Limnological Quality: Seasonality Assessment and Potential for Contamination of the Pindaré River Watershed, Pre-Amazon Region, Brazil

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Abstract: Watersheds are defined as a set of lands where water drainage occurs through rivers and their tributaries. A large quantity of water resources exist in the state of Maranhão, Brazil, where rivers and their basins must meet environmental quality standards defined by the limits set out in national environmental council (CONAMA) legislation 357/05 for physicochemical and microbiological parameters, including parasites. Multivariate statistical techniques were applied to study the temporal and spatial variations in water quality of a segment of the Pindaré River. The data set included nine parameters for three sampling points over eleven months. Principal component analysis grouped the monitored sampling points into four clusters and identified electrical conductivity, temperature, total dissolved solids (TDS), pH, salinity, and Escherichia coli as being associated with the dry season and nitrite, nitrate, and turbidity as being associated with the rainy season. Three principal components explained 83.80% of the data variance during the rainy and dry seasons. The evaluated correlations indicated that during the rainy season, nitrite (~0.18 mg L\(^{-1}\)) and turbidity (~46.00 NTU) levels were the highest, but pH was at its lowest (~6.61). During the dry season, TDS (~155.00 mg L\(^{-1}\)) and pH (~8.10) were highest, and E. coli bacteria was more abundant.

Keywords: fecal coliforms; parasites; principal component analysis; river; water quality

1. Introduction

Rivers are formed by natural water sources that are liable to biological, chemical, and anthropological influences, and yet river water often represents a source for human water supply. Such influencing factors allow us to understand the importance of water for our bodies and especially in our cells [1].

Watersheds are defined as a set of lands where water drainage occurs through rivers and their tributaries, and are always formed in higher relief regions through water distribution [2]. A large
quantity of water resources exist in the state of Maranhão, Brazil, which cover an area of 325,650 km² and can be distinguished as subterranean waters, watersheds, and lacustrine basins [3]. The state of Maranhão is particular because it contains a large portion of the northeastern hydrographic basins, of which the Pindaré River basin is recognized for its large area and its hydrological potential [4].

The Pindaré River basin covers an area of ~36,680 km², a large part of which is within the state of Maranhão. The Pindaré River is divided into three regions: alto (high), médio (middle), and baixo (low) [4]. The main tributary is the Mearim River, which extends ~686 km in length. However, the water quality of the rivers and the wider basin depends on factors related to water preservation; therefore, there should be a periodic water quality control assessment to indicate the existence of pollution [5].

Water pollution relates both to environmental and anthropogenic factors. The latter includes domestic, industrial, and hospital wastes among others, which are often discharged without adequate treatment and, thus, contaminating directly streams and spring with chemical and biological agents [6,7].

According to the resolution of national environmental council (CONAMA), legislation 357/05 [8] was passed in 2005 and states that the waters of the Pindaré River basin are classified as fresh and underground waters class II. The river must therefore meet the respective standards and be suitable for recreational purposes (e.g., swimming, water skiing, agricultural irrigation, and fishing).

As observed in many places, people acquire diseases through transmission, causing damage to their health and the economy [9]. Water contains indicators of fecal pollution, which are thus of paramount importance for determining its quality. Bacteriological tests for total coliforms, fecal coliforms, and fecal streptococci are those most commonly used [9,10] to detect pathogenic bacteria, which are present in animal and human feces. Total coliforms, even in low quantities, can still be found in water, already Fecal coliforms are considered transitory because they should not be habitually found in the water. The correlation between the pathogenic intestinal bacteria and the coliforms that are found in the water have been reported to, are approximately similar [11].

Among the parasitic microorganisms that are notable for being found in aquatic environments, some of the more recurrent include Giardia lamblia, Entamoeba coli, and Entamoeba histolytica. These are associated with gastrointestinal diseases and are still considered a serious public health problem because they can be found just as much in surface water as in drinking water, thus indicating the level of fecal contamination [12,13]. The main sources of contamination for these enteroparasites is the intake of raw foods or water contaminated by cysts [14].

Based on this information, bacterial contamination is an essential factor indicating water quality. The existence of enteroparasites represents a risk because they can be transmitted to humans, and their effect can be particularly unfavorable when they reach a population undergoing socioeconomic development [15].

Multivariate statistical methods such as cluster analysis (CA) and principal component analysis (PCA) have been widely used as unbiased methods in the analysis of water quality data to obtain useful information such as a reduced number of latent factors/sources of pollution, and to assess of temporal and spatial variations [16–20].

In the whole northeast region of Brazil, where the state of Maranhão is located, there are only two well-defined seasons throughout the year, the dry season, characterized by long periods without rain and intense heat, and rainy season, characterized by months of heavy rain. In the tropics, knowledge about the timing and amount of natural water flows is important, as nutrients can affect sensitive coastal ecosystems and adjacent settlements. The high permeability of aquifers, combined with high discharges during heavy rains, lead to close connectivity between natural waters and the coastal zone. The change between dry and rainy seasons can lead to a temporal variability in the volume of water discharge of associated nutrient flows. Nutrient flows are mainly controlled by the flow of waste materials and present a high variability over time [21].

Basic sanitation in the region of the Alto Alegre do Pindaré is scarce and household and hospital wastes often seep through sewers, thus allowing contamination by chemical agents and
microorganisms [21]. Thus, by bearing in mind the use of local rivers by people and animals, the aim of this study is to evaluate the quality of water of the Pindaré River in terms of seasonality and physicochemical, microbiological, and parasitological parameters. To achieve this, we use multivariate statistical analyses of the similarities and dissimilarities among the sampling points due to spatial and temporal variations in the conditions of the river.

2. Materials and Methods

2.1. Study Area Description

The municipality of Alto Alegre do Pindaré is located in the middle region of the Pindaré River (Figure 1). The municipality was created by law 6167 of November 10, 1994, which separated it from the municipality of Santa Luzia. Alto Alegre do Pindaré is located in western Maranhão State and has a territorial extension of 1,932,289 km² (03°87′78″ S, 45°84′12″ W) and an estimated population of 31,312 inhabitants in 2016 [4,22,23].

Sampling was carried out at points where there is a greater concentration of human activity (fishing, bathing, etc.) within the municipality. The samples were collected on the banks of the Pindaré River at three points: P1, which is located near an old pottery (03°39′54″ S, 45°50′30″ W); P2, which is located in the central bathing and fishing balneary (03°39′44″ S, 45°50′34″ W); P3, which is located in the canoes’ balneary (03°39′45″ S, 45°51′25″ W). Sites were sampling four sampling times, which covered 11 months from the dry season in 2017 to the rainy season in 2018 (Figure 2).
The water samples were collected in 500 mL polyethylene vessels, rinsed with sample water and then stored in a thermal box for transportation to the Laboratories of Microbiology, Environment, and Parasitology. In the Laboratory, all sample vessels were refrigerated (4 °C), to afterwards filtered through 0.45-m cellulose acetate membranes (Millipore) before analysis.

2.2. Monitored Parameters

Eight physicochemical parameters (pH, temperature, turbidity, total dissolved solids (TDS), salinity, electrical conductivity, nitrate, and nitrite) were selected for analysis based on a literature review and knowledge of the region. Samples were analyzed according to the methodologies of the American Public Health Association (APHA, 2012) [25], with the exception of nitrate and nitrite, which were performed according to the protocol provided by the manufacturer of the Veromar® kit, and complemented by photometric analysis (Spectroquant, Merk - Model Prove 600). The pH was measured by a digital pH meter (KASVI - model K39-2014B) and the turbidity, total dissolved solids, salinity, electrical conductivity were analyzed by multiparameter probe (Horiba – Model U52G). The maximum permitted values for each parameter according to the CONAMA legislation 357/05 are as follows: pH 6.0–9.0; electrical conductivity < 100 µS cm⁻¹; TDS < 500 mg L⁻¹; turbidity < 100 NTU; salinity < 0.5 ppt; nitrite < 1 mg L⁻¹ and nitrate < 10 mg L⁻¹.

The COLItest® kit was used for the microbiological assays. The water samples were collected in sterile flasks, then the indicator was added to 100 mL of sample and homogenized. Samples were subsequently placed in an incubator for 24 h at 37 °C. After the presence of total fecal coliforms was demonstrated, sowing was performed in Eosin Methylene Blue (EMB) agar to identify the bacteria belonging to the group of fecal and thermotolerant coliforms (Escherichia coli (E. coli), UFC/100 mL). The flasks containing the yellowish colored broth were then taken to a laboratory with UV radiation...
for fluorescence observation. The indol test was performed to verify the presence of \textit{E. coli}, whereby an aliquot of the broth was added to tubes and three drops of the reagent, occurring in the formation of a red halo, determined the positivity of the sample.

For parasitological analysis, 50 mL of each water sample was used as per the sedimentation method by centrifugation. After 60 minutes of sedimentation, the supernatant was discarded from the sample and the pellet was homogenized before being transferred to a Falcon tube. The sample tube was placed in a centrifuge for one minute at 1600 rpm. Subsequently, the supernatant was discarded from the tube and the pellet was added to a slide by adding one drop of lugol. This was then homogenized with the tip of the cover slip and the slide was observed at ×10 and ×40 magnification using an optical microscope [26].

2.3. Multivariate Statistical Method: PCA

PCA is a multivariate statistical technique that is designed to reduce large datasets into new, uncorrelated variables called principal components. PCA mainly involves six major steps [27]: (1) start by coding the variables \(x_1, x_2, \ldots, x_p\) to have zero means and unit variance, and standardize the variables to ensure they have equal weighting for further analysis; (2) calculate the covariance matrix; (3) calculate the correlation matrix; (4) calculate the eigenvalues and the corresponding eigenvectors; (5) rank eigenvalues and corresponding eigenvectors by the order of numerical values and discard components that interpret a small part of the total variance in the dataset; (6) develop the variable loading matrix to infer the principal parameters.

3. Results

3.1. Descriptive Measures of River Water Quality Data

To compare the significant differences of the mean values at \(p < 0.05\), one-way analysis (ANOVA) and the Tukey’s test and Fischer LSD’s multiple range test were applied (Tables 1 and 2) to the results by using Origin Pro 8.0 v. 80724-B724 software. PCA was applied to the experimental data in order to visualize the differences among the samples, and the results were analyzed using the Minitab 17 statistical software program for Windows. For the physicochemical analyses, the results were expressed as the mean and standard deviation (±SD). The data were evaluated through ANOVA in consideration of physicochemical parameters (temperature, electrical conductivity, TDS, turbidity, salinity, nitrite, and nitrate) and microbiological parameters during dry and rainy periods, where for the means different at each point (P1, P2 and P3), different letters (a, b and c) were attributed, so that at the points where the means have equal letters, it can be stated that there was no statistical difference between the data observed.

\[
\begin{array}{|c|c|c|c|}
\hline
\text{Parameters} & \text{P1} & \text{P2} & \text{P3} \\
\hline
\text{pH} & 8.13 \pm 0.15a & 8.10 \pm 0.10a & 7.06 \pm 0.40b \\
\text{EC (\(\mu\)S cm\(^{-1}\))} & 363.33 \pm 6.65a & 230.33 \pm 3.51b & 231.00 \pm 6.53b \\
\text{Turb. (NTU)} & 32.34 \pm 1.50a & 31.30 \pm 2.06a & 37.74 \pm 0.70b \\
\text{Sal. (ppt)} & 14.15 \pm 0.010a & 0.15 \pm 0.005a & 0.17 \pm 0.01a \\
\text{TDS (mg L\(^{-1}\))} & 185.00 \pm 2.00a & 140.66 \pm 2.08b & 142.66 \pm 2.51b \\
\text{Temp (\(^\circ\)C)} & 26.66 \pm 2.08a & 27.00 \pm 1.00a & 24.00 \pm 1.00a \\
\text{NO}_3^{-} (\text{mg L}^{-1}) & 5.54 \pm 0.05a & 2.79 \pm 0.03b & 2.78 \pm 0.02b \\
\text{NO}_2^{-} (\text{mg L}^{-1}) & 0.08 \pm 0.015a & 0.07 \pm 0.005a & 0.07 \pm 0.017a \\
\text{Escherichia coli (UFC/100 mL)} & 2.68e^{-5}a & 3.10e^{-6}b & 2.04e^{-6}c \\
\hline
\end{array}
\]
Table 2. Evaluation of the physicochemical and microbiological parameters in the three sampling points analyzed in the rainy season.

| Parameters        | P1                      | P2                      | P3                      |
|-------------------|-------------------------|-------------------------|-------------------------|
| pH                | 6.80 ± 0.040a           | 6.61 ± 0.010b           | 6.72 ± 0.025c           |
| EC (µS cm⁻¹)      | 210.66 ± 1.52a          | 208.66 ± 2.51a          | 200.00 ± 2.00b          |
| Turb. (NTU)       | 44.51 ± 0.41a           | 48.56 ± 0.39b           | 47.63 ± 0.06c           |
| Sal. (ppt)        | 0.10 ± 0.020a           | 0.08 ± 0.025a           | 0.12 ± 0.015a           |
| TDS (mg L⁻¹)      | 84.00 ± 1.00a           | 102.00 ± 1.00b          | 101.00 ± 1.00b          |
| Temp (°C)         | 24.00 ± 1.00a           | 26.33 ± 0.57b           | 24.00 ± 1.00a           |
| NO₃⁻ (mg L⁻¹)     | 8.57 ± 0.02a            | 9.09 ± 0.03b            | 6.89 ± 0.03c            |
| NO₂⁻ (mg L⁻¹)     | 0.20 ± 0.015a           | 0.17 ± 0.02a            | 0.16 ± 0.020a           |
| E. coli (UFC/100 mL) | 7.80e⁻⁵a              | 1.20e⁻⁶b               | 3.50e⁻⁵c               |

* Values in mean (n = 3). Means followed by the same letter in the same line do not differ statistically from each other by the Tukey test (p < 0.05). EC (electrical conductivity), Turb. (turbidity), Sal. (salinity), TDS (total dissolved solids), Temp (temperature), NO₃⁻ (nitrate), NO₂⁻ (nitrite).

The comparative test between the averages of all parameters was performed using the Tukey test (p < 0.05). For the set of analyses (physical, chemical, and microbiological), a PCA was applied to the mean values of the replicates (n = 3) to identify possible correlations between the data and to group them according to seasonal influence.

PCA was used to investigate the possible correlations between all of the studied variables and to evaluate hypothetical models for the classification of the sampled points. Initially, an assessment of the relationships between the nine variables that were related to the two studied periods was performed by PCA based on a correlation data matrix, in which the entire dataset was auto-scaled for all variables.

3.2. Box Plots of Water Quality Parameters

Figure 3 presents box plots of the individual water quality parameters that illustrate the temporal variations corresponding to the two seasons as determined from the PCA. These were prepared by combining the data for all parameters in correspondence to each season for a given parameter. The median, lowest, and highest values for a given period were determined for each parameter by analyzing the data for all points for the specific periods. The line across the box shows the median concentration. The vertical lines that extend from the bottom and top of the box correspond to the lowest and highest observations.

3.3. PCA of River Water Quality Data

Figure 4 shows the sampling points analyzed during the rainy and dry periods. Due to the dispersion of the points around PC1, the points P1, P2, and P3 presented similarities in relation to the studied season; for the points analyzed during the dry period, points P1, P2, and P3 did not present a tendency to group, thus confirming the distinction between them. The correlation between the periods is also shown in relation to the PC1 (x-axis), where the points analyzed during the rainy period and to the right of the x-axis are arranged, where the data of points P1, P2, and P3 were analyzed for the dry period. Table 3 shows the loadings of the variables for the first three principal components.
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### Table 3. Loadings of the variables for the first three principal components.

| Variables  | PC1* | PC2* | PC3* |
|------------|------|------|------|
| pH         | 0.41 | 0.08 | -0.03|
| Conductivity | 0.32 | 0.55 | 0.07 |
| Turbidity  | 0.42 | 0.06 | -0.17|
| Salinity   | 0.33 | -0.25| 0.36 |
| TDS        | 0.41 | 0.21 | 0.06 |
| Temperature| 0.22 | 0.12 | -0.68|
| Nitrate    | -0.13| 0.09 | 0.57 |
| Nitrite    | -0.40| 0.20 | -0.06|
| E. coli    | 0.17 | -0.71| -0.13|

* Significant correlation (>0.4).

Figure 5a,b presents the results of the parasitological analysis in the rainy and dry season.
Table 3. Loadings of the variables for the first three principal components.

| Variables       | PC1 * | PC2 * | PC3 * |
|-----------------|-------|-------|-------|
| pH              | 0.41  | 0.08  | -0.03 |
| Conductivity    | 0.32  | 0.55  | 0.07  |
| Turbidity       | -0.42 | 0.06  | -0.17 |
| Salinity        | 0.33  | -0.25 | 0.36  |
| TDS             | 0.41  | 0.21  | 0.06  |
| Temperature     | 0.22  | 0.12  | -0.68 |
| Nitrate         | -0.13 | 0.09  | 0.57  |
| Nitrite         | -0.40 | 0.20  | -0.06 |
| Escherichia coli| 0.17  | -0.71 | -0.13 |
| Eigenvalue      | 5.07  | 1.04  | 1.00  |
| Total variance  | 56.40 | 16.30 | 11.10 |
| Cumulative variance (%) | 56.40 | 72.70 | 83.80 |

* Significant correlation (>0.4).

Figure 5a,b presents the results of the parasitological analysis in the rainy and dry season.

Figure 5. Parasitological analysis, (a) frequency in dry and rainy seasons, (b) for the three points analyzed in the rainy and dry season.

4. Discussion

During the dry period (Table 1), pH was only relatively low at sampling Point 3 (7.06). The electrical conductivity data of the evaluated sampling points were different and decreased from Point 1 (366.33 µS cm\(^{-1}\)) to Points 2 and 3 (231.00 µS cm\(^{-1}\)). When comparing the data obtained in different seasonal periods, an increase in electrical conductivity during the dry period in comparison to the rainy period was verified. The analyzed samples were above that established for natural waters (100 µS cm\(^{-1}\)) according to the CONAMA legislation 357/05. An increase in electrical conductivity during the dry season was evident, which agreed with [28] who found that electrical conductivity decreased during the rainy season due to high ion-dilution.

TDS ranged from 185.00 mg L\(^{-1}\) to 142.66 mg L\(^{-1}\) during the dry period, and values were below the limit acceptable in legislation 357/05 (500 mg L\(^{-1}\)). TDS are relatively proportional to electrical conductivity because the latter is related to the number of dissolved solids [28–30].

The temperature results indicate the degree of heating that the water was exposed to through the solar radiation, and vary with season but can remain stable in the same regions [28]. The temperatures obtained from the two seasons were within the range considered to be normal, with an average of 25.88 °C obtained for the dry period and 24.77 °C for the rainy season. According to legislation 357/05, the temperature of natural waters in Brazil should not exceed 40 °C.

In the analysis of turbidity, the sample values ranged from 32.00 to 37.00 NTU during the dry season and 44.00 to 48.00 NTU during the rainy season, which fell within the limits defined by
legislation 357/05 (maximum of 100 NTU). The turbidity increased during the rainy season when there was likely to have been larger inputs of sediment even without water flow and moisture exchange through the soil [31].

The pH of the sampled waters during the rainy season remained close to neutral (6.80), whereas the pH ranged from 7.06 to 8.13 (i.e., basic pH) during the dry season. All pH values were within the values permitted by legislation 357/05 (i.e., pH 6–9). For natural waters, carbonate and bicarbonate ions control the pH at higher levels [28].

The salinity values obtained during both seasons were within the standards defined by legislation 357/05 (0.5 ppt). Salinity varied from 0.14 ppt to 0.17 ppt during the dry period and from 0.08 ppt to 0.12 ppt during the rainy season. Hence, although the salinity range was relatively higher during the dry season, values did not exceed the limit established for salinity. According to [32] and [33], salinity is a consequence of the accumulation of solutes found in soils; however, the dilution of solids in water is proportionally associated because they are concentrated.

Nitrite levels varied from 0.07 to 0.08 mg L\(^{-1}\) during the dry period and 0.16 to 0.20 mg L\(^{-1}\) during the rainy season, and were within the limit set by the CONAMA 357/05 legislation (up to 1.0 mg L\(^{-1}\)). While nitrate values ranged from 2.78 to 5.54 mg L\(^{-1}\) during the dry season and from 6.89 to 9.09 mg L\(^{-1}\) during the rainy season, they also did not exceed the maximum limit of 10.00 mg L\(^{-1}\). The nitrate concentration varied during the rainy season and was considered to be elevated. According to the National Water Agency (ANA, 2018) [34], high concentrations of nitrate in water pose a hazard to humans and can also present a risk to aquatic life due to its high toxicity. Elevated nitrate can lead to potentially lethal diseases such as infant methemoglobinemia because it is transformed into nitrite in the blood and an antagonism between nitrite and oxygen exists.

The microbiological evaluation revealed that fecal coliforms were present in all water samples at all points. According to the test, which was considered to be sensitive, it was possible to observe the presence of bacteria such as *E. coli*, which in turn provide an indication of the water quality. Contamination by fecal coliforms was expected for the rainy season samples because when it floods, the river breaches its banks and water comes in contact with embankments that have been contaminated by animal and human defecation. According to legislation 357/05, fecal coliform levels should not exceed 1000 CFU/100 mL, but the concentrations of all water samples exceeded this limit. This leads to the assertion that there is contamination of the river water, which remains quite high during the dry period. According to the study by [35] regarding the microbiological quality of river water, our findings are worrying in so much as they suggest the widespread contamination by fecal coliforms between the evaluated sampling points.

Figure 5a presents the results of the parasitological analysis, which shows that three types of parasitic species were observed in this study: two types of protozoa (*Entamoeba coli* cyst and *Giardia lamblia* cyst) and a helminth (*Trichuris trichiura* eggs). Of these etiologic agents, protozoa were the most frequent in the water samples of [36]. Of the three types observed in our study, only *E. coli* is not pathogenic to humans; however, it is considered to provide a marker of environmental contamination [37]. It can be seen from Figure 5b that during the dry season, all sampling points were associated with parasitic forms, and up to two distinct species were observed at Points 1 and 2. In the rainy season, a parasitic form was found in the samples from Points 2 and 3, whereas this was not the case for Point 1. This decrease in the presence of parasites during the rainy period may have been due to the increased river levels.

Some aspects that we observed include the fact that although the presence of *Trichuris trichiura* eggs are due to fecal contamination, they are not understood to be found in river water because they require oxygen and temperature of 30 °C for their development. According to a study of on aquatic parasitosis [38], parasites were still present in river water samples in municipality of Tefé—Amazonas, between October and June because the temperature and humidity conditions were ideal such that they could survive in the external environment. According to [39,40], Brazil is a country in which
published studies related to parasites in water are rare, which makes it difficult to ascertain the relative magnitude of parasite contamination.

The box plots in Figure 3 indicate seasonal influence in the river flow rates as well as changes in pollution loading. However, pH, conductivity, turbidity, TDS, nitrate, nitrite, salinity, and *E. coli* showed substantial variations between the two seasons and quite different individual patterns. For example, Figure 3a illustrates the considerable change in pH between the rainy season to the dry season, which corresponds at least partially to the fact that rainfall inputs can increase the acidity of surface water [41].

Turbidity concentrations varied between the rainy season and dry season due to heavy rain bringing more sediment from upstream agricultural land as well as from the erosion of river banks [19]. The magnitude of the levels of conductivity, TDS, and salinity all increased from the rainy season to the dry season. This was due to the fact that during the dry season, the river flow rate was relatively low and the pollution loading from landfill and non-point sources resulted in higher levels of these parameters. Already in the rainy season, the river flow increases substantially reducing these levels considerably. As for nitrates and nitrites levels, they showed low concentrations in the dry period and high concentrations in the rainy period, which may have been due to the use of in situ sanitation systems and, consequently, the runoff of soil residues and other contaminants to the water table. Furthermore, the box plots of the *E. coli* concentrations imply that there was a higher effect of pollution sources during the dry season.

The PCA of the water quality data from the three sampling points was performed and corresponding eigenvalues obtained. Following the methods of Boyacioglu & Boyacioglu (2008) [42], eigenvalues greater than 1 were retained. It was found that the first three eigenvalues collectively explained > 83% of the variance of the original data set. This confirmed the strong applicability of PCA to the dataset. Based on the theory of PCA [27,43], the projections of water quality parameters on the axes of principle components (PCs) are called loadings and represent the correlation coefficients between the PCs and variables. Table 3 shows the loading of the retained PCs. According to [44], factor loadings were classified as either ‘strong’ (>0.75), ‘moderate’ (0.75–0.50), or ‘weak’ (0.50–0.30) for absolute loading values.

PC1 corresponded to 56.40% of the variance and was weakly (<0.5) contributed by pH, turbidity (negative), TDS, and nitrite (negative), whereas it was moderately (0.5–0.7) contributed by conductivity and *E. coli* (negative). These water quality parameters also showed similar positive or negative correlations (Table 2). PC1 represents parameters that indicate organic pollution associated with anthropogenic pollution sources. Table 2 shows the correlation coefficients of all parameters, the total explained variance, and the variance accumulated by the first three PCs.

Figure 4 shows the PCA based on the physicochemical and bacteriological parameters in relation to the two studied periods. We found that the sampling points that were measured during the rainy season presented high levels of nitrite (*r* = 0.403) and turbidity (*r* = 0.419) and low pH levels (*r* = 0.411) and TDS (*r* = 0.415). Sampling Point 1 during the dry period exhibited high pH and TDS values, but low levels of turbidity and nitrite. Sampling Points P2 and P3 during the dry period presented high salinity (*r* = 0.366) and *E. coli* (*r* = 0.176) values. The first two dimensions of the PCA for all parameters explained 72.60% of the variance. Four clusters could be highlighted: (1) containing the sampling points P1, P2, and P3 during the rainy season; (2) containing only sampling point P1 during the dry period; (3) containing the sampling point P3 during the dry period; (4) sampling point P2 during the dry period. In general, the correlations between all the analyses performed and the sampling points, we determined that there was a significant difference between the studied periods.

Dissolved organic matter at relatively high concentrations consumes large amounts of oxygen and undergoes anaerobic fermentation processes, which leads to the formation of ammonia and organic acids. When hydrolysis of these acidic materials does not occur, an increase of water pH could result [19], as indicated by the positive loading of pH in Table 3. Furthermore, the negative loadings of turbidity, nitrate, and nitrite agree with other studies [45] and the negative correlation of these
parameters with anthropogenic pollution sources. PC2 was moderately contributed by conductivity and strongly contributed by *E. coli* (negative). PC3 was moderately contributed by both temperature (negative) and nitrate, possibly due to an increased flow of nutrients from leachate and other sources during rainfall.

PC1 exhibited a weak loading in relationship to seasonality. PC2 had a strong negative loading on *E. coli*, whereby there was a negative correlation of this bacteria with the rainy season. PC3 had a positive loading on nitrate (associated with leachate, domestic wastewater, and agricultural runoff) and a negative loading on temperature. In general, inorganic pollutants primarily affect conductivity, whereas TDS is affected by both organic and inorganic pollution. A moderate positive loading of PC3 on nitrate suggests the impact of fertilizer use and agriculture activity, which often leach into runoff during the rainy season, and weak negative loading of nitrite in PC2. Nitrate ion (NO$_3^-$) is biochemically reduced to nitrite (NO$_2^-$) by denitrification processes under anaerobic conditions. In the rainy season the highest nitrate concentration and the lowest concentration of microorganisms, and bacteria (e.g., *E. coli*) were found, suggesting the action of these microorganisms to produce nitrite is not as significant (when associated with the principal component analysis) as the production of nitrate from various sources of contamination.

5. Conclusions

The samples analyzed indicated changes in physical-chemical, microbiological and parasitological parameters, thus indicating poor preservation of the river, leading to its contamination, making it unsuitable for use. The results obtained for physical and chemical parameters for pH, salinity, turbidity, electrical conductivity, temperature and total dissolved solids remained within the maximum limits allowed by CONAMA nº 357/05. Only the levels of nitrite and nitrate remained above the established standards, thus being classified as harmful to human health and aquatic life. The analysis of main components showed that the physicochemical parameters pH, turbidity, total dissolved solids and nitrite were the most sensitive regarding seasonality. As for *E. coli* bacteria, it was possible to correlate the highest incidence of this contaminant to the dry period, specifically related to Points 2 and 3.

Microbiological results indicate the high level of contamination of the river by fecal coliforms, since all results were positive, indicating that the values were not in accordance with the legislation. Therefore, we know that basic sanitation in the region is scarce and that domestic and hospital waste often reaches the Pindaré River basin allowing contamination by chemical and mainly pathological agents, such as intestinal parasites investigated in this study.

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