season (March 2023). To minimize the influence of precipitation on the results, since rainfall can alter the natural composition of water resources, it was established that no precipitation should occur within 24 h before each sampling.

### *2.3. Presence of Enterobacteria*

The detection of fecal coliforms, *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa*, and *Enterococcus* were performed using the Quanti-Tray/2000 system [16]. This system enables semi-automated bacterial quantification through the Most Probable Number (MPN) method.

For quantification, the process began with sample preparation by filtering 100 mL of water into a sterile container. The powdered Enterolert reagent [16] was then added, followed by agitation until completely dissolved, ensuring no foam formation. The solution was transferred into Quanti-Tray, a card containing 97 wells of different sizes to ensure uniform distribution after sealing. Each sample was incubated under specific conditions as follows: *Enterococcus*: 24 to 28 h at 41 °C, *Pseudomonas aeruginosa*: 24 h at 38 °C, and *Escherichia coli* (*E. coli*) and total coliforms: 24 h at 35 °C. After incubation, samples were analyzed under a 365 nm ultraviolet (UV) lamp. Fluorescent wells indicated the presence of coliforms, which were then counted and recorded using Idexx's MPN counting software (version 1).

The detection of *Salmonella* spp. followed a different technique, using the Compact Dry Salmonella SL test [17], which consists of ready-to-use plates for microbiological detection and quantification. This test was performed once to determine the presence of the bacteria. Before applying the sample to the plate, it underwent a pre-enrichment step. A 25 mL sample was pipetted into 225 mL of buffered 1% peptone saline solution, serving as a culture medium for bacterial growth and enrichment. The sample was homogenized for approximately 60 s using a stomacher homogenizer, left at room temperature for one hour, and then incubated at 36 °C for 16 to 20 h. In the second stage, the pre-enriched sample was applied to the detection plate by adding 0.1 mL of the sample to one section and 1 mL of sterile water to another section, approximately 1 cm from the plate's opposite edge. The plate was then incubated at 41 to 43 °C for 24 h. The identification of *Salmonella*

was determined by a color change on the plate, shifting from purple to yellow. This color transition indicated the presence of the bacterium in the sample.

### *2.4. Carbon and Nitrogen Analyses*

Carbon and nitrogen analyses were performed in triplicate to ensure the quality, accuracy, and reproducibility of the collected data. Thus, three analyses were conducted for each sampling station. To determine the concentrations of dissolved organic carbon, dissolved inorganic carbon, total dissolved carbon, and total dissolved nitrogen, a Total Organic Carbon Analyzer [18] was used. This equipment employs Shimadzu's combustion catalytic oxidation method at 680 °C, enabling the efficient detection of low-molecular-weight organic compounds. During the analysis, carbon is oxidized to carbon dioxide, which is then detected by a non-dispersive infrared (NDIR) sensor. The total organic carbon concentration is obtained by subtracting the inorganic carbon content from the total carbon in the sample. For nitrogen analysis, the instrument must be equipped with the TNM-L (Total Nitrogen Measurement Unit) [18], which utilizes catalytic thermal decomposition at 720 °C followed by chemiluminescence detection to quantify the total nitrogen present in the sample.

### *2.5. Ion Analysis*

Ion analysis was carried out in triplicate using an ion-exchange chromatograph. The chromatograph used was the Dionex Easion [19]. The equipment is equipped with a chemically inert dual-piston pump and operates through chemical suppression, a method that reduces the conductivity of the eluent, thereby decreasing noise and increasing measurement sensitivity. The detector is a thermally controlled conductivity detector, ensuring greater consistency in peak area measurements.

### *2.6. Statistical Analyses*

To evaluate the relationship between the physicochemical water parameters and socioeconomic indicators, correlation analyses were performed. The intensity and direction of the correlations were represented by a color gradient, with blue tones indicating positive correlations and red tones indicating negative correlations between the variables. Additionally, a principal component analysis (PCA) was conducted to identify patterns of joint variation among the physicochemical water parameters and socioeconomic indicators. The PCA allowed for dimensionality reduction and visualization of the relationships between variables, highlighting the main factors responsible for the observed variation in the watersheds studied. The data were processed and analyzed using R software (version 4.5.0) [20].

## 3. Results

### *3.1. Physicochemical Parameters of Stream Water and Social Indicators*

Figure 3a presents the correlations between the physicochemical variables of the streams and the socioeconomic indicators of the watersheds. Carbon-related variables, such as dissolved organic carbon (DOC), total dissolved carbon (TDC), dissolved inorganic carbon (DIC), and total dissolved nitrogen (TDN), showed a strong positive correlation with each other and with electrical conductivity. Notably, all carbon variables are positively correlated with the number of inhabitants (HAB). Conversely, per capita income (PCI) shows a negative correlation with all carbon variables and with HWW (households without access to sewage treatment). A negative correlation was observed between pH and the HWW indicator (households without access to sewage treatment), suggesting that a lack of basic sanitation is associated with more acidic waters. Additionally, a negative corre-

lation was found between the number of inhabitants (HAB) and per capita income (PCI), indicating that areas with higher population density tend to have lower income levels.

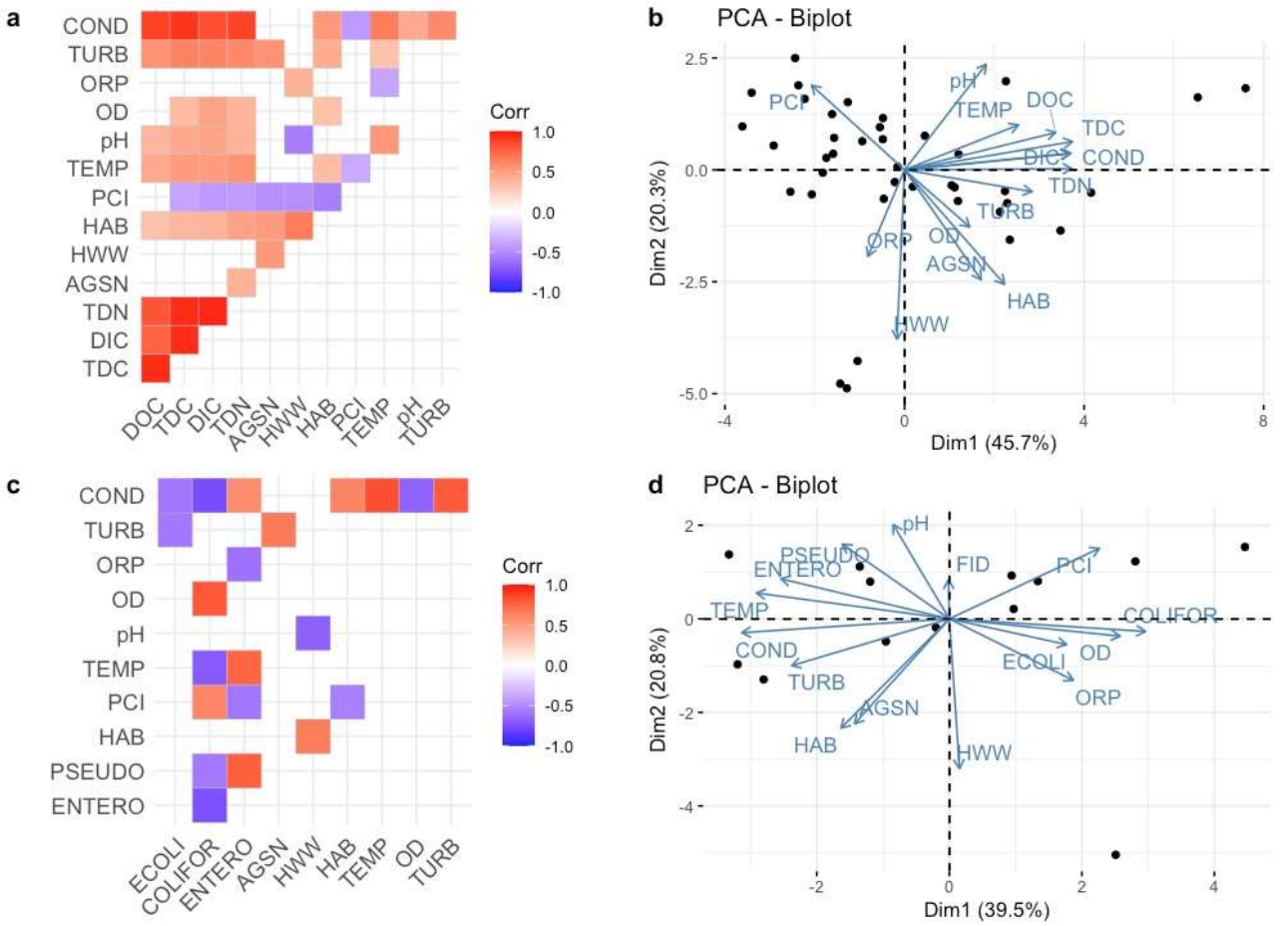


**Figure 3.** Correlation and principal component analysis (PCA) of physicochemical water parameters, socioeconomic indicators, and enterobacteria in the watersheds studied. (**a**) Correlation between physicochemical water parameters and socioeconomic indicators (**b**) PCA of physicochemical water parameters and socioeconomic indicators. (**c**) Correlation between physicochemical water parameters (pH, DO, ORP, and electrical conductivity), enterobacteria (*Enterococcus*, *E. coli*, *Pseudomonas aeruginosa*, and total coliforms), and socioeconomic indicators. (**d**) PCA of the relationships between physicochemical water parameters, Enterobacteria, and socioeconomic indicators. DOC—dissolved organic carbon, TDC—total dissolved carbon, DIC—dissolved inorganic carbon, TDN—total dissolved nitrogen, pH, DO—dissolved oxygen, ORP—oxidation-reduction potential, and electrical conductivity, HAB—number of inhabitants, PCI—per capita income, HWW—households without basic sanitation, and AGSN—subnormal clusters). The intensity and direction of the correlations are represented by a color gradient, indicating negative (blue) or positive (red) relationships.

Figure 3b presents a principal component analysis (PCA), where Axes 1 and 2 account for 66% of the data variation, integrating physicochemical parameters of urban streams and socioeconomic indicators of the watersheds. It is observed that dissolved organic carbon (DOC), total dissolved carbon (TDC), dissolved inorganic carbon (DIC), and total dissolved nitrogen (TDN), which represent organic and inorganic matter dissolved in water, are clustered with social indicators such as subnormal clusters (AGSN) and the number of inhabitants (HAB). Additionally, in the lower section of the figure, an association is observed between dissolved oxygen (DO), oxidation-reduction potential (ORP), households without sewage treatment (HWW), HAB, and AGSN. This suggests that areas with a higher number

of inhabitants, a greater presence of subnormal clusters, and a lack of basic sanitation tend to show elevated DO and ORP levels.

### *3.2. Enterobacteria and Social Indicators*

The correlation analysis conducted between microbiological and physicochemical variables in water and the social indicators of the watersheds revealed strong positive and negative correlations. Since they are indicators of fecal contamination, the positive correlation of total coliforms (COLIFOR), Enterococcus, and *E. coli* with dissolved oxygen (DO), temperature (TEMP), and conductivity (COND) reinforces this association. On the other hand, a negative correlation was observed between total coliforms (COLIFOR) and *E. coli* with conductivity (COND), turbidity (TURB), temperature (TEMP), and per capita income (PCI). From a socioeconomic perspective, the strong positive correlation between subnormal clusters (AGSN) and households without basic sanitation (HWW) indicates that areas with higher population density and more subnormal clusters also exhibit a greater lack of sewage treatment. Regarding physicochemical parameters, the correlation between temperature (TEMP) and pH suggests that, under certain conditions, an increase in temperature may be accompanied by a slight decrease in pH, possibly due to enhanced biological activity. Additionally, the strong positive correlation between dissolved oxygen (DO) and oxidation-reduction potential (ORP) suggests that oxidation processes in water are associated with higher levels of available oxygen (Figure 3c).

Figure 3d presents a principal component analysis (PCA), where axes 1 and 2 together explain 64.4% of the data variation, integrating physicochemical and microbiological water variables with social indicators. On the right side of the PCA, per capita income (PCI), total coliforms (COLIFOR), and *E. coli* (ECOLI) are grouped, suggesting that watersheds with higher per capita income exhibit greater presence of fecal contamination indicator bacteria. On the left side, variables related to population density, such as subnormal clusters (AGSN) and the number of inhabitants (HAB), are associated with indicators of poorer water quality, such as *Enterococcus* (ENTERO) and *Pseudomonas aeruginosa* (PSEUDO).

## 4. Discussion

The first hypothesis of this study was that areas with higher social vulnerability would exhibit poorer water quality in streams. The results support this hypothesis, as social indicators such as population (HAB), informal settlements (AGSN), and households without access to basic sanitation (HWW) showed positive correlations with all carbon and nitrogen parameters. This suggests a high presence of organic load in the water, a consequence of the improper discharge of sewage into water bodies. These findings can be attributed to the inefficiency of sanitation infrastructure, which primarily affects areas with greater social vulnerability. Uncontrolled demographic growth in urban centers often forces lower-income populations to settle in floodplain areas of streams, where, due to a lack of infrastructure, sewage is frequently discharged directly into water bodies. This practice impacts both hydrology and water quality [5,21]. This scenario is further reinforced by the negative correlation between per capita income (PCI) and dissolved carbon parameters, suggesting that higher-income areas tend to have better access to sanitation and more effective environmental management practices [10]. Conversely, areas with less access to sanitation infrastructure tend to have more acidic waters due to the decomposition of organic matter from sewage, as reported by Machado [22]. Thus, the results not only confirm the initial hypothesis but also emphasize the need for public policies focused on social inclusion and the expansion of sanitation services to mitigate the negative impacts of urbanization on water bodies.

The second hypothesis of this study was that areas with higher social vulnerability would show a greater presence of enterobacteria. The results also supported this hypothesis, as *Enterococcus* (ENTERO) and *Pseudomonas aeruginosa* (PSEUDO) were positively correlated with population size (HAB) and informal settlements (AGSN). In other words, these two bacteria were more prevalent in densely populated areas with lower residential infrastructure. Additionally, *Enterococcus* (ENTERO) showed a negative correlation with per capita income (PCI), indicating that higher-income areas had lower levels of *Enterococcus*. According to Carneiro [7], enterobacteria are reliable indicators of fecal contamination, reinforcing the link between inadequate sanitation infrastructure and high population density, where direct discharge of wastewater into urban streams is common. A study [23] highlights that *Pseudomonas aeruginosa* is an antibiotic-resistant bacterium, posing a significant public health risk. An unexpected result was the positive correlation between per capita income (PCI) and the presence of total coliforms (COLIFOR) and *E. coli* (ECOLI) in certain watersheds. Although this contradicts the initial expectation, it may be explained by specific factors, such as improper sewage disposal in wealthier regions with low population density but limited sanitation infrastructure [10]. Additionally, seasonal and climatic conditions may have influenced these variations. Factors such as temperature and precipitation can affect bacterial survival and proliferation, as noted by Galán-Relaño [8]. These findings highlight the need for further investigation into the factors influencing these relationships and correlations.

Our results support recent findings in the literature indicating that the degradation of water quality in urban areas is not only associated with high population density and the lack of sanitation but is also influenced by multiple stressors, such as unplanned urban growth and the impacts of climate change [24–27]. Studies highlight those tropical streams, particularly in urban regions, are subject to simultaneous stressors that affect both biota and ecosystem processes [27]. Additionally, a study demonstrated that the combination of low income, high population density, and inadequate sewage infrastructure leads to widespread contamination, including by emerging pollutants such as antidepressants [10]. Similarly, our data indicate that areas with higher levels of social vulnerability exhibited the highest concentrations of dissolved carbon and enterobacteria, suggesting an organic overload linked to the absence of sewage collection and treatment. The inclusion of analyses across different seasons could enhance understanding of temporal variability, since studies have reported significant differences in pollutant loads between dry and rainy seasons [6,28]. These findings underscore the importance of public policies that promote equitable access to sanitation and the continuous monitoring of water quality in the face of environmental and urban change.

These findings become even more relevant in light of climate projections for the region, which indicate an increase in the frequency and intensity of rainfall events [27,29,30]. The intensification of precipitation, combined with inadequate urban infrastructure, is likely to exacerbate diffuse contamination processes and the transport of pollutants into water bodies, particularly in areas with high social vulnerability [10,27]. Such extreme events may further deepen inequalities in access to clean water, highlighting the urgent need for adaptive strategies that integrate urban planning, basic sanitation, and climate change mitigation efforts.

The results of this study point to the need for integrated policy interventions aimed at mitigating the impacts of social vulnerability on water quality in urban environments. Prioritizing the expansion and modernization of sanitation infrastructure—particularly in areas with limited access to sewage collection and treatment—emerges as a key strategy for reducing both organic and microbiological contamination in aquatic ecosystems [10,31,32]. These interventions should be aligned with territorial planning policies that discourage

settlement in environmentally sensitive areas, where the absence of infrastructure amplifies pollutant loads [33,34]. In parallel, the implementation of systematic and continuous water quality monitoring programs could enhance the capacity to detect and respond to contamination events, especially in the context of increasing climate variability [27]. Such measures would not only contribute to achieving national policy targets, including those outlined in the Brazilian National Sanitation Plan (PLANSAB), but also reinforce commitments to international agendas, such as the Sustainable Development Goals (SDGs), particularly SDG 6 (Clean Water and Sanitation) and SDG 3 (Good Health and Well-being). Collectively, these actions would promote more resilient and equitable urban systems in the face of rapid urbanization and projected climate change scenarios.

Despite the associations identified between social vulnerability indicators and water quality parameters, this study has some limitations that warrant consideration. One key limitation is the potential influence of confounding factors that were not explicitly controlled for, such as land use patterns and industrial discharges [35,36]. These factors can significantly contribute to the degradation of water quality and may interact with social variables in complex ways, potentially obscuring or amplifying observed correlations. For instance, watersheds with similar levels of social vulnerability may differ substantially in terms of industrial activity, which could independently influence levels of carbon, nitrogen, and microbial contamination. Additionally, while our study focused on spatial variability, the lack of temporal replication—particularly the absence of dry season data—may limit the generalizability of the findings across different hydrological conditions. Future research should aim to incorporate multivariate models that account for these additional sources of variability, along with seasonal sampling, to better isolate the effects of social determinants from other environmental stressors.

## 5. Conclusions

Despite significant advancements in sewage collection and treatment over recent decades, our findings underscore the persistence of critical gaps in wastewater management, even in one of Latin America's most urbanized and industrialized regions—the São Paulo Metropolitan Region (RMSP). The study demonstrates that socioeconomic variables, particularly per capita income (PCI), population (HAB), the extent of informal settlements (AGSN), and access to sanitation (HWW), remain key determinants of water quality in urban streams. These factors are positively correlated with elevated concentrations of carbon and nitrogen compounds, as well as with microbiological indicators such as *Escherichia coli* (*E. coli*), highlighting ongoing fecal contamination in areas marked by high social vulnerability. Importantly, our results confirm that the urban water quality degradation observed is not uniform but rather spatially heterogeneous, disproportionately impacting communities with inadequate infrastructure and limited access to essential services. These populations often reside in flood-prone areas with minimal urban planning, where sewage is frequently discharged directly into nearby water bodies. Such dynamics create a cycle of environmental degradation and social inequality, which is further exacerbated by unplanned urban expansion and insufficient enforcement of land-use regulations. While this study provides valuable insights into the relationships between social indicators and water contamination, it also reveals the need for future research to explore these patterns in greater depth and across broader spatial and temporal scales. Comparative analyses between regions with varying levels of social vulnerability, including areas with more robust infrastructure and environmental governance, could yield important contrasts that enhance our understanding of vulnerability-resilience dynamics in urban streams. Additionally, considering the increasing unpredictability of precipitation patterns and the intensification of extreme weather events due to climate change, future studies should

incorporate seasonal and interannual variability to evaluate how climate factors modulate pollutant dynamics. Our findings underscore the urgency of advancing integrated and equitable public policies that go beyond infrastructure provision and promote sustained environmental monitoring, social inclusion, and climate adaptation. Only through such multi-scalar and cross-sectoral approaches will it be possible to promote urban water security and achieve the objectives set forth by national frameworks such as the Brazilian National Sanitation Plan (PLANSAB) and global agendas such as the United Nations Sustainable Development Goals—particularly SDG 6 (Clean Water and Sanitation) and SDG 11 (Sustainable Cities and Communities). By bridging the gap between environmental science and social equity, this study contributes to the growing body of evidence that calls for systemic and just urban water management practices in the face of rapid urbanization and global environmental change.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/limnolrev25020021/s1; Enterobacterias dates and Physicochemicals.

**Author Contributions:** Conceptualization, R.H.T. and R.d.F.B.; methodology, R.H.T. and L.C.S.; software, R.H.T. and B.G.-P.; investigation, B.G.-P., R.R.S.D., J.M.S.d.J., I.G.O., and G.S.S.A.; resources, R.d.F.B. and R.H.T.; writing—original draft preparation, B.G.-P.; writing—review and editing, R.H.T. and J.M.S.d.J.; supervision, R.H.T.; project administration, R.d.F.B. and R.H.T.; funding acquisition, R.H.T. and R.d.F.B. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** This study did not require institutional approval, as it did not involve humans or animal experimentation (CEUA UFABC—Decreto Federal no. 6029/07).

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The original contributions presented in this study are included in the Supplementary Materials. Further inquiries can be directed to the corresponding author.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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