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Original Research

Biomonitoring the public supply reservoir in Camaquã – RS using *Tradescantia pallida* var. *purpurea* and *Escherichia coli*

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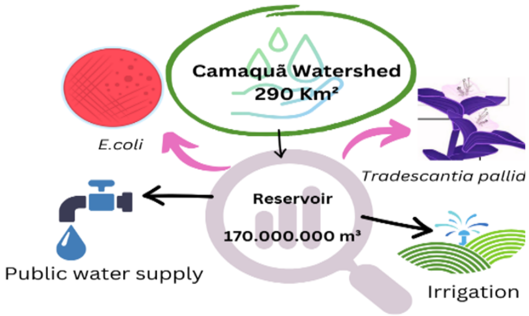

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Abstract: Water plays a central role in sustainable development, encompassing social, economic, and environmental dimensions: social, economic and environmental. The Arroio Duro Dam, located in the municipality of Camaquã - RS, is a reservoir that provides water for the public supply of approximately 60,000 inhabitants, in addition to enabling recreational activities and serving as a source of irrigation for agriculture. This work aims to evaluate the environmental quality of the Arroio Duro Dam using the determination of genotoxicity through pollen abortion in pollen grains of the bioindicator plant species *Tradescantia pallida* var. *purpurea* and the health quality bioindicator *Escherichia coli*. Preliminary results from two collections indicate high genotoxicity of the samples in relation to the negative control ($p<0.05$). Analyzes of the sanitary conditions of the Arroio Duro Dam indicated that the water is appropriate for the assigned uses, within the limits established by Brazilian legislation. This work highlights the importance of bioindicators in the integrated determination of environmental quality, generating results that assist in the integrated management of water resources in their multiple uses.

Keywords: Environmental monitoring, genotoxicity, water resources, environmental pollution.

Introduction

The Arroio Duro Dam is a reservoir with a capacity of 170 million cubic meters of water, distributed for public supply to around 60,000 inhabitants of the city of Camaquã (RS) [1] and during harvest periods it provides water for irrigated rice around 16,000 hectares and 19,000 hectares of irrigated soybeans [2].

The dam's multiple uses influence its physical, chemical, and biological characteristics, potentially compromising its suitability for public supply and posing risks to public health. Thus, biomonitoring of public water supply sources is of fundamental importance for assessing water quality, identifying polluting sources and ensuring the preservation of water resources for their priority uses. In this sense, Resolution 357 of the National Environment Council (CONAMA) of 2005 [3] establishes five classes of water, parameters and limits that must be met for their uses.

Among the parameters established by CONAMA are health bioindicators, such as the *Escherichia coli* bacterium, which is used as an indicator of fecal contamination, helping to identify the risk of spreading waterborne diseases. However, there are various risks to human health and environmental preservation that fall outside the parameters established by CONAMA. In this sense, ecotoxicological studies stand out for their ability to measure the integrated effects of different pollutants on the ecosystem. One of the methods for assessing exposure to environmental pollution and its potential impact on living organisms is based on the use of genotoxicity bioassays, which can be carried out using bioindicator plant species, such as those of the genus *Tradescantia L.* (*Commelinaceae*). These plants are capable of showing changes in their DNA, even when exposed to low levels of contamination [4, 5].

With these bioindicators, it is possible to analyze a water source globally, establishing relationships between activities outside the watercourses and how these activities are influencing water quality [6]. This underscores the importance of conducting analyses that integrate traditional standards with genotoxicity tests. The aim of this study was therefore to assess the environmental conditions of the Arroio Duro Dam public supply reservoir, using the bioindicators *Escherichia coli* and *Tradescantia pallida* var. *purpurea*.

Experimental Section

Study Area

Arroio Duro belongs to the Camaquã River Basin, which is part of the Pampa biome (South of Brazil). Its source is located between the municipalities of Chuvisca and Dom Feliciano, whose economy is based on agriculture, with a predominance of tobacco crops (*Nicotiana tabacum*). The water from the Arroio Duro is collected in a 290 km² basin and stored in a reservoir located in the municipality of Camaquã (30°49'29.4"S, 51°50'50.6"W) (Figure 1). After being distributed for supply and irrigation, the Arroio Duro flows into its mouth in the Patos Lagoon.

Figure 1. Arroio Duro Dam.

Water Samples Collection

Two water samples were taken in September 2022 (sample 1) and February 2023 (sample 2). The water was collected according to the methodology described in the Standard Methods for the Examination of Water and Wastewater [7]. At each sampling, 6 L of water were collected using a bucket attached to the end of a cable, the samples were preserved in previously sanitized plastic bottles and transported to the laboratory.

Water Genotoxicity

The plants came from a collection kept in a specific garden. We used 20 branches of *Tradescantia pallida* var. *purpurea* (10 to 15 cm long) containing flower buds at a stage before anthesis, all collected on the same day and at the same time. The branches were adapted in distilled water for 24 hours, then exposed to water from the Arroio Duro dam for 8 hours and finally recovered for 28 hours in distilled water [8]. In addition, for the negative control, 20 branches were exposed only to distilled water. After the recovery period, the flower buds were fixed in a solution of absolute ethanol: acetic acid (3:1 v:v) for 24 hours and then stored in 70% ethyl alcohol under refrigeration (4 °C) until analysis.

To prepare the slides and analyze the pollen grain mother cells, the flower buds were dissected and the anthers macerated and stained with 1% acetic carmine. On each slide, 300 pollen grains were counted. Ten slides were analyzed for each collection and another 10 for the negative control, giving a total of 7,000 pollen grains in each repetition.

The pollen grains were analyzed under 400x magnification and the pollen abortion frequencies (AP) were expressed as AP/100 (number of abortive pollen grains in 100 cells). The pollen abortion frequencies met the assumptions of normality and were submitted to Student's t-test for independent samples. The tests were carried out using the Statistical Package for the Social Sciences (SPSS) version 25 (SPSS Inc., Chicago, IL, USA), with a significance level set at 5%.

Microbiological Analysis

To assess the sanitary conditions of the dam, using *Escherichia coli* bacteria as an indicator, the Colilert® (IDEXX) defined substrate technique was used. According to the manufacturer's recommendations and the Standard Methods for Examination of Water and Wastewater [7]. The samples were packaged under refrigeration and taken immediately to the laboratory, where they remained under refrigeration until the moment of analysis, respecting the limit of a maximum of 24 hours between collection and analysis.In the laboratory, under aseptic conditions, 100 ml aliquots were collected in sterile flasks, the defined substrate was applied and dilutions were made as shown in Figure 2.

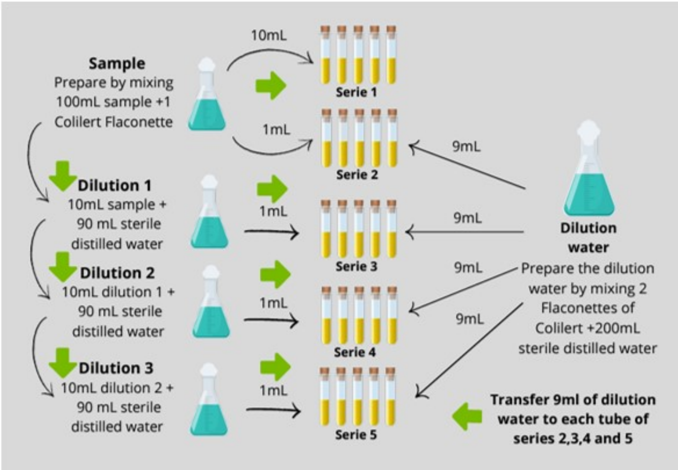

Figure 2. Sample preparation and dilution.

The samples were then incubated at 35 °C for 24 hours. After incubation, a reading was taken, determining the presence of total coliforms by changing the color of the solution in the tubes from transparent to yellow. The presence of total coliforms was confirmed, the presence of *E. coli* was verified by observing the blue fluorescent coloration of the tubes under ultraviolet light (366 nm), as shown in Figure 3.

After determining the number of tubes with a positive result for *E. coli*, in each sample dilution series, three dilutions were chosen, constituting the critical series formed by three digits used to consult the most likely number of *E. coli* in 100 ml of sample (MPN/100 ml). The critical series is composed from the most diluted series that presents all positive tubes, complementing it with the following two series. After consulting the NMP, whenever necessary, the correction factor is applied, multiplying the result according to the dilution factor of the first dilution used to compose the critical series.

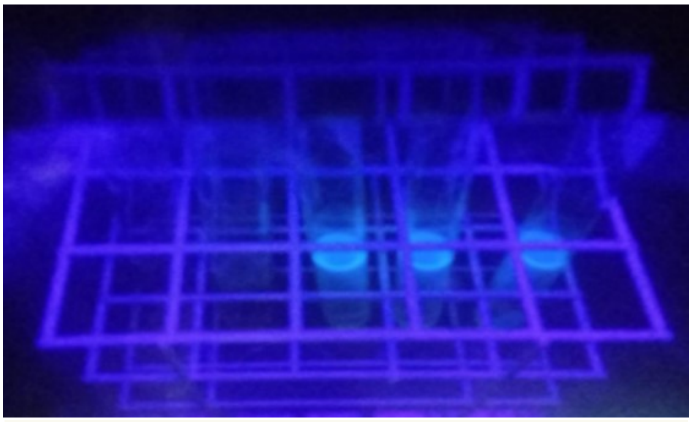

Figure 3. Determination of the presence of *E. coli* using the defined substrate. On the left, two negative test tubes are observed, as they do not show blue fluorescence when exposed to ultraviolet light, indicating the absence of *E.coli.* On the right are three positive tubes, with typical blue fluorescence when exposed to ultraviolet light, indicating the presence of *E. coli.*

Results and Discussion

Water from the Arroio Duro Dam caused genetic changes in the germ cells of T. *pallida* var. *purpurea* manifested by the occurrence of pollen abortion (Figure 4). In collection 1, the frequency of pollen abortion recorded was 22.83 ± 9.18 and in collection 2 7.11 ± 1.60 and there was no difference in the comparison of genotoxicity between collections 1 and 2 ($p>0.05$). The negative control presented a mean frequency of 2.16 ± 1.86 and differed from the samples in both collections ($p<0.05$) (Table 1). Although there is no basal frequency for pollen abortion in *Tradescantia*, the differences observed between treatments demonstrate that the genotoxic pollutants present in the water of the Arroio Duro Dam caused damage to the reproductive cells of T. *pallida* var. *purpurea*.

Table 1. Genotoxic potential and microbiological analysis.

	Pollen abortion	E. Coli NMP/100 ml
Sample Collection 1	22,83 ± 9,18	2,0
Sample Collection 2	27,11 ± 1,60	4,5
Negative control	2,16 ± 1,86	

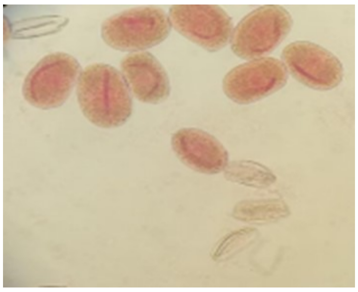

Figure 4. Pollen grain of *Tradescantia pallida* var. *purpurea* viable (colored) and aborted pollen grain (pale) 400x.

The results of the genotoxicity test on *Tradescantia* suggest that these changes are likely caused by exposure to diffuse pollution sources, such as complex mixtures from the extensive use of pesticides and fertilizers predominantly applied to tobacco crops in the region [9]. In a study similar to this, [10] monitored the public water supply from the Cantareira System Dam (SP) and found high levels of genotoxicity, which were also attributed to diffuse sources of pollution.

Sanitary bioindicators such as *E. coli* are used in conjunction with physical and chemical parameters to classify water resources, allowing the establishment of water quality goals that must be achieved to ensure safety in the intended activities. This framework is provided for in the CONAMA Resolution nº 357 of March 17, 2005 (Table 2).

Table 1. Classification of water bodies, limits and uses.

Class	Quality specifications (*Escherichia coli* /100 mL)	Intended for
Class 1	Below 200	Supply for human consumption after simplified treatment, protection of aquatic communities, primary contact recreation, irrigation of vegetables that are consumed raw, protection of aquatic communities with Indigenous Lands.
Class 2	Below 1000	Supply for human consumption after conventional treatment, irrigation of vegetables, fruit plants and parks, gardens, sports and leisure Fields, with possible direct contact by humans, aquaculture and fishing activities.
Class 3	Below 2500	Supply for human consumption after conventional or advanced treatment, irrigation or tree cultivation, cereal and fodder, amateur fishing, secondary contact recreation, animal drinking.
Class 4	Not determined	Navigation and landscape Harmony.

Comparing the limits established by CONAMA Resolution nº 357 of March 17, 2005 for E. coli (Table 2) with the results found in the two collections, we can infer that the sanitary conditions of the Arroio Duro Dam are appropriate for the assigned uses, being within the limits established for class 1.

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This bioindicator makes it possible to evaluate contamination of fecal origin, mainly from domestic effluents and animal husbandry without proper treatment. Taking only the result of sanitary conditions, the water present in the dam is considered suitable, through treatment, to be offered for public supply, in addition to the protection of aquatic communities, primary contact recreation, irrigation and other uses. However, there are other possible forms of contamination, industrial effluents, pesticides, among others that can compromise human health and other uses of the reservoir's water, thus, the health bioindicator is just one of the parameters required for classification and the class of the dam. It can only be established in conjunction with other parameters such as dissolved oxygen, biochemical oxygen demand, turbidity, among others.

CONAMA Resolution 357 [3] establishes that: "Possible interactions between substances and the presence of contaminants not listed in this Resolution, capable of causing harm to living beings, must be investigated using ecotoxicological, toxicological tests, or other scientifically recognized methods". In this sense, the assessment of water quality through bioindicator plants of genotoxicity provides warnings about the environmental risks that water can pose, revealing effects that extend to human health [8, 11]. Although preliminary, the results found indicate that the water from the reservoir, despite meeting the established quality criteria, may present risks to human health and the biodiversity present, due to various substances, not originating from domestic effluents, which have genotoxic potential.

Given the wide range of pollutants that can contaminate water reservoirs, assessing water quality solely through traditional parameters is inadequate. Given that *E. coli* is usually the standard for monitoring watersheds, classifying and delimiting uses, the absence of contamination from feces, which classifies the watershed as excellent for human supply after simplified treatment, may hide risks to human health from other sources of contamination, generating a false sense of security.

This perspective is evident when observing the high levels of genotoxicity observed in the Camaquã dam, indicating the presence of risks to human health that persist after water treatment. These findings highlight worrying implications for the health of populations supplied by these water resources, since continued exposure to contaminants can lead to significant risks, including waterborne diseases and genetic damage. Thus, we reaffirm that the combined use of different bioindicators, such as health and genotoxicity bioindicators, offers a more comprehensive analysis of water quality and potential sources of pollution.

Therefore, the data reinforce the urgency of implementing effective environmental management actions, which are essential to mitigate sources of contamination, preserve the quality of water resources and protect public health. Continuous monitoring, combined with the implementation of sustainable public policies, must be a priority to ensure the safety and well-being of communities dependent on these systems.

Conclusions

This study demonstrates that combining health and genotoxicity bioindicators offers a comprehensive perspective on water quality and potential pollution sources. Studies like this provide information that contributes and highlights the need for environmental management actions, aiming to improve the quality of water resources.

Authors Contribution

D. C. da Silva: Methodology, Formal analysis; C. D. Woloski: Methodology, Formal analysis; G. S. Laguna: Methodology, Formal analysis; M. E. F. Gonçalves: Methodology, Formal analysis; B. N. F. Machado: Methodology, Formal analysis; C. N. Wille: Writing; review and editing; L. R. Nogueira: Writing; review and editing. All authors have read and agreed to the published version of the manuscript.

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