Papers

Statistical Analysis of Bathing Water Quality Parameters in a Lagoon Influenced by Estuarine Dynamics

Daniele Martin Sampaio

Rubia Flores Romani

Cícero de Coelho Escobar

Pascal Silas Thue

Hartur Xavier Pinheiro

Maicon Oliveira Luiz

Keywords

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Abstract

This study investigates Lagoa dos Patos's bathing water quality parameters, located in Pelotas, focusing on assessing the influence of estuarine conditions. Statistical analysis was conducted on water quality data from 2015 to 2022, with parameters including Escherichia coli, turbidity, organic matter, phosphorus, and chlorides. Two principal components were extracted using the Kaiser criterion and cumulative variance threshold, revealing significant correlations between chloride concentrations and various water quality parameters. The results indicate that salinity, represented by chlorides, plays a critical role in influencing water quality, particularly in brackish water conditions. Notably, Escherichia coli concentrations showed a reversal of correlation direction at higher salinity levels, suggesting potential limitations in using the microbiological indicator in estuarine environments. Furthermore, significantly higher levels of organic matter were observed in brackish water, likely due to anthropogenic inputs such as effluent discharge. These findings highlight the importance of considering alternative water quality indicators in estuarine areas, as traditional microbiological markers may not fully reflect the environmental dynamics of these systems. This study provides valuable insights for decision-makers in managing water quality and public health in coastal and estuarine regions. Furthermore, this research aligns with several Sustainable Development Goals, notably Goal 6 (Clean Water and Sanitation), by improving water quality and addressing pollution. It also supports Goal 14 (Life Below Water) by examining the impact of environmental factors on coastal ecosystems, Goal 3 (Good Health and Well-being) by highlighting the importance of water quality for public health, and Goal 13 (Climate Action) due to the influence of environmental changes on salinity.

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¹ Universidade Federal de Pelota – UFPEL, Pelotas, RS, Brazil. dmartinsampaio@gmail.com

² Universidade Federal de Pelota – UFPEL, Pelotas, RS, Brazil. fgrubia@yahoo.com.br

³ Universidade Federal de Pelota – UFPEL, Pelotas, RS, Brazil. cicero.escobar@gmail.com

⁴ Universidade Federal de Pelota – UFPEL, Pelotas, RS, Brazil. pascalsilasthue@gmail.com

⁵ Universidade Federal de Pelota – UFPEL, Pelotas, RS, Brazil. hartur.eng@icloud.com

⁶ Universidade Federal de Pelota – UFPEL, Pelotas, RS, Brazil. maicon.oliveiraaluiz@gmail.com

INTRODUCTION

Transitional ecosystems, including estuaries, lagoons, and coastal lakes, are complex humanenvironmental systems that offer a wide range of societal benefits, including both commercial and non-commercial values (Newton et al. 2023). The interactions between two environments make the estuarine system unstable on both temporal (i.e., tidal cycle, seasonal) and spatial (lower-middle-upper estuary) scales (Fan et al., 2023; Li et al., 2024). The activities carried out impose numerous pressures and impacts on these environments, causing rapid degradation of these ecosystems (Partha et al., 2024).

Belarmino et al., (2024) showed that a longterm decline in the functional structure of fish assemblages in a subtropical estuary, attributed to a mix of local abiotic factors (such as salinity and temperature), global climatic phenomena, and anthropogenic impacts (including changes to the estuarine mouth's morphology and its sea connection). Significant vectors of threats to these ecosystems include organic matter and contaminant loads primarily due to the release of domestic effluents (Costa; Carreira, 2018). Freshwater and small tributaries impacted by fecal contamination may play an essential role in disseminating or introducing waterborne pathogens in seawater and brackish recreational sites, posing a threat to the health of beachgoers (Leal et al., 2024).

In this context, water quality in recreational areas emerges as a central concern for both public health and environmental preservation (Hirai; Porto, 2016). In Brazil, the assurance of water quality is regulated by guidelines set forth in the environmental legislation Resolution Conselho Nacional do Meio Ambiente (National Environment Council, CONAMA) nº. 274/2000, which defines the criteria and indicators of water quality that must be monitored to ensure bathing water quality (CONAMA, According to this regulation, sanitary conditions are assessed by detecting microbiological indicators of fecal pollution—specifically, Escherichia coli (E. coli), which belongs to the coliform group. However, although it is widely employed in various countries due to its

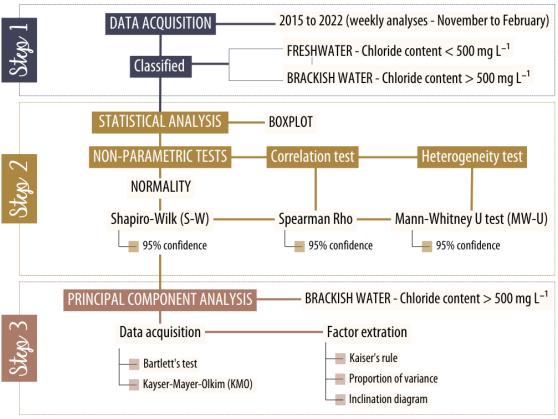
specificity and ease of quantification (Frena et al., 2019), several studies have shown that the decay rate of pathogens may be significantly different from that of E. coli (Greaves et al., 2020). This is mainly due to the complex physical and chemical variables that can affect the survival of microorganisms in these systems. Studies reported influences in the quantification of E. coli in estuarine surface waters according to variations in parameters such as pH, salinity, and dissolved oxygen (Zeki et al., 2021)

Temporal and spatial salinity variations can influence coastal water stratification, density, and suspended sediments (Zhang et al., 2021b) and also biological habitats by structuring an estuary's microbial communities (Zhang et al., 2021a). In these environments, salinity levels change notably due to the mixing of tidal flows and seasonal variability in the amount of freshwater transported (Shih et al., 2021). The extent of seawater intrusion in brackish and transitional lakes induces changes in the salinity gradient due to reverse flow and habitat heterogeneity (physicochemical gradient), which directly affects species diversity and abundance across different levels of the trophic chain (Obolewski; Glińska-Lewczuk, 2020). Lew et al. (2023) report that E. coli levels are associated with organic carbon content, pH, and, to a lesser extent, dissolved oxygen, which are typical of freshwater lakes, highlighting the influence of salinity in transitional areas. Therefore, it is crucial to understand the limitations of using E. as a bioindicator ofpollution, environmental dynamics can affect reliability. In this complex context, the primary objective of this study is to assess the influence of salinity variations in the Patos Lagoon (PL), in the city of Pelotas - RS, on bathing water quality parameters.

METHODS

The methodology was divided into three main stages (Figure 1). The method employed was the analysis of secondary data, which involves using previously collected and published data and requires a careful examination of the data's provenance (Creswell, 2009).

 $\label{eq:Figure 1-Flowchart} Figure \ 1-Flowchart \ of the \ proposed \ method, \ divided \ into \ three \ main \ steps: \ data \ acquisition \ (blue), \\ statistical \ analysis \ (brown), \ and \ principal \ component \ analysis \ (rose)$



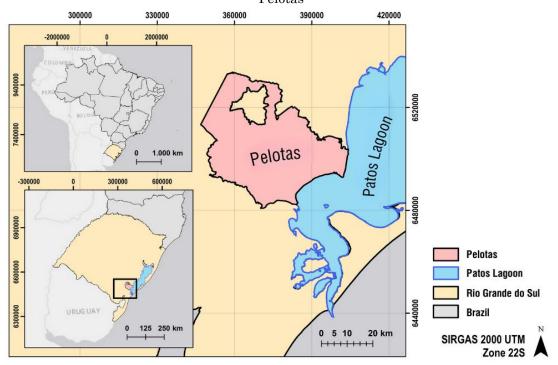
Source: The authors, 2025.

STUDY AREA

The Patos Lagoon (PL), located on the subtropical coast of Rio Grande do Sul, southern Brazil (Figure 2), stands out as one of the largest coastal lagoons in the world, with an average length of 250 km, an average width of 40 km, and a surface area of 10,360 km² (Kjerfve, 1986). The upper and middle portions of the PL are predominantly composed of freshwater, while the southernmost section hosts an estuary, covering approximately 10% of the total area, with brackish waters (Seeliger, 2001). The Patos Lagoon Estuary (PLE) is a microtidal estuary (tidal range <1 m) in southern Brazil that receives freshwater from the Patos Lagoon and discharges into the Southwestern Atlantic

Ocean (Weissheimer; Colling, 2024). The estuary covers an area of approximately 900 km2, with most of it consisting of shallow sandflats (<2 m deep) (Odebrecht et al., 2017). The balance between freshwater discharge and marine intrusions from the Atlantic Ocean is regulated by the Rio Grande channel and governed by wind direction, which determines seasonal salinity variations. The Rio Grande channel has a funnel-like geometry towards the ocean, with the narrowest section at the inlet, approximately $500 \, \mathrm{m}$ wide (Santa-Rosa; Schettini, 2024). Rainy periods promote freshwater discharge, resulting in lower salinities, while drier periods favor the intrusion of saltwater into the estuary (Marques et al., 2010; Möller et al., 2001).

Figure 2 – Location and Mapping of Pelotas and Patos Lagoon - location of Rio Grande do Sul in Brazil; location of Pelotas in Rio Grande do Sul; detailed view of the Patos Lagoon in the proximity to Pelotas



Source: The authors, 2025.

The Patos Lagoon (PL) is a crucial ecosystem that has significant social, ecological, economic, and touristic influence on the numerous surrounding cities. However, despite its significant ecological and socio-economic value, the PL faces multiple anthropogenic threats, such as chemical contamination and the input of sediments, nutrients, and organic matter due to the discharge of effluents drained from urban areas (Abreu et al., 2010; Medeiros et al., 2005; Pereira et al., 2018).

DATA ACQUISITION

To characterize the water quality of the Patos Lagoon (PL), five physical, chemical, and microbiological parameters were determined following the recommendations of APHA (2005): (i) Organic Matter, measured via potassium permanganate oxidation; (ii) Chloride content, determined titrimetrically using Mohr's method; (iii) Escherichia coli, analyzed through microbial reproduction using the Colilert method; (iv) Phosphorus, determined spectrophotometrically, based on acid digestion of the sample followed by treatment with ascorbic acid (quantification at 470 nm); and (v) Turbidity, measured using the nephelometric method.

All water quality data were provided by the Serviço Autônomo de Saneamento de Pelotas (Pelotas Autonomous Sanitation Service, SANEP), the municipal agency responsible for basic sanitation services in the city. The data cover the period from 2015 to 2022 and were collected during bathing suitability campaigns, which include weekly analyses conducted from November to February, totaling 107 results.

Samples were collected from the Patos Lagoon in 2-liter polypropylene bottles at a distance of approximately 1 meter from the shore, simulating the conditions of a bathing individual, at a depth between 15 and 30 cm. For bacteriological analysis, 100 mL graduated glass bottles were used, with samples taken directly from the water body. The samples were transported in refrigerated coolers, ensuring that the temperature was maintained at around 4°C and protecting them from direct sunlight. Analyses were conducted within a maximum of 24 hours upon arrival at the laboratory, with bacteriological tests performed immediately.

To analyze the influence of salinity on water quality parameters, the data were classified into two groups based on chloride content: low salinity (freshwater, <5%) and high salinity (brackish water, $\geq 5\%$). This classification followed Resolution No. 357/2005 (CONAMA, 2005).

STATISTICAL ANALYSIS

The collected data were subjected to statistical analysis using Jamovi, version 2.6 (Jamovi, 2025), a free, cross-platform software (available for Linux, Mac, Windows, and ChromeOS) that utilizes the R programming language. First, basic descriptive statistics were conducted, followed by the Shapiro-Wilk (S-W) test. Since the data did not follow a normal distribution at the 95% confidence, non-parametric tests were adopted. The Mann-Whitney U test (MW-U) was performed to determine significant differences in lagoon water quality between periods of low and high chloride concentrations, with a 5% significance level.

The identification of determinants of water quality variability in the brackish section of the Patos Lagoon was based on multivariate statistical modeling using Principal Component Analysis (PCA).

The evaluation of data adequacy and verification of assumptions for PCA were initially conducted through Bartlett's test of sphericity. This test provides a statistical significance assessment, indicating whether the correlation matrix contains significant correlations between at least one pair of variables. A statistically significant result (p<0.05) suggests sufficient correlations among variables, justifying the continuation of the adequacy analysis. Subsequently, the Kaiser-Meyer-Olkin (KMO) method was applied, comparing the magnitude of observed correlation coefficients with partial correlation coefficients, resulting in a KMO index. Values below 0.5 were discarded based on the guidelines of Hair et al. (2009).

The number of extracted factors was determined using three criteria: (i) the eigenvalue criterion, also known as Kaiser's criterion, which considers only components with eigenvalues greater than one, meaning that

each factor must explain a variance greater than that of a single variable; (ii) the variance percentage criterion, which estimates a threshold of 60% of the cumulative variance explained by the components; and (iii) the scree plot criterion, which uses the scree plot—a graph of eigenvalues plotted against the number of factors in the order of extraction. The shape of the resulting curve is used to evaluate the cutoff point, which is identified as the point of inflection.

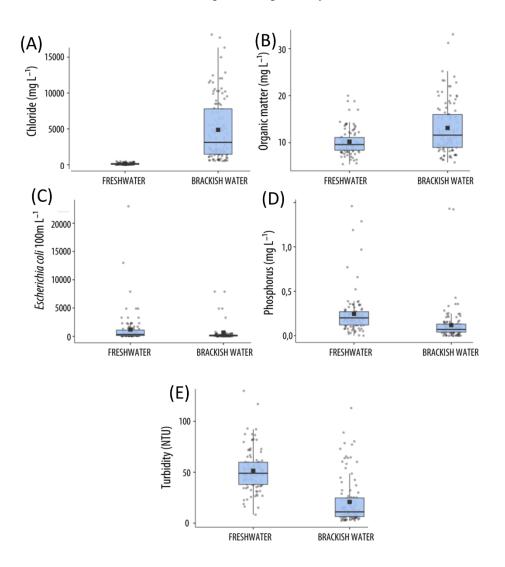
RESULTS AND DISCUSSION

Physicochemical and microbiological characterization

The results of the LP bathing water quality monitoring campaigns for the studied period (2015 to 2022) (Figure 3). It is observed that, due to the large number of samples, outliers are present in all five evaluated parameters. This occurrence may indicate pollution events related to climatic conditions, industrial discharges, or agricultural runoff. These events should be regarded as a warning signs of the sensitivity and vulnerability of this and other similar environments to human interference (e.g., domestic effluent discharge, sugarcane irrigation, fertilization, and milling effluents) (Costa et al., 2018).

As shown in Figure 3-C, E. coli in brackish water exhibited a fewer number of outliers compared to the other parameters. Relationships between runoff, precipitation, and bacterial contamination are site-specific and depend on the physiographical characteristics of each catchment (Colaiuda *et al.*, 2021). Desta *et al.* (2024) reported that, in marine beaches, higher mean salinity levels were predicted to result in lower *E. coli* levels.

Figure 3 – Water quality parameters results under fresh and brackish water conditions - (a) chlorides, (b) organic matter, (c) Escherichia coli, (d) phosphorus, and (e) turbidity. The box limits indicate the 25th and 75th percentiles; the vertical lines indicate the 90th and 10th percentiles; the points indicate the obtained data. The median and the mean are shown by a horizontal line and a square, respectively



Source: The authors, 2025.

When verifying the normality of the data using the S-W test, presented in Table 1, it was found that none of the datasets followed a normal distribution. Thus, the non-parametric MW-U test was applied to compare the parameters under the two different conditions of the LP. Corroborating the findings discussed above, this test revealed a statistically significant difference in the concentrations of all

analyzed parameters between the two water conditions of the PL (p < 0.05) – Table 1. These results provide evidence of the impacts of the lagoon's estuarine condition and chloride input, as they indicate that $E.\ coli$, phosphorus, and turbidity values are significantly lower in brackish water conditions compared to freshwater conditions.

Table 1 – Summary of Mann-Whitney U Tests Comparing Water Quality Parameters of the Patos Lagoon Under Two Salinity Conditions

Category	Chlorides (mg L-1)	Organic Matter (mg L-1)	E. coli (100mL ⁻¹)	Phosphorus (mg L-1)	Turbity (NTU)
freshwater	94.6 (31.5 - 490)	9.6 (5.4 - 20)	330 (0 - 23000) *	0.2 (0 - 1.46) *	49 (8.29 - 130) *
brackish water	3127 (535 - 18150) *	11.6 (5.8 - 33) *	130 (0 - 2300)	0.07 (0 - 1.43)	11.1 (2.38 - 113)
freshwater (S-W)	<.001	<.001	<.001	<.001	<.001
brackish water (S-W)	<.001	<.001	<.001	<.001	<.001

Median values (min - max)

The results are consistent with those reported by Kataržytė et al. (2018), who found empirical evidence showing that water turbidity influences the variability of *E. coli* concentration in the Curonian Lagoon, located in the southeast of the Baltic Sea. The presence of suspended particulate matter in water has been shown to reduce UV light transmission through shading (refraction, reflection, or scattering of UV radiation), resulting in less inactivation of microorganisms by UV radiation (Lew et al., 2023; Walters et al., 2014), leading to higher E. coli concentrations in more turbid waters. Furthermore, E. coli, along with other microorganisms, tends to attach to suspended particles, creating an environment conducive to proliferation (Jin et al., 2004). In addition, the adhesion of bacteria to sediment particles affects both the transport and subsequent fate of bacteria in the water column (Lew et al., 2023).

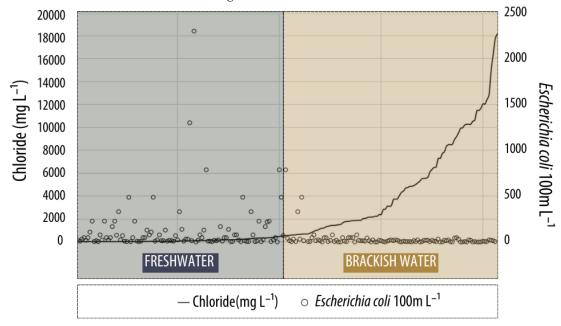
Regarding phosphorus, Korajkic *et al.* (2018) demonstrate the presence of phosphorus and nitrogen forms in the aquatic environment necessary for the survival and reproduction of microorganisms, including *E.coli*. Nutrients that normally originate lake catchments accumulate in sediments, which supports higher bacterial biomass (Pearman *et al.*, 2020). The

gastrointestinal tract of animals and humans that constitute a natural environment for *E.coli* is a copiotrophic habitat characterized by an abundant supply of nutrients (Lew *et al.*, 2023); therefore, the transition to aquatic habitats poses a challenge for these bacteria (Zhang *et al.* 2022).

difference The significant in the microbiological index between fresh brackish water can be observed in Figure 4. The inverse relationship observed, where higher chloride concentrations are associated with lower E. coli levels, is confirmed by the correlation (Figure 5), suggesting possible interactions between chemical and microbiological factors. Salinity, represented by chlorides, plays a crucial role in aquatic ecology, influencing the survival and behavior of microorganisms. Based on the combined data from the study by DeVilbiss et al. (2021) and pioneering studies (Anderson et al., 1979; Carlucci; Pramer, 1960; Hanes; Fragala, 1967), which analyzed the microbiological parameter Escherichia coli, supported by microorganism growth in Colilert at different salinity levels, as salinity reaches brackish concentrations, there is an exponential decrease in the survival rates of E. coli.

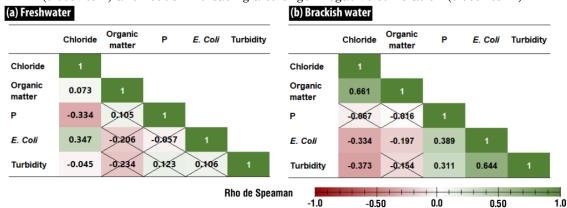
^{*}The concentration is significantly higher compared to the other category (p < 0.05). Source: The authors, 2025.

Figure 4 – Variation in chloride concentration (—, solid line) and Escherichia coli index (o, circles) in the waters of Patos Lagoon, segmented between freshwater and brackish water. The left section (gray) represents freshwater, where chloride concentration is relatively low and E. coli levels vary minimally. The right section (beige) represents brackish water, showing a drastic increase in chloride concentration and a gradual but substantial rise in the E. coli index



Source: The authors, 2025.

Figure 5 – Correlograms for water quality parameters in Patos Lagoon with (a) Freshwater and (b) Brackish Water conditions. Cell values represent Spearman's rank correlation coefficients (Rho), and crossed-out cells indicate values that are not statistically significant at the 0.05 level. Cell colors represent the strength of the correlation, with greener indicating a stronger positive correlation (closer to 1) and redder indicating a stronger negative correlation (closer to -1)



Source: The authors, 2025.

As shown in Figure 5, among the parameters that exhibited a significant correlation in both ranges, turbidity and organic matter maintained the same correlation direction with chlorides, but with a stronger correlation at higher chloride concentrations. This was not the case for E. coli, the focus of this study, as it maintains its absolute value in both ranges, but with an inversion of direction at higher salinity levels. Fulke et al. (2024) observed a strong negative correlation for fecal coliforms and total coliforms with salinity (-0.97).

Xu et al. (1982) were pioneers in observing the persistence of $E.\ coli$ in aquatic environments in the Viable But Not Cultivable (VBNC) state under various salinity and temperature conditions. The VBNC state represents an adaptation of Gram-negative bacteria, such as $E.\ coli$, allowing them to maintain metabolic activity without demonstrating colony growth. Oliveira et al. (2021) highlighted that osmotic stress due to salinity at the mouth of the Corea River is a factor that promotes the induction of the VBNC

state in *E. coli* samples. This finding supports the results presented in the homogeneity test (Table 1) and the graph in Figure 4, showing the interference of chloride content. This can be explained by the fact that this compound acts as a disinfectant agent, inactivating the microbiological indicator. To better visualize the relationship of the parameters in brackish water conditions, Principal Component Analysis was performed.

PRINCIPAL COMPONENT ANALYSIS

PCA was applied to the data from the brackish water condition of the LP. To assess the suitability of these data for factor analysis, the Kaiser-Meyer-Olkin (KMO) and Bartlett tests were conducted (Table 2).

Table 2 – Sampling adequacy measure by Bartlett's sphericity test and the KMO method

Parameter	Bartlett	KMO
Global	< 0.001	0.545
Chlorides	-	0.551
Organic Matter	-	0.524
Phosphorus	-	0.522
$E.\ coli$	-	0.540
Turbidity	-	0.784

Source: The authors, 2025.

Two principal components were extracted based on the application of the Kaiser criterion (eigenvalue greater than 1) and the cumulative variance criterion (threshold of 60% cumulative variance); therefore, the explanatory power of the model is adequate (Table 3).

Table 3 – Initial eigenvalues

Component	Eigenvalue	% of Total Variance	% Cumulative
1	2.243	44.87	44.9
2	1.458	29.16	74.0
3	0.860	17.19	91.2
4	0.257	5.13	96.3
5	0.183	3.65	100.0

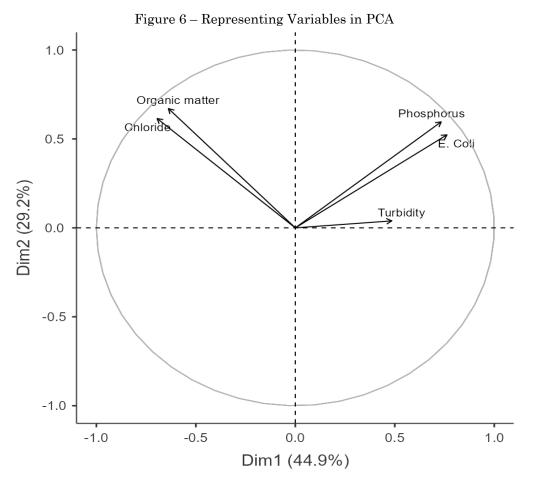
Source: The authors, 2025.

The component extraction test using the scree plot indicated that up to 4 factors could be extracted (inflection point: the curve of the individual variance of each factor becomes horizontal or experiences a sharp decline). This happens because, as a general rule, the scree test results in at least 1, and sometimes 2 or 3 additional factors compared to the Kaiser criterion (eigenvalue > 1) (Hair et al., 2009; Matos; Rodrigues, 2019).

The two identified components represent 74% of the total variance, while the first component alone accounts for nearly 45% of the

variance (Table 3). The loadings of each principal component (PC), were classified according to the criteria as "strong," "moderate," and "weak," corresponding to loading values of > 0.70, 0.70 - 0.50, and 0.50 - 0.30, respectively.

The first component was characterized by a strong positive loading of *E. coli* and phosphorus, and a moderate positive loading of turbidity. The second component explained 29% of the total variance and was dominated by a strong positive loading of chlorides and organic matter (Figure 6).



Source: The authors, 2025.

The results align with the correlations obtained in the previous sections, as they highlight the interaction between the group formed by chlorides with the *E. coli* group. Under specific environmental conditions, induced by the VBNC state in response to salinity represented by chlorides, the detection of low levels or even the non-detection of microorganisms in brackish water does not necessarily indicate their absence or low concentration in this environment. Additionally, in the brackish water condition, significantly higher values of organic matter were observed, possibly resulting from anthropogenic activities and effluent discharge into the estuary. The low concentration of E. coli found and the influence indicated by the results suggest a potential limitation of this organism as a bioindicator of water quality.

FINAL CONSIDERATIONS

The analysis conducted in this study highlights the influence of natural processes typical of estuarine areas on the bathing water quality indicators. The findings suggest that salinity

besides affecting microbiological input, indicators such as E. coli, also influences other water quality parameters. These results emphasize the need for a broader perspective in evaluating water quality in estuarine environments, considering factors beyond traditional microbiological markers. This study can be a valuable tool for decision-makers in managing public health and environmental quality.

This research aligns with several SDGs, notably Goal 6 (Clean Water and Sanitation), by improving water quality and addressing pollution. It also supports Goal 14 (Life Below Water) by examining the impact of environmental factors on coastal ecosystems, Goal 3 (Good Health and Well-being) by highlighting the importance of water quality for public health, and Goal 13 (Climate Action) due to the influence of environmental changes on salinity.

Nevertheless, future research should focus on identifying potential alternative indicators for water quality in estuarine areas, taking into account the unique environmental dynamics of these ecosystems. Additionally, further studies could investigate the interaction between salinity and other water quality parameters,

such as nutrients and organic matter, to develop a more comprehensive understanding of estuarine health. Exploring the use of biomarkers or other ecological indicators may also provide insights into the resilience of estuarine environments under varying salinity conditions.

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AUTHORS CONTRIBUTION

Daniele Martin Sampaio: Conceptualization, Methodology, Project administration, Writing original draft, Writing - review & editing; Hartur Xavier Pinheiro: Conceptualization, Writing – original draft, Writing – review and Oliveira editing; Maicon Luiz: Project administration, Writing original draft, Writing – review and editing; Cícero de Coelho Escobar: Conceptualization, Supervision, Writing – review and editing; Pascal Silas Thue: Supervision, Writing – review and editing; Flores Romani: Conceptualization, Rubia Supervision, Writing – review and editing.



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