SEASONAL ASSESSMENT OF WATER QUALITY IN AN URBAN STRETCH OF THE NEVES RIVER, NORTHEAST BRAZIL

AVALIAÇÃO SAZONAL DA QUALIDADE DA ÁGUA EM UM TRECHO URBANO DO RIO NEVES, NORDESTE DO BRASIL

Matheus Fernando Rocha Fonseca de Araujo[1]
Maria Betania Cunha Pereira[2]
Kawany da Silva Santos[3]
Maria Rafaela da Silva Sousa[4]
Barbara Lohanny Silva Alves[5]
Rafael Costa Leite[6]
João Gustavo Mendes Rodrigues[7]
Guilherme Silva Miranda[8]

ABSTRACT

Introduction: Rivers constitute one of the main sources of freshwater for living beings. However, they suffer constant environmental impacts that may compromise their quality. **Objective:** Therefore, this study aimed to evaluate the seasonal bacteriological quality of the Neves River in São Raimundo das Mangabeiras, Maranhão, Brazil. **Methodology:** Water samples were collected monthly at four points for six months, between 2023 and 2024, including the dry and rainy seasons. In the field, the temperature and pH of the water were measured, and an ecological evaluation protocol was used at each point. Water samples were used to quantify mesophilic bacteria, total and thermotolerant coliforms, and to assess the presence of *Escherichia coli* (*E. coli*),

[1]Graduação em Ciências Biológicas (Licenciatura). Instituto Federal do Maranhão. São Raimundo das Mangabeiras. Maranhão. Brasil. E-mail: matheusfernado26@gmail.com. ORCID: https://orcid.org/0009-0003-9604-1973.

[2]Graduação em Zootecnia. Instituto Federal do Maranhão. São Raimundo das Mangabeiras. Maranhão. Brasil. E-mail: mariabetaniacp@gmail.com. ORCID: https://orcid.org/0009-0003-6661-0370.

[3]Graduação em Zootecnia. Instituto Federal do Maranhão. São Raimundo das Mangabeiras. Maranhão. Brasil. E-mail: kawanysilvasantos71@gmail.com. ORCID: https://orcid.org/0009-0007-0004-1047.

[4]Graduação em Ciências Biológicas (Licenciatura). Instituto Federal do Maranhão. São Raimundo das Mangabeiras. Maranhão. Brasil. E-mail: rafaela.sousa@acad.ifma.edu.br. ORCID: https://orcid.org/0009-0002-4886-7319.

[5]Graduação em Zootecnia. Instituto Federal do Maranhão. São Raimundo das Mangabeiras. Maranhão. Brasil. E-mail: alvesbarbara647@gmail.com. ORCID: https://orcid.org/0009-0004-6555-911X.

[6]Graduação em Ciências Biológicas (Licenciatura). Professor na Universidade Estadual do Maranhão, Departamento de Ciências Biológicas. Coelho Neto. Maranhão. Brasil. E-mail: rafael.loreto12@gmail.com. ORCID: https://orcid.org/0000-0003-3486-9639.

[7]Doutor em Parasitologia. Centro Universitário de Balsas (UNIBALSAS). Balsas. Maranhão. Brasil. E-mail: gustavorodrigues_98@hotmail.com. ORCID: https://orcid.org/0000-0003-0920-4977.

[8]Doutor em Parasitologia. Professor do Instituto Federal do Maranhão. São Raimundo das Mangabeiras. Maranhão. Brasil. E-mail: mirandagsbio@gmail.com. ORCID: https://orcid.org/0000-0003-3763-2776.

Salmonella, and coagulase-positive *Staphylococcus* under laboratory conditions. Results: It was demonstrated that 50% of the evaluated points had a high degree of environmental impact. Water temperature ranged from 27.5°C to 29.8°C, and pH from 6.1 to 7.0, without major oscillations between the seasons. However, during the rainy season, the highest number of mesophilic bacteria, coliforms (total and thermotolerant), and the greatest presence of *E. coli* (58% of the evaluated samples) were detected. The presence of *Salmonella* or coagulase-positive *Staphylococcus* was not detected. **Conclusion**: Therefore, it was verified that some urban stretches of the Neves River have low water quality, mainly during the rainy season. Thus, it was recommending the urgent adoption of public policies to minimize the environmental impact on this river.

Key words: coliforms; *Escherichia coli*; environmental impact; water quality.

RESUMO

Introdução: Os rios constituem uma das principais fontes de água doce para os seres vivos. No entanto, sofrem impactos ambientais constantes que podem comprometer sua qualidade. **Objetivo:** Portanto, este estudo teve como objetivo avaliar a qualidade bacteriológica sazonal do rio Neves em São Raimundo das Mangabeiras, Maranhão, Brasil. **Metodologia:** Amostras de água foram coletadas mensalmente em quatro pontos durante seis meses, entre 2023 e 2024, incluindo as estações seca e chuvosa. Em campo, foi medida a temperatura e o pH da água e utilizado um protocolo de avaliação ecológica em cada ponto. As amostras de água foram utilizadas para quantificar bactérias mesófilas, coliformes totais e termotolerantes, e para avaliar a presença de *Escherichia coli* (*E. coli*), *Salmonella* e *Staphylococcus* coagulase-positivo em condições de laboratório. **Resultado:** Foi verificado que 50% dos pontos avaliados apresentaram um alto grau de impacto ambiental. A temperatura da água variou de 27,5°C a 29,8°C, e o pH de 6,1 a 7,0, sem grandes oscilações entre as estações. No entanto, durante a estação chuvosa, foi detectado o maior número de bactérias mesófilas e coliformes (totais e termotolerantes), e a maior presença de *E. coli* (58% das amostras avaliadas). Não foi detectada a presença de *Salmonella* ou *Staphylococcus* coagulase-positivo. **Conclusão:** Portanto, foi constatado que alguns trechos urbanos do rio Neves apresentam baixa qualidade da água, principalmente durante a estação chuvosa. Assim, recomenda-se a adoção urgente de políticas públicas para minimizar o impacto ambiental neste rio.

Palavras-chave: coliformes; *Escherichia coli*; impacto ambiental; qualidade da água.

INTRODUCTION

Water is essential for the survival of living beings, because it is fundamental to the chemical reactions underlying metabolism[1]. Although water covers 70% of our planet, only 0.77% is available for consumption[2]; therefore, it is urgent to ensure its quality.

Rivers are the main sources of freshwater that supply cities; however, with the constant advancement of urbanization and increased pollution, the quality of these ecosystems is suffering[3]. River waters become unsuitable for multiple uses when their chemical, physical, and microbiological parameters do not comply with legislation[4,5]. Among these parameters, the presence of pathogenic microorganisms in water bodies is particularly concerning, as they can cause serious diseases in humans and animals. These microorganisms are generally excreted in the feces of infected hosts and ingested through contaminated water or food[6].

Bacteria, especially coliforms, are the main microorganisms evaluated to monitor river water quality[7]. The high quantity of coliforms, especially thermotolerant, indicates strong fecal contamination in water bodies, as well as the possible presence of enteric pathogenic bacteria, such as *Escherichia coli* (*E. coli*) and *Salmonella*[8]. These bacteria are responsible for acute cases of diarrhea, septicemia, and vomiting[9,10]. In addition to these fecal-origin microorganisms, the evaluation of pathogenic bacteria from other sources is strongly recommended[14]. This group of bacteria includes the genus *Staphylococcus*, which is responsible for skin, ear, eye, and urinary system infections[11,12]. The severity of infections can vary according to the individual's immunity as well as the virulence factors (e.g., toxins) exhibited by some *Staphylococcus* species and strains[13].

Therefore, it is essential that river water quality is constantly evaluated to assess the presence and quantity of these pathogenic microorganisms, to prevent serious diseases in the population and animals. The Neves River, located in the municipality of Raimundo das Mangabeiras, state of Maranhão, has been subjected to constant anthropogenic pressures over the years, which can compromise the water quality for the supply and leisure of the population living in this region. However, no study has evaluated the microbiological conditions of this river. To address this issue, we aimed to verify the seasonal microbiological quality of urban stretches of the Neves River.

MATERIALS AND METHODS

STUDY AREA

The municipality of São Raimundo das Mangabeiras (Latitude: 7° 1' 20" South, Longitude: 45° 28' 53" West) is located in the Southern Mesoregion of Maranhão, covering an area of 3521.5 km² and with a population of 18.672 inhabitants[14]. The predominant biome is the Cerrado, and its climate is characterized as tropical and sub-

humid dry, with two well-defined periods: a rainy season (November–April) and a dry season (May–October).

The city developed along the banks of the Neves River, which currently supplies water to the local population. Thus, this river has been of fundamental importance for the development of São Raimundo das Mangabeiras, providing water for consumption, leisure, and fishing. However, over the years, the river's water volume has been decreasing due to anthropogenic activities. Burning, deforestation, and waste dumping into its bed have contributed to negative environmental impacts.

SAMPLE COLLECTION AND ENVIRONMENTAL CHARACTERIZATION OF THE RIVER

Water samples from the Neves River were collected monthly over six months (2023/2024), with three collections during each season (dry and rainy). Four collection points were selected along the river according to the type of environment (visually preserved or not) and multiple uses of the river at each point.

The first point (P1=7°01'52.4"S 45°30'57.5"W) is located within the properties of the Federal Institute of Maranhão (IFMA), in an area with preserved riparian forest. The second point (P2=7°02'15.1"S 45°30'03.7"W) is located below the bridge on the federal highway (BR-230, KM 319), where herds of cattle use it for watering. The third point (P3=7°02'01.2"S 45°28'32.8"W) is characterized as the main bathing area used by residents. The fourth point (P4=7°02'09.5"S 45°27'58.0"W) is in a transition area between the Neves River and the Cachoeira River (figure 1).

Figure 1 – Collection points located in the urban stretches of the Neves River, Maranhão, Brazil.
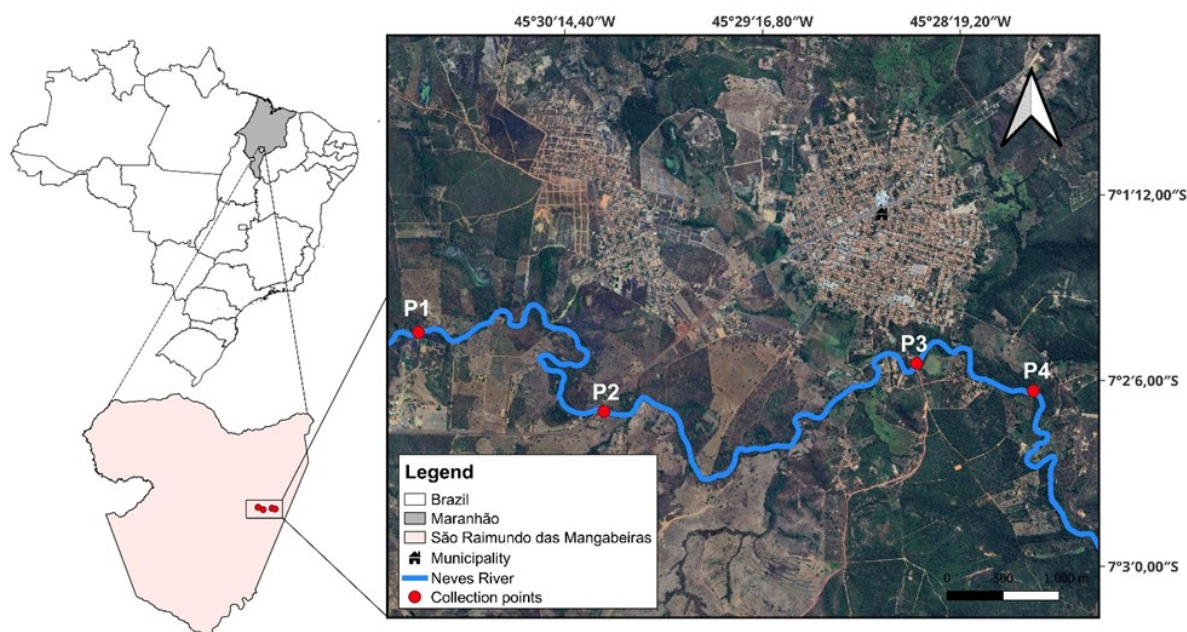
All points were assessed using the Rapid Ecological Assessment Protocol (REAP) proposed by Callisto et al. to classify the environmental conditions of each selected stretch[15]. Field measurements of the water temperature and pH were performed using portable equipment (Quimis, São Paulo, Brazil).

MICROBIOLOGICAL ASSESSMENTS

Glass bottles with pre-sterilized caps were used for water collection. These bottles were immersed against the current at a depth of 30 cm, as recommended by the Brazilian Ministry of Health [16]. A 2 L water sample was collected from each point, and two drops of dechlorinator (sodium thiosulfate) (Nutricon, São Paulo, Brazil) were added to eliminate the possible presence of residual chlorine and chloramine. Subsequently, the samples were stored in thermal boxes containing ice and transported to the laboratory for microbiological analysis.

All glassware sterilization procedures, culture media preparation, and sample processing for bacteriological analyses were performed according to previously established protocols[17]. A 100 mL aliquot of water, in triplicate, at concentrations of 1:1, 1:10, and 1:100, was used for each bacteriological assay. Mesophilic bacteria were enumerated using PCA agar, coliforms were quantified using the multiple tube technique, and the presence of *E. coli* and *Salmonella* was verified using eosin methylene blue agar (EMB), and enteric agar (HE) and xylose-lysine agar (XLD), respectively[17,18]. The presence of coagulase-positive *Staphylococcus* was verified using Baird-Parker agar, followed by coagulase, catalase, and Gram staining[19]. All prepared culture media were initially tested for their efficacy using standard bacterial strains (ATCC®).

The obtained results were compared with the standards established in the National Environmental Council (CONAMA) Resolution No. 274 for waters classified as primary contact recreation[20], and with the CONAMA Resolution No. 357 for Class 2 waters intended for human consumption after treatment, as well as recreation and irrigation[4].

DATA ANALYSIS

Data on colony numbers (mesophilic and coliform) for each season (dry and rainy) were evaluated for normality using the Shapiro-Wilk test. The obtained means or medians for each colony count were compared between the dry and rainy seasons and among the river sections using two-way ANOVA or Kolmogorov-Smirnov tests, depending on data normality. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using STATA version 11.1 (Stata Corp., College Station, TX, USA) and GraphPad Prism 8 (GraphPad Software, Irvine, CA, USA).

RESULTS

Overall, the assessed points in the Neves River were classified as "bad, good, or excellent," according to the rapid ecological assessment protocol[15]. Specifically, we found that point 1 (Figure 2) was classified as "good" (score 70/100), presenting partially preserved riparian vegetation, low levels of litter, absence of oil in the water, and visible aquatic fauna. Although point 2 (Figure 2) was also classified as "good" (score 50/100), we observed cattle pastures, landslides, litter accumulation, and sewage channels. Point 3 (Figure 2) presented the lowest ecological quality (score 30/100), and it was classified as "bad." At this point, we observed the presence of residences and businesses, landslides on the banks, litter in the water and on the banks, a bridge, sewage channels, reduced riparian vegetation, and limited visible aquatic fauna. Finally, point 4 (Figure 2) exhibited the best ecological quality (score 75/100). At this location, we observed the absence of construction and sewage channels, no oil in the water, low levels of litter, partially preserved riparian vegetation, and abundant visible aquatic plants and fauna. However, we found muddy sediments with a strong ammonia-like odor.

Figure 2 – Collection points. A - Point 1, located at the Federal Institute of Maranhão. B - Point 2, located downstream of the federal highway (BR-230). C - Point 3, located at the main recreational area for residents of São Raimundo das Mangabeiras. D - Point 4, located in a transition zone with the Cachoeira River. E - Wooden bridge located at point 3. F - Presence of litter at point 2.
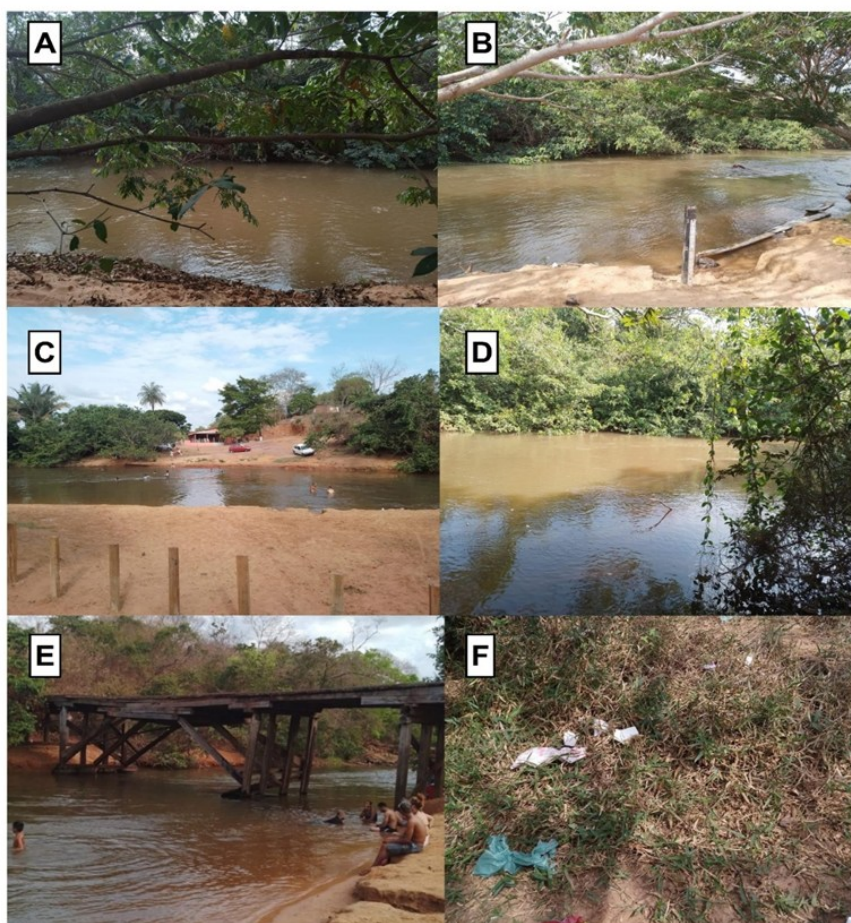
Regarding physical parameters, our data showed that water temperature ranged from 27.5°C (rainy season) to 29.8°C (dry season), and pH ranged from 6.1 to 7.0 (rainy season) (table 1). Little variation was observed between the dry and rainy seasons, or among the collection points, with no statistically significant differences.

Table 1 – Seasonal variation of the average values of physical parameters of water in four urban stretches of the Neves River located in São Raimundo das Mangabeiras, Maranhão, Brazil.

Dry season		
Points	**°C**	**pH**
1	29.2±2	6.6±0.2
2	29.8±3	6.4±0.1
3	28.7±3	6.7±0.4
4	28.5±3	6.3±0.2
Rainy season		
Points	°C	pH
1	28.4±2	7±0.2
2	27.8±2	6.8±0.2
3	27.5±3	6.7±0.1
4	27.5±3	6.1±0.1

Point 1, located at the Federal Institute of Maranhão. B - Point 2, located downstream of the federal highway (BR-230). C - Point 3, located at the main recreational area for residents of São Raimundo das Mangabeiras. D - Point 4, located in a transition zone with the Cachoeira River. pH: potential hydrogen.

Our bacteriological data revealed the presence of mesophilic bacteria, total coliforms, and thermotolerant coliforms at all collection points during both seasons (table 2). Overall, mesophilic bacteria count ranged from 6.2×10^2 (dry season) to 2.0×10^4 colony forming units (CFU)/100 mL (rainy season). For total coliforms, this variation ranged from 2.4 (dry season) to 886 most probable number (MPN)/100 mL (rainy season). Finally, thermotolerant coliforms varied from 3.4 (dry season) to <1100 MPN/100 mL (rainy season) (table 2). Therefore, our findings revealed that the highest quantities of these bacteria occurred during the rainy season, particularly at point 4 ($p<0.05$). Points 2 and 3 also showed a statistically significant increase in coliforms during the rainy season ($p<0.05$) (table 2).

Table 2 – Seasonal variation of the mean number of colony forming units (CFU/100 mL) of mesophilic bacteria and most probable number (MPN/100mL) of total (TC) and thermotolerant (TTC) coliforms found in four urban stretches of the Neves River, located in the city of São Raimundo das Mangabeiras, Maranhão, Brazil.

Dry season			
Points	**Mesophilic bacteria**	**TC**	**TTC**
1	1.4×10^3	2.4±1.2	3.4±0.2
2	8.9×10^2	9.0±4.2	7.0±2.3
3	7.6×10^2	9.8±3.3	5.2±1.9
4	6.2×10^2	6.1±3.0	4.2±1.3
Rainy season			
Points	Mesophilic bacteria	TC	TTC
1	3.3×10^3	376±160	382±259
2	1.7×10^3	148±82	813±187*
3	1.3×10^3	438±136	813±187*
4	2.0×10^4	886±113*	>1100*#

Point 1, located at the Federal Institute of Maranhão. B - Point 2, located downstream of the federal highway (BR-230). C - Point 3, located at the main recreational area for residents of São Raimundo das Mangabeiras. D - Point 4, located in a transition zone with the Cachoeira River. *Statistically significant (dry period vs rainy period). # value in disagreement with CONAMA (2005) legislation[4].

Of the 12 samples collected during the dry season, *E. coli* was detected in three (25%), distributed at points 2 and 4. During the rainy season, we observed an increase in the presence of this bacterium, corresponding to approximately 58% of the samples analyzed (table 3). In the present study, *Salmonella* spp. and coagulase-positive *Staphylococcus* spp. were not detected. However, it is important to note that we identified coagulase-negative *Staphylococcus* at point 1 in one sample collected during the dry season.

Table 3 – Seasonal assessment of the presence of *Escherichia coli* in four urban stretches of the Neves River, located in the city of São Raimundo das Mangabeiras, Maranhão, Brazil

Dry season				
Months	**Point 1**	**Point 2**	**Point 3**	**Point 4**
August	Negative	Negative	Negative	Positive[#]
September	Negative	Negative	Negative	Negativo
October	Negative	Positive[#]	Negative	Positive[#]
Rainy season				
Months	Point 1	Point 2	Point 3	Point 4
August	Negative	Positive[#]	Negative	Positive[#]
September	Positive[#]	Positive[#]	Positive[#]	Positive[#]
October	Negative	Negative	Positive[#]	Negativo

Point 1, located at the Federal Institute of Maranhão. B - Point 2, located downstream of the federal highway (BR-230). C - Point 3, located at the main recreational area for residents of São Raimundo das Mangabeiras. D - Point 4, located in a transition zone with the Cachoeira River. # in disagreement with CONAMA (2005) legislation[4].

DISCUSSION

In this study, we characterized, for the first time, the seasonal bacteriological profile associated with the ecological characteristics of the Neves River in Southern Maranhão. Our findings demonstrated that most urban stretches of this river exhibited some level of environmental impact, based on the presence of a high quantity of potentially pathogenic bacteria.

According to the ecological protocol proposed by Callisto et al., we verified that 25% of the evaluated points presented a score classified as 'poor' (point 3), exhibiting profound anthropic alterations[15]. Point 2 also presented characteristics similar to point 3; however, according to the general analysis of the protocol proposed by Callisto et al., this point received a classification considered 'good,' despite the score being low (50 points)[15]. However, the protocol proposed by Callisto et al. may present some limitations, as it does not accurately reflect all variables that can influence the ecological conditions of a river[15]. Climatic factors and socio-environmental aspects specific to each region are not fully considered in this method, which reinforces the need to complement it with other environmental analyses for a more comprehensive assessment of the quality of aquatic ecosystems.

Another approach to assess the environmental impact in urban river stretches is to analyze their physical parameters, which can provide valuable information about the water quality status and its potential for the growth of pathogenic bacteria. Regarding temperature, we observed a variation from 27.5°C (rainy season) to 29.8°C (dry season). These results are within the common range for aquatic environments, indicating adequate thermal conditions for the development of aquatic fauna [21].

Regarding pH, we observed a maximum variation from 6.1 to 7.0 (rainy season), which is generally acceptable for aquatic ecosystems with the characteristics of the Neves River[4]. Furthermore, these pH values are generally favorable for the growth of most bacteria, including pathogenic ones[22].

Indeed, in our bacteriological analyses, we detected the presence of mesophilic bacteria and coliforms (total and thermotolerant) in all evaluated water samples. However, during the rainy season, the number of these bacteria increased significantly, especially the coliform group. This increase can be attributed to excessive rainfall, which may have transported organic matter and other pollutants from the terrestrial environment into the river, potentially favoring increased bacterial growth[23]. The presence of domestic sewage discharged directly into this river also explains the high number of coliforms found in our study[24]. The presence of coliforms indicates fecal contamination; however, values up to 1000 CFU/100 mL are permitted in freshwater bodies[25]. Although point 4 presented the best environmental characteristics according to the protocol proposed by Callisto et al., this location showed coliform values exceeding 1000 CFU/100 mL during the rainy season[15]. These data reinforce that the environmental protocol proposed by Callisto et al. represents only a superficial characterization of a highly dynamic environment[15]. Significantly high coliform counts were also identified in water samples from the Mearim River (Maranhão state)[26] and Lontra River (Tocantins state)[27], thus confirming the presence of environmental impacts in other Brazilian hydrographic basins. Furthermore, in the current study, it is important to emphasize that point 4 receives all the water from the other evaluated points and from the Cachoeira River, which may explain the high contamination of fecal origin at this location. Therefore, we recommend that further studies be conducted to evaluate the water quality of tributaries that flow into the Neves River, such as the Cachoeira River.

Although the presence of coliforms in water is not directly associated with the induction of diseases, their detection suggests the occurrence of potentially pathogenic bacteria such as *E. coli*[9]. In our study, we detected *E. coli* at all evaluated points, mainly during the rainy season, which agrees with our data regarding the increase in coliforms during this period. Similar to our findings, a high frequency of *E. coli* was also observed in the Mearim River[26] and Lontra River[27]. *Escherichia coli* causes various diseases, such as gastroenteritis, urinary tract infections, and intestinal infections[9]. The pathogenic strains of *E. coli* responsible for inducing intestinal infections are called diarrheagenic strains and have been identified in several diarrhea outbreaks associated with the ingestion of contaminated water in various regions of the world[28]. Although the *E. coli* strains found in this study were not genotyped, the high presence of this bacterium represents a serious public health concern to the population of São Raimundo das Mangabeiras, who use water from the Neves River.

In addition to *E. coli*, we detected the presence of coagulase-negative *Staphylococcus* at point 1 during the dry season. Bacteria of the genus *Staphylococcus* are divided into two groups based on their coagulase activity: coagulase-positive and coagulase-negative[12]. The presence of coagulase-positive bacteria is indicative of the species *Staphylococcus aureus*, an opportunistic pathogen responsible for skin infections, pneumonia, endocarditis, and septicemia[29]. Although considered less virulent, coagulase-negative *Staphylococcus* can also pose a public health risk as they

can cause infections in immunocompromised individuals, such as children and the elderly[30]. However, the detection of coagulase-negative *Staphylococcus* in this study represents a limited risk and is not directly associated with environmental impacts in the Neves River.

Finally, it's essential to note that we do not use filtration methods to concentrate bacteria from the water samples, nor do we employ positive and negative controls in each bacteriological assessment. These methodological limitations may affect the accuracy of our results, suggesting that the water quality of the Neves River could be worse than presented in this current study.

Therefore, we verified that the Neves River presents environmental impacts along its urban stretches, which may be associated with the high contamination of the waters by potentially pathogenic bacteria, especially during the rainy season. We believe that the data generated in this study can clarify the current situation regarding the environmental contamination of the Neves River, in addition to proposing new environmental impact control measures.

CONCLUSION

Our study revealed that some urban stretches of the Neves River exhibit inadequate water quality, including the presence of pathogenic bacteria, such as *E. coli*. Among the evaluated points, the transition zone between the Neves and Cachoeira rivers showed the highest rates of bacterial proliferation, especially during the rainy season. Therefore, we suggest that constant microbiological monitoring of the Neves River waters is needed and that public measures and population awareness actions should be adopted for its preservation. In addition, to better evaluate the microbiological status of the Neves River, it's necessary to employ the most robust methods for bacteriological detection in further work.

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ACKNOWLEDGMENTS