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FIRST SURFACE WATER MONITORING CONDUCTED AT THE SÃO DONATO BIOLOGICAL RESERVE (REBIO)

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Abstract: Surface water monitoring is an essential tool for environmental assessment, as the quality of water resources comprehensively reflects the biological, ecological, physical, and chemical conditions of a study area. With the aim of assessing water quality—which is correlated with purification capacity—the first systematic water monitoring was carried out in 2024 and 2025 at the São Donato Biological Reserve, a state conservation unit that recently celebrated the 50th anniversary of its legal establishment. The characterization comprised field and laboratory stages, during which the physicochemical and biochemical properties of samples collected at three strategic points adjacent to Highway BR-472—a road that cuts through the reserve and demarcates the municipalities of Itaquí and Maçambará in this section—were evaluated. The acidity of the collected samples indicates that the pH of the surface waters is balanced; analysis of temperature and dissolved oxygen showed that the water bodies formed around the aqueducts beneath BR-472 operate aerobically and occasionally anaerobically, some parameters are influenced by agricultural activity in the region, such as turbidity and COD. BOD and water hardness showed values considered appropriate for a balanced aquatic environment. Overall, the high environmental quality of the analyzed samples was confirmed, demonstrating that the protected wetland within this conservation unit fulfills the function of mitigating any imbalances that occur in the hydrograph of the analyzed area.

Keywords: Wetland. Environmental Quality. Conservation Unit.

Introduction

The year 2026 began with a warning from the United Nations (MADANI, 2026); the document titled “*Global Water Bankruptcy: Living Beyond Our Hydrological Means in the Post-Crisis Era*” reflects growing concern regarding the planet’s available water resources. The term “Bankruptcy” has sparked debates across the globe; the document warns that the planet is operating beyond its supported water capacity, suggesting that the “planetary boundary” of the water cycle has been exceeded, pointing to what is termed “*irreversibility on a human scale*.”

The 72-page document mentions the term “wetlands” 38 times throughout its text, classifying this type of ecosystem as “*shock absorbers*” of the water cycle. The document repeatedly mentions the preservation and restoration of these ecosystems as one of the strategies to reverse the global water crisis.

The *Natural Resources Conservation Service (NRCS)*, a U.S. environmental agency, highlights several benefits associated with wetlands, including water storage, improved water quality, erosion control, a source of natural products, and habitat for flora and fauna, as discussed in *live streams* with members of this important U.S. environmental agency (NRCS, 2023). In Australia, in the state of Queensland, a “Wetlands” scheme was designed to support a consistent reference classification of lacustrine, palustrine, and fluvial wetlands, as well as some intertidal wetlands above mean sea level (*Queensland Wetlands Program*, 2023).

The management of these ecosystems includes surface water monitoring, which consists of scientific methodologies involving laboratory activities, fieldwork, and geospatial data analysis—essential tools for assessing water quality in these areas and understanding *the “water cycle”* of these ecosystems within their respective watersheds.

Despite the great importance of surface water monitoring in ecosystem management, in the half-century since the São Donato Biological Reserve (Rebio São Donato) was legally established, no research focusing on this topic had been conducted until now; therefore, this scientific study can be considered the first systematic characterization of surface waters in the São Donato Biological Reserve. Therefore, the objective of this academic study is to present and discuss the data obtained in this field and laboratory research, correlating the results with concepts associated with the purification capacity of *wetlands*.

Methodology

This study, conducted by the State University of Rio Grande do Sul (UERGS) in São Borja, in collaboration with the Secretariat of the Environment and Infrastructure (SEMA), represents the first systematic monitoring of the region, coinciding with the unit’s 50th-anniversary celebrations.

The planning phase includes verifying and calibrating portable equipment used in the field; in addition, the solutions used in laboratory procedures are prepared, and the glassware and equipment necessary for project execution are cleaned. The following is a description of the monitored parameters.

pH

A Kasvi portable pH meter was used. The pH meter’s measurement range is 0–14, and its accuracy is ± 0.1 . Data were collected in the field, with three separate samples measured at each collection point (triplicate).

Dissolved oxygen and temperature

To assess dissolved oxygen (DO) parameters, an AK88 multisensor kit was used, calibrated prior to each use. The parameter was preliminarily obtained *in situ* by measuring three different samples for each monitored collection point (P1, P2, and P3), resulting in nine samples (3 x 3). The values acquired by this device in the field were expressed as percentage of saturation (%) and temperature ($^{\circ}\text{C}$). Therefore, the second *“ex situ”* stage consisted of converting these values to mgO_2/L , performed using an online converter.

Turbidity

The turbidity of the samples was measured in the field using a Del Lab turbidimeter. This analysis was performed by collecting three different water samples for each monitoring point (triplicate).

Conductivity

The conductivity of the collected water samples was measured *ex situ*, immediately after returning from the fieldwork, using an MS TECNOPON mCA150 conductivity meter. Each sample was analyzed in triplicate.

Chemical Oxygen Demand (COD)

To determine this parameter, the Manual of Laboratory Procedures and Techniques for Water and Sanitary and Industrial Sewage Analysis from the Polytechnic School of the University of São Paulo (POLI-USP, 2004) was used, employing the Open Reflux method. This methodology involves oxidizing organic/inorganic matter in an acidic medium (H_2SO_4) using a strong oxidizing agent, potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$). The reaction is catalyzed by silver sulfate (Ag_2SO_4) and heat, in a digestion process lasting approximately 2 hours. After digestion, and allowing the solution to cool, titration of the excess dichromate is performed against a solution of Ammonium Ferrous Sulfate ($\text{Fe}(\text{SO}_4)_2(\text{NH}_4)_2$), to then determine the amount of oxidizing agent consumed in the reaction. The result is expressed in oxygen equivalents. The ferroin indicator was added after the flask had cooled to prevent its deactivation.

Natural Ammonia Nitrogen

The determination method used for this parameter was preliminary distillation/titrimetric titration according to POLI-USP (2004). The sample was buffered to pH 9.5 using borate buffer to prevent the hydrolysis of thiocyanates and other organic compounds. Next, the solution was distilled for approximately 30 minutes, and the distilled content was collected in a boric acid absorbent solution. The pH of the boric acid solution was determined in a volumetric flask, measured, and compared with a reference solution (blank). In the event of a significant pH difference (), the solution was subjected to potentiometric titration using sulfuric acid.

Biochemical Oxygen Demand (BOD)

To determine BOD, the incubation method (20°C, five days) was used in accordance with NBR 12614/1992. The process consisted of diluting three samples from each monitored site. After five days of incubation, the measurement was performed again. The results are evaluated and undergo a critical analysis; in most cases, the estimate obtained consists of the arithmetic mean calculated from the three dilutions performed.

Hardness

Water hardness was estimated using the methodology described in POLI-USP (2004), in which the chelating agent ethylenediaminetetraacetic acid disodium salt (EDTA) forms a complex when in contact with certain metals. If a small amount of Erythrosine Black T indicator is added to the aqueous solution containing calcium and magnesium ions, within a pH range of 10 ± 0.1 , the solution turns pink. With the addition of EDTA, the calcium and magnesium ions are complexed by it, and the solution turns blue, indicating the endpoint of the titration.

Description of the study area

The São Donato Biological Reserve is a state conservation unit located in western Rio Grande do Sul, established by State Decree No. 23,798 in 1975, covering an area of 4,392 ha (official boundary). Upon reaching the 50th anniversary of its legal establishment, a gap in scientific knowledge regarding the monitoring of its surface wa-

ter quality was identified, which served as a major motivator for conducting the research, initiated in 2024 and completed in 2025 (field and laboratory work). Located on the Western Border, between the municipalities of Itaquí and Maçambará, the reserve is divided by Highway BR-472. Figure 1 shows the location of the São Donato Biological Reserve in the municipalities of the Western Border of Rio Grande do Sul.

Hydrography

Figure 2 shows the main elements that make up the hydrography of the study area; its main course consists of a canal built with funds from the Pro-várzea program implemented in the 1980s, a period during which the drainage of various wetlands in Rio Grande do Sul was encouraged and financed. This canal has become the main boundary between anthropized areas and the preserved area within the reserve. In addition to this drainage route, there are several aqueducts beneath BR-472, designed to drain water accumulated in Maçambará (a municipality at a higher elevation) toward the municipality of Itaquí (at a lower elevation).

Field and laboratory work

The study consisted of a physical-chemical and biochemical characterization of samples collected monthly between April 2024 and March 2025 (12 collections in total). Three strategic points were selected along BR-472, on the Itaquí side:

- **Point 1 (P1):** Canal (lotic/flowing environment).
- **Point 2 (P2):** Spring fed by an aqueduct under BR-472 (lentic/still environment).

- **Point 3 (P3):** Water body with intermediate flow generated by an aqueduct under BR-472, existing small channel.

Figure 3 shows the locations of the three sampling points defined in this study.

Figure 4 shows photographs of the three locations chosen for sampling.

Characterization and discussion of results

The results obtained in the field and in the laboratory are organized in tabular format in Appendix A. The authors of this scientific publication consider that the approach taken by the U.S. environmental agency *Natural Resources Conservation Service (NRCS)* represents a highly relevant method for classifying and analyzing wetlands, consistent with the objectives of the research conducted. With the exception of COD, all other parameters estimated in this scientific study are recommended in the *“National Water Quality Handbook (NWQH)”* published in September 2003 (USDA, 2003), regarding water quality monitoring in natural environments. The analysis is presented below.

The pH of most samples was below 7, indicating acidity. Results below pH 7 are expected in wetlands; the decomposition of organic matter by bacteria and fungi releases CO_2 , which creates acidic aquatic environments. The most stagnant monitoring point (P2) yields the lowest pH values; therefore, it is a location where the effect of organic matter decomposition is most pronounced.

Dissolved oxygen is the most important gas for aquatic life; it has a physical relationship with temperature and a biochemical relationship with the microbiological activity

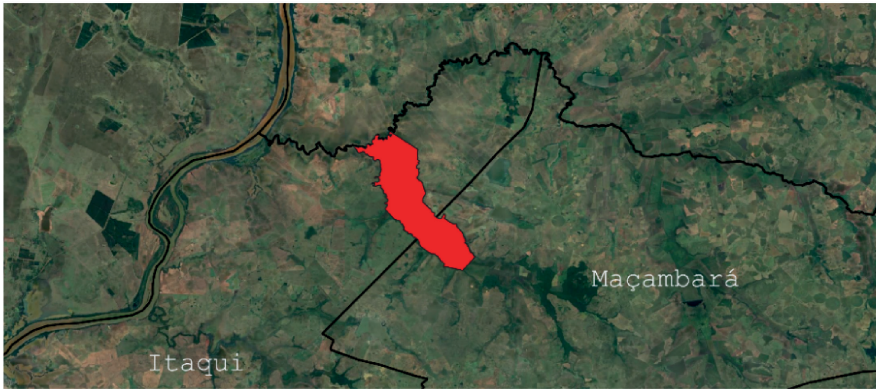


Figure 1. Location of the São Donato Biological Reserve.

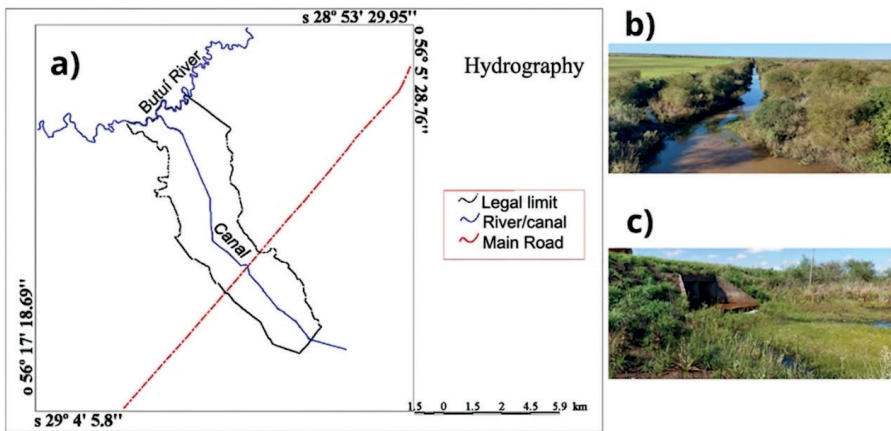


Figure 2. Items: a) Map showing the main watercourses in the São Donato Biological Reserve. b) Photograph of the canal taken from the bridge located on BR-472, the highway that crosses the reserve. c) Photograph of one of the aqueducts located beneath BR-472.

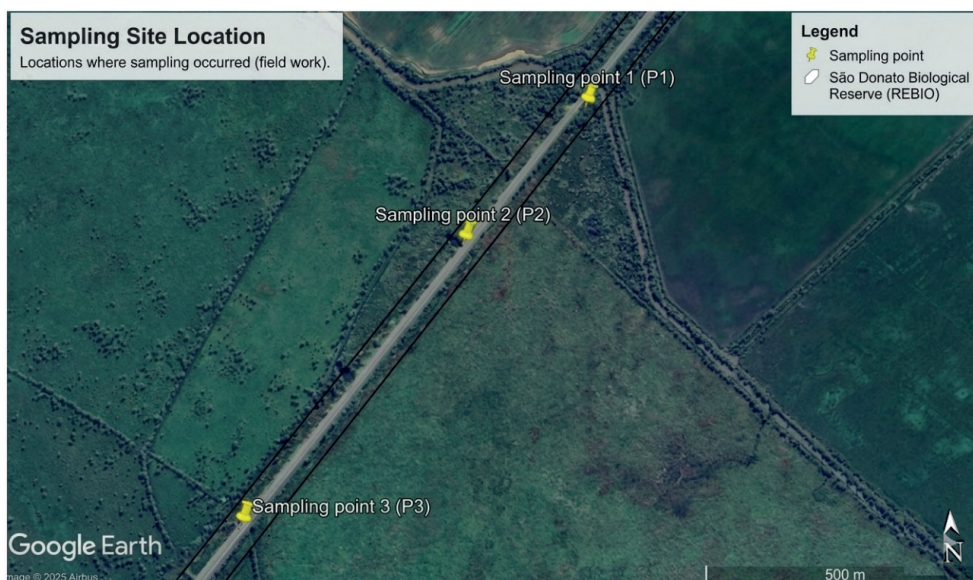


Figure 3. Location of the sites where surface water samples were collected along the BR-472 highway.



Figure 4. Surface water monitoring sites: a) View from the bridge overlooking the channel (P1); b) View from BR-472 overlooking the spring (P2); c) View from inside the reserve overlooking point 3 (P3).

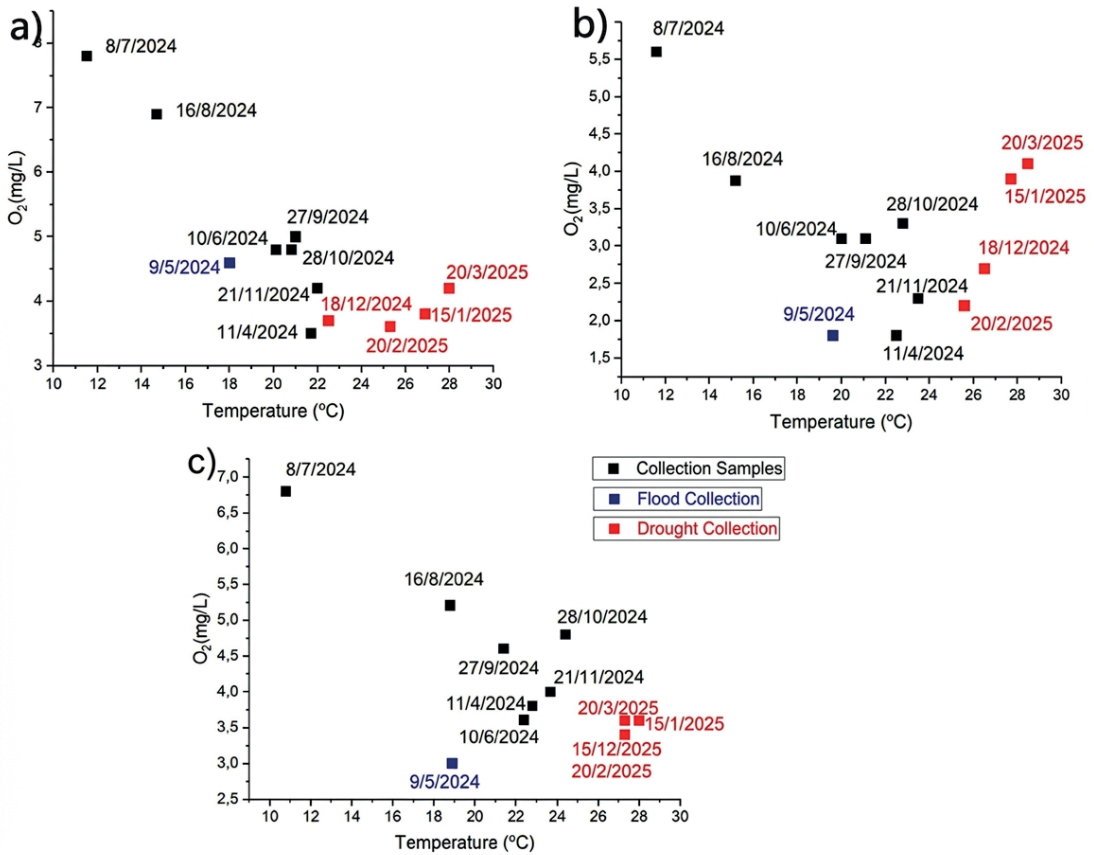


Figure 5. Plots showing the relationship between temperature and dissolved oxygen in the 12 samples collected during the study: a) correlations obtained at Point 1 (P1), b) correlations obtained at Point 2 (P2), c) correlations obtained at Point 3 (P3).

of the sites evaluated. The July collection, the coldest, yielded the highest dissolved oxygen values; a lower temperature implies greater solubility of dissolved oxygen in the samples and lower biochemical activity of microorganisms. The correlations between O_2 and temperature allow us to identify distinct biochemical mechanisms vital to aquatic life. Figure 5 illustrates these relationships.

Temperature and dissolved oxygen play a key role in denitrification, a biochemical process in which bacteria convert nitrates (NO_3^-) into gaseous nitrogen (N_2) in an anaerobic environment, using organic matter as an energy source. Klein et al. (2017) demonstrated that their short-term experimental results confirm the potential increase in denitrification with rising temperature, driven by reduced oxygen availability in the upper sediment layer, which contributes to understanding the variability of denitrification in freshwater.

Hou et al. (2015) evaluated the removal of nitrogen compounds through the anaerobic oxidation of ammonium, a microbial process that removes ammonia (NH_3) and nitrite (NO_2^-) from the nitrogen cycle, converting them into gas (N_2). The rates of this process were more closely associated with bacterial abundance than with bacterial diversity. Among all the environmental variables measured, temperature was a key environmental factor, driving a latitudinal distribution of the composition, biodiversity, and activity of the bacterial community.

Therefore, when oxygen levels decrease at the monitored sites, anaerobic conditions in the aquatic environment perform the important task of reducing nitrate levels in the water, helping to purify it. This microbiological activity is particularly valued when it occurs during the summer agricul-

tural cycle, as crop management ultimately contributes to increased nitrate levels in watercourses, which can be balanced by the presence of wetlands in agricultural watersheds. Monitoring point P2, due to its lower oxygenation, is certainly the location where the anaerobic phenomenon is most pronounced. Dissolved oxygen measured in the field was estimated from samples collected at a depth of approximately 20 cm, where oxygenation is highest; the bottom exhibits lower oxygenation levels. The samples collected on April 11 and May 9 correspond to the lowest oxygen levels; the water body selected for monitoring (P2) was certainly operating in anaerobic mode. Figure 6 illustrates this phenomenon at P2.

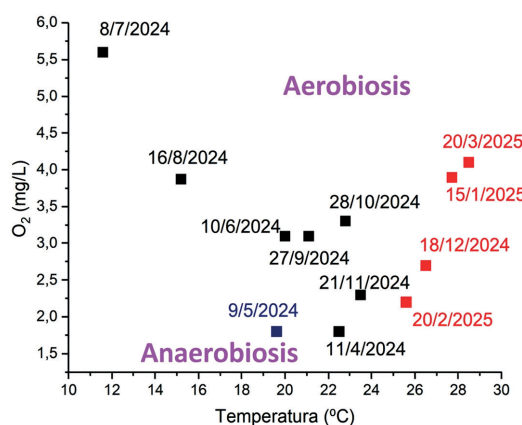


Figure 6. Representation of aerobic and anaerobic modes at monitoring point 2 (P2).

Turbidity values fluctuate significantly, which is natural since this parameter is affected by weather conditions, particularly precipitation. However, it is evident that monitoring points P1 and P3 represent the locations with the highest turbidity; Rebio's geographic analysis, particularly regarding land use for agricultural activities, demonstrates that these are the locations where the impact of agriculture is most evident. The NWQH identifies excessive algae and sediments as a potential problem when this parameter rises.

Water body P2, the most stagnant (lentic) location, exhibited lower turbidity, has a lower sediment content, and offers conditions more favorable to the development of macrophytes. Photographic records show a greater presence of macrophytes in P2; however, their moderate presence, covering a limited portion of the water body, indicates a state of equilibrium. Eutrophic environments are characterized by low dissolved oxygen, high nutrient loads, and uncontrolled macrophyte growth.

Analysis of dissolved oxygen and turbidity suggests that the São Donato wetland is far from conditions that favor the eutrophication of water bodies—characterized by high nutrient levels and low oxygen—and the evaluation of these parameters demonstrates an inverse relationship; therefore, the existence of the São Donato wetland inhibits the feared phenomenon of water body eutrophication, contributing to the balance of aquatic biota in the Butuí River watershed, within which it is located.

Conductivity is a property that can be associated with Total Dissolved Solids (TDS); therefore, it can be correlated with the wetland's ability to act as a natural filter. The results clearly show that monitoring point P3 has the highest conductivity, indicating a higher concentration of TDS.

The range obtained throughout the study at the three monitored points varies between 78 and 378 $\mu\text{s}/\text{cm}$, a range lower than that recorded in the *wetland* study conducted by **Joseph et al.** (2010), which obtained a range of 469–548 $\mu\text{s}/\text{cm}$ measured at fifteen sampling points located in the “*Lake Waco Wetlands*.”

Thus, it is believed that the filtration capacity of the area upstream of the monitored points is efficient, corresponding to the section belonging to the municipality of Maçambará (water flow occurs from Maçambará to Itaquí). Therefore, the conductivity values obtained in the São Donato Biosphere Reserve are lower than those in other scientific studies conducted in *wetlands* abroad, indicating a lower TSS content in the assessed conservation unit, which is an indication of environmental balance.

Studies using *wetlands* as treatment systems demonstrate that the efficiency of removing Chemical Oxygen Demand (COD) and ammonia (NH_4^+ -N) is higher in spring and summer compared to fall and winter. **Haiming et al.** (2011) report removal rates of 10.72–19.34 mg l^{-1} and 0.18–0.90 mg l^{-1} of COD and ammonia, respectively, in a pilot-scale *wetland* system.

In the characterization conducted at Rebio São Donato, the first five samples were collected in the fall and winter, and the remaining seven samples were collected in the spring and summer. The arithmetic means of COD obtained from these two periods are 19.9 and 36.9 mg/L , respectively. Therefore, it is believed that the wetlands are more efficient during the period when COD levels are higher, which is consistent with the basic principle that a greater concentration gradient generates greater biochemical removal efficiency.

An inverse relationship was identified in the analysis of the BOD obtained, using the arithmetic means of the same periods (samples); the average BOD of the fall and winter samples was 3.9 mg/L , while the spring and summer samples averaged 2.9 mg/L .

The COD/BOD ratios obtained—5.1 and 12.7, respectively—indicate that the inert fraction of the samples increases considerably during spring and summer. This increase may be associated with agricultural activity, which requires soil management. In both cases, the biodegradability of the collected samples can be considered low. The wetland area upstream of the sampling points is equivalent to a biological treatment plant, which explains these relationships, yielding samples in which the biological fraction is low, since the water has already spent a considerable residence time in the upstream wetland, which exceeds 1,000 ha in area.

Most of the titrations obtained in this study, aimed at determining total water hardness, measured in mg/L of CaCO_3 , fall within the classification range known as moderate hardness, which varies from 50–150 mg/L. Two factors may be associated with this parameter: phytoplankton formation and bioaccumulation in organisms.

The “Water Quality Manual for Aquaculture” (de Oliveira, 2000) establishes the range of 55 to 200 mg of Ca and Mg/L as good water with a mineral salt content suitable for aquatic life; values below 20 mg/L represent a nutrient limitation in phytoplankton formation. Therefore, the mineral salt content in the analyzed samples is adequate for phytoplankton formation.

Studies conducted many decades ago had already established an inverse relationship between toxicity and water hardness. For example, a study evaluating zinc toxicity in fish (“guarus”) concluded that zinc toxicity increased as water alkalinity and hardness decreased (Gianotti, 1986). Therefore, the moderate hardness present in the aquatic biota of the study area is con-

sidered beneficial for this important fish spawning site in western Rio Grande do Sul, as it can reduce the toxicity of potential contaminants by inhibiting the bioaccumulation of metals in fish.

Bioaccumulation depends on many abiotic factors (such as site-specific water quality, including hardness or pH) and biotic factors (such as lipid content, age, or sex of the organism) that must be considered in bioaccumulation assessments (Schäfer, 2015).

The method used in this study to estimate natural ammonia nitrogen did not prove to be sensitive, as the level of contamination by this pollutant is low. Do not confuse this parameter with total ammonia nitrogen, which requires digestion of the samples.

Conclusions

The water pools formed by the aqueducts beneath BR-472 operate aerobically and occasionally anaerobically, which certainly alters the biochemical mechanisms of water purification and may influence phytoplankton formation and fish behavior.

The mineral salts in the collected samples are at levels considered adequate for phytoplankton formation, which is regarded as the basis of the food chain in aquatic environments; this is one of the positive aspects confirmed by laboratory analyses.

The COD/BOD ratios indicate that the water analysis could be divided into two periods: one associated with the agricultural cycle (spring and summer), characterized by low biodegradability of the samples, and a non-agricultural period (fall and winter), during which the biodegradability of the samples increases.

Overall, the results obtained from the first systematic monitoring of surface waters conducted in the São Donato Biological Reserve demonstrated that it is a balanced environment; the sampling points allowed for the assessment of the water flowing from the municipality of Maçambará, and then to Itaquí; this is a watershed influenced by the agricultural cycle, which underscores the importance and necessity of preserving the ecosystem protected by state environmental legislation.

The NRCS recommends that this type of work be conducted every five years, which creates a need for active, decentralized research groups located near the study areas that possess a genuine capacity for monitoring the target ecosystems.



Monitoramento de águas superficiais da Rebio São Donato

Apresentação exibida no VI SEREEI realizado no Instituto Farroupilha nos dias 3 e 4 de outubro de 2025 em São Borja. Consiste na primeira apresentação do primeiro estudo sistemático de águas superficiais realizado na Reserva Biológica do São Donato localizada na Fronteira Oeste do Rio Grande do Sul.

Disponível em: <https://youtu.be/8JvwiX4a1Cc>

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Anexo A – Resultados obtidos no trabalho de campo e laboratorial.

	11/04/2024			09/05/2024			10/06/2024			08/07/2024			16/08/2024			27/09/2024		
	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
pH	6,7	6,7	7,1	6,9	6,5	6,8	7,3	6,7	7	7,5	6,6	7,4	7	6,7	7,2	6,6	6,4	6,8
OD (mg/L)	3,5	1,8	3,8	4,6	1,8	3	4,8	3,1	3,6	7,8	5,6	6,8	6,9	3,9	5,2	5	3	4,6
Turbidez (NTU)	90,8	27,2	66,4	38,1	24,9	23,5	50,1	21,3	62,3	30,7	20,9	16,1	47,3	22,7	37,7	150	28,4	95,3
Temperatura (°C)	21,7	22,5	22,8	18	19,6	18,9	20,1	20	22,4	11,5	11,6	10,8	14,7	15,2	18,8	21	21,1	21,4
Condutividade (µs/cm)	170	122	327	159	140	183	175	124	378	170	78	358	117	103	174	113	129	177
DQO (mg/L)	30,2	40,2	24,2	19,2	31,6	22,3	11,8	21,9	18,8	9,1	15,7	4,5	18,8	16	14,6	42,9	27,5	49,2
DBO (mg/L)	3,7	4,9	4,6	2,3	4	3,9	2,9	5,2	5	4,7	5,5	4,6	3	2	1,8	3,7	3,9	5,3
Dureza (mgCaCO ₃ /L)	67,9	55,3	97	46,6	48,5	53,4	63,6	46,5	104,5	86,5	28,5	96,9	45,6	37	65,5	58	47,5	63,6
Nitrogênio Amoniacal (mgN-NH ₃ /L)	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1

	28/10/2025			21/11/2024			18/12/2024			15/01/2025			20/02/2025			20/03/2025		
	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
pH	6,4	6,2	6,9	6,6	6,8	7,1	6,6	6,6	7,1	6,4	6,7	7	6,2	6	6,4	7,2	7,3	7,3
OD (mg/L)	4,8	3,3	4,8	4,2	2,3	4	3,7	2,7	3,4	3,8	3,9	3,6	3,6	2,2	3,4	4,2	4,1	3,6
Turbidez (NTU)	62,5	25,4	88,4	123	38,4	118	42,3	36,3	69,6	39,2	45,8	137,7	43,2	18,3	26	41	24,9	38,1
Temperatura (°C)	20,8	22,8	24,4	22	23,5	23,7	22,5	26,5	27,3	26,9	27,7	28	25,3	25,6	27,3	28	28,5	27,3
Condutividade (µs/cm)	152	145	262	142	176	205	137	125	189	148	142	318	134	153	173	151	144	187
DQO (mg/L)	39,4	22,7	19,2	69,3	61,4	75,3	24,4	33,1	16	21,8	22,2	79,9	37,8	30,5	21,4	23,6	32,7	24,8
DBO (mg/L)	4	4,3	1,7	4,1	6	6,3	0,5	0,8	1,2	1	2	4	2,5	3,7	3,2	0,3	1	1
Dureza (mgCaCO ₃ /L)	66,4	62,6	72	53,3	72,9	61,7	68,3	57	68,3	63	60,8	105,8	58,5	61,9	66,4	70,9	73,1	84,4
Nitrogênio Amoniacal (mgN-NH ₃ /L)	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1