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WATER QUALITY AND GENOTOXICITY IN FISH SUBMITTED TO THE WATERS OF THE CAVEIRAS RIVER IN OUTLOOK AREAS OF THE GUARANI HEREFER

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Abstract: The water quality of confined aquifers, such as the Guarani, depends on the geological matrix that contains it and the quality of the recharge. Outcrop areas need attention, especially when close to potentially polluting urban centers or industrial complexes. This work evaluated DNA damage in fish of the species: *Rhamdia quelen*, submitted to the waters of the Caveiras River in areas of upwelling of the Guarani Aquifer. The physical-chemical and microbiological analyzes evaluated samples from five different points of the river. For the genotoxic analyses, the fish were exposed to water at one point, at different times. Genotoxicity was evaluated by the Micronucleus Test and Comet Assay techniques. Statistical analysis was performed using the non-parametric Kruskal-Wallis analysis of variance ($p<0.05$). The results of water analysis and genotoxicity indicate an accelerated process of degradation of the Caveiras River in the region in contact with the outcrop of the Guarani Aquifer, after the urban contribution.
Keywords: Recharge, Contamination, Jundiá, Micronucleus Test and Comet Assay.

INTRODUCTION

The physical-chemical and biological characteristics of river water are the result of the influence of climate, geology, vegetation, as well as human occupation and land use in the hydrographic basin. Contributions from human activities, such as depositing and dumping of waste, can change aspects of water quality, compromising aquatic life and restricting the possible uses of water resources (DORIGON, STOLBERG and PERDOMO 2008; FERRARO, 2009).

As a result of these residues, an increasing number of chemical compounds, foreign to the environment, called xenobiotics, have negatively influenced the dynamics of ecosystems as a whole (OOST, BEYER and

VERMEULEN, 2003). The consequences of these contaminations of water resources are serious and must be evaluated through analysis with biochemical and biological parameters (BRASIL, 2005).

With an approximate area of 2400 km² and 130 km of extension of the main thalweg, the Caveiras River Hydrographic Basin is located in the mountainous region of Santa Catarina, being the second largest sub-basin of the Canoas River Hydrographic Basin. The Caveiras river, mainly in the urban area, runs through areas of outcrops of the Guarani Aquifer. It receives industrial effluents from a brewery and urban effluents from the city of Lages, mainly through the rivers: Ponte Grande (24km²) and Carahá (30 km²) (RAFAELI NETO, 1994).

In Santa Catarina, the main areas of aquifer outcrop occur in the central portion of the state and are located in the west and south of the Domo de Lages (FREITAS, CAVE and MACHADO, 2003). These outcropping bands occupy about1.780 km², and whether they constitute areas of high vulnerability to contamination, being formed by sandstones from the Botucatu formation (GOMES, FILIZOLA and SPADOTTO, 2006; ZANATTA, ANDRADE, COITINHO, 2008). In the rest of the state, the Guarani Aquifer is covered by rocks from the Serra Geral Formation, which makes it less vulnerable to contamination (ZANATTA, ANDRADE, COITINHO, 2008).

The present work aims to evaluate the water quality of the Caveiras River in the area of contact with the Botucatu sandstone, using physical-chemical, microbiological and genotoxic analyzes to establish the effects of probable polluting compounds in the waters of the Caveiras River, on fish of the species: *Rhamdia quelen*.

MATERIAL AND METHODS

The collection points on the Caveiras River and nearby spring (Fig. 1) are areas of upwelling and consequent recharge of the Guarani Aquifer. Water collections were divided into two stages: in the first, water was collected at five different points along the river every 15 days, from April to August 2014; in the second moment, genotoxic analyzes were carried out in fish of the species: *Rhamdia quelen* (jundiá), that were submitted to the waters of point 4.

The five collection points sampled in the Caveiras river basin comprise four points in the stretch of contact between the river and the Botucatu sandstone: a point prior to the municipal water collection (Point 1 - Latitude: 27°51’55.95”S and Longitude: 50°12’13.77”W) and close to the boundary of the municipalities of Lages and Panel; one point after the contribution of the Ponte Grande River (Point 2 - Latitude: 27°50’41.90”S and Longitude: 50°18’35.13”W) and after the contribution of industrial effluents from a brewery; one point after the contribution of the Carahá River (Point 3 - Latitude: 27°51’28.73”S and Longitude: 50°20’27.56”W) and another at the end of the Botucatu sandstone, already with the appearance of Basalt (Point 4 - Latitude: 27°52’48.40”S and Longitude: 50°22’56.59”W). A control point was selected (Point 5 - Latitude: 27°52’18.13”S and Longitude: 50°11’47.12”W), using a spring located in the area of the Brazilian Institute of the Environment (IBAMA), close to Point 1. It must be noted that the Botucatu sandstone is already cut by the Caveiras Riverin the municipality of Panel, however, upstream of Point 1 the river cuts through the basalt area.

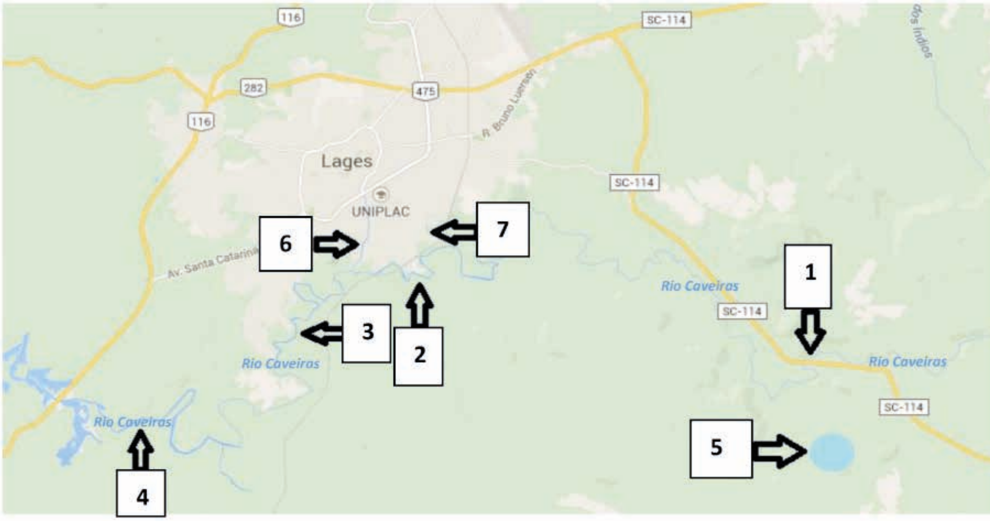

Figure 1: Collection points on Caveiras River (1, 2, 3, 4). Rising at 5. Numbers 6 and 7 represent the Carahá River and Ponte Grande, respectively.

Water collections for the physical-chemical and microbiological analyzes were carried out from April to August 2014. As for the collections, at point 4, for exposing the fish to the river waters, they were carried out from April to May 2014.

The collections were carried out in accordance with the Technical Manual for the Collection of Water Samples of the Public Ministry of Santa Catarina (MPSC, 2009), in accordance with the determinations of the United States Environmental Protection Agency (EPA, 2007) and the American Health Association. (APHA, 2005), which are the basis of the ANA/MMA criteria (CETESB, 2011).

Analyzes of pH, DO and water temperature parameters were carried out in the field, at the collection sites, with a YSI multiparameter probe.

For the genotoxicity experiment, 80 juvenile fish of the species were used: *Rhamdia quelen* (Jundiá), approximately three months old, average weight of 17.3g and average length of 9.57cm. The project was approved by the Committee for Ethics in Animal Experimentation at UDESC (CETEA) under protocol 1.76.12. The animals were previously acclimatized for a period of 30 days with water from the Pisciculture Laboratory of the Agroveterinary Center (CAV) of ''Universidade Estadual de Santa Catarina" (UDESC). The fish were packed in two 250-liter boxes, with approximately 200 liters of water in each. The boxes were cleaned daily, with the siphoning of food and feces residues, as well as the replacement of the siphoned water, which was around 30% of the total volume of each container. For water changes, there were two 500-liter containers that were used to store the water collected in the river and two 500-liter containers for laboratory water.

The fish were divided into two large groups, one group (Control) was kept in waters from the Pisciculture Laboratory of CAV/UDESC and the other group (Test) in waters collected from the Caveiras River. The study was subdivided into four different times (Time I - day zero, 30 days after fish acclimatization and before separation into two groups; Time II - seven days after time I, seven days exposed to river waters; Time III - 14 days after time II, 21 days of exposure; and Time IV – seven days after time III). At each time (I, II and III and IV) blood was collected from 20 fish, 10 from the control group and 10 from the test group. After time III, the 10 remaining animals in the Test group, which were kept in waters of the Caveiras River, were removed and placed in the same conditions as the control group for another seven days, and the animals' blood was collected again, this being time IV. During all stages the animals had the same nutritional diet and the same handling conditions.

The animals were slaughtered with a lethal dose of benzocaine hydrochloride. (28 mgL^{-1}). Promptly, blood collection was performed with the aid of a heparinized syringe. The aliquots of blood collected (5 µL) of each animal were stored in centrifuge microtubes of the "eppendorf" type containing 500 µL of fetal bovine serum (FBS). Immediately after collection, the samples were subjected to the comet assay and micronucleus test techniques.

For the comet assay, 100 nuclei were analyzed in triplicate by fluorescence microscope in a blind test, totaling 300 nuclei analyzed per animal and a total of 24,000 cells analyzed in all. Cells were classified into five predefined damage classes (0, 1, 2, 3, and 4) which correspond to size and nucleoid/tail ratio, with 0 (class A, no apparent damage, < 5%), 1 (small damage, from 5 to 20%), 2 (medium damage, from 20 to 40%), 3 (maximum damage, from 40 to 95%) and 4 (total damage with nucleus destroyed or in apoptosis, > 95%). Damage scores were obtained by the formula Score = (0. (A) + 1.(B) + 2.(C) + 3.(D) + 4.(E))/Number of cells counted.

As a standard genotoxic substance for in vitro exposure (positive control), hydrogen peroxide was used (H_2O_2) in the concentration: 5µM. In 10 µL of the whole blood and fetal bovine serum mixture was added 1µL de H_2O_2. The tube was left at room temperature (20 to 25°C) for 30 minutes. After this period, the Comet Assay technique was used. Statistical analysis was performed using the non-parametric Kruskal-Wallis analysis of variance test with significance level $p < 0.05$.

For the micronucleus (MN) test, smears were performed with whole blood from each animal, in triplicate. After staining with 5% Giemsa, the number of MN on each slide was analyzed under an optical microscope under 1000x magnification, with a total of 1000 cells per slide, totaling 3000 cells per animal and 240,000 nucleated cells in total.

RESULTSAND DISCUSSION

The pH values (Table 1) obtained at the different points are in accordance with the CONAMA 357/05 resolution for fresh waters classified as classes II and III, which is from 6.0 to 9.0. A desirable situation, since Paiva (2004) showed that values chronically above or below the biological limits, as well as strong short-term changes, result in the inhibition of metabolic processes, in the reduction of species of animal and plant organisms or in the reduction of the power of self-debug.

According to Siqueira, Aprile and Miguéis (2012), a small pH variation along the rivers reveals an excellent buffering capacity by the ecosystem. In this sense, it is positively observed that the pH values found at the collection points vary from a slightly acidic range, mainly due to the decomposition of organic matter, to a slightly alkaline range. This fact may be related to the increasing rainfall that leads to a greater dilution of dissolved compounds. These statements also agree with the findings of Marchesan et al (2009), Esteves (1998) and Carvalho and Siqueira (2011).

Temperatures (Table 1) were low due to the collection interval being in the autumn/winter period, with water temperature variations being part of the natural climate regime. On the contrary, the rise of temperature in a body of water is indicative of industrial discharges. It must be noted that the temperature was determined at approximately the same time for all points.

CONAMA Resolution 357/05 establishes the maximum turbidity value of 100 UNT (Nephelometric Turbidity Units) for class II water bodies. The results obtained (Table 1) are in compliance with the legislation. However, the values of the color parameter are not in conformity in point 3, that is, after the contribution of the Carahá River.

For OD, CONAMA resolution 357 (2005) establishes the minimum concentration of 5 mg $O_2.L^{-1}$ for class II and 4 mg $O_2.L^{-1}$ for class III rivers. The Caveiras River showed good oxygenation at the sampled points (Table 1), with the exception of Point 3 where the amount of dissolved oxygen was less than 4 mg $O_2.L^{-1}$. This may be due to large releases of domestic sewage effluents into the Carahá River, since Point 3 is directly influenced by this river. For Bottin et al (2007), in the process of stabilizing the organic matter from these effluents, the bacteria make use of oxygen in their respiratory processes, causing a reduction in the levels of dissolved oxygen.

As evidenced in the analysis results of the Caveiras River, mainly at Point 3, Bottin et al (2007), in a limnological evaluation study of the Lajeado Passo do Índios microbasin, Chapecó, Santa Catarina, reaffirms that the excess of organic matter influences in obtaining low DO values, as well as the contribution of effluents with large discharges of organic load from domestic and industrial discharges.

Based on quality criteria published by the US Environmental Protection Agency (EPA, 2007), the ranges of DO concentrations with the respective aquatic communities that can support such DO levels are: from 0 to 2 mg $O_2.L^{-1}$ is insufficient to support aquatic life; from 2 to 4 mg $O_2.L^{-1}$ only a few species of fish can survive; from 4 to 7 mg $O_2.L^{-1}$, it is acceptable for warm water fish; de 7 to 11 mg $O_2.L^{-1}$ It is ideal for cold water fish. In relation to this determination, the Caveiras River at all points, with the exception of Point 3, has acceptable indexes for fish from warm waters and ideal for cold waters.

For Lima et al (2006), the Biochemical Oxygen Demand (BOD5) has always been characterized as one of the main parameters in the evaluation of water quality. It is defined as the amount of oxygen required to oxidize biodegradable organic matter under aerobic conditions, that is, it evaluates the amount of dissolved oxygen (DO) in mg $O_2.L^{-1}$, which will be consumed by aerobic organisms when degrading the organic matter present in the water.

Table 1 also shows the BOD_5 values. With the exception of Point 5, referring to the source, all other points had BOD_5 values above the recommended by law, with emphasis on Points 2, 3 and 4, where altered values were obtained. These points are directly influenced by the urban region of the municipality of Lages, which is characterized by the release of large amounts of organic material in the form of untreated sewage into the Ponte Grande (Point 02) and Carahá (Point 03) rivers. This influence can also be observed in the high concentration of total and thermotolerant coliforms in Points 2, 3 and 4, showing that these rivers function as recipients of untreated domestic effluents. However, at Point 4 a decrease in values is observed in relation to Points 2 and 3, probably due to the decomposition of organic matter.

Higher OD values and lower DBO_5 indicate that the Caveiras river has a visible purification capacity, but that this is not enough to guarantee that values compatible with what CONAMA resolution 357/2005 establishes - maximum value of 5 mg $O_2.L^{-1}$ for DBO_5 in Class II and 10 mg rivers $O_2.L^{-1}$ for class II riversI.

Since the BOD5 parameters were the most altered in relation to all the analyzes carried

POINTS AVERAGE TESTS	Point 1	Point 2	Point 3	Point 4	Point 5
pH	7,16	6,92	6,85	6,87	6,28
Water temperature	12,76	13,43	13,73	13,41	10,55
Dissolved oxygen	9,46	6,71	3,30	7,61	8,37
Turbidity	14,88	42,46	47,69	20,50	18,34
DBO_5	11,01	17,65	15,85	14,66	2,39
DQO	30,56	50,83	40,59	38,90	5,8
Detergents and Surfactants	< 0,01	0,025	0,089	0,143	< 0,01
Total solid	48,75	90,75	113,63	75,88	52,5
Conductivity	32	61,72	109,36	78,16	29,70
Chloride	5,93	10,41	10,83	9,10	4,95
Total alkalinity	17	26,50	40	27,50	17,13
Color	16,25	30,63	39,38	24,25	23
Total hardness	12,43	16,40	20,12	17,46	11,86
Fluoride	< 0,02	0,220	0,326	0,279	< 0,02
Sodium	1,53	6,33	10,04	6,74	1,60
Potassium	1,47	1,87	2,68	2,07	1,01
Total phosphate	0,06	0,18	0,39	0,18	0,09
Nitrate	0,56	0,89	1,14	1,64	0,45
Calcium	2,82	3,86	4,93	4,04	2,73
Magnesium	1,10	1,44	1,54	135	1,14
Copper	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Zinc	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Iron	0,93	1,23	1,16	1,15	1,09
Manganese	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Total coliforms (NMP/100mL)	4224,63	86113,75	122262,50	12559,50	8394,88
Thermotolerant coliforms (E. coli -NMP/100mL)	666,18	37065,13	19345,44	1533,4	802,94

Table 1: Average found in the physical-chemical and microbiological analyzes in 8 different collections in the five points surveyed.

out, Guimarães and Nour (2001) point out that higher values, in a body of water, are caused by discharges of predominantly organic origin. They also point out that the presence of a high content of organic matter can lead to the complete extinction of oxygen in the water, causing the disappearance of fish and other forms of aquatic life.

According to CONAMA Resolution 357 of 2005, fresh water for use in class III secondary contact recreation, a limit of 2500 thermotolerant coliforms per 100 mL must not be exceeded. Therefore, the waters of the Caveiras River, in the path observed in Points 2, 3 and 4, are above the standards established in the resolution, which can be classified, in these points, as class IV, allowing their use only for navigation and landscape harmony, according to the same resolution. Frinhani and Carvalho (2010) in a monitoring of water quality in the Tigre River, Joaçaba, SC, state that high concentrations of total and thermotolerant coliforms are mainly due to the large amount of raw sewage released into the river body. The same is evident for the Caveiras river.

It is important to emphasize that there were no significant variations in relation to the analyzed metals (Table 1).

Regarding the genotoxicity tests, the animals, both in the control and test groups, were kept at temperatures ranging from 22 to 28 °C, with constant mechanical aeration and a biological filter composed of porous clay.

In observing the rates of DNA breaks identified by the comet assay (Table 2 and figure 2), it can be seen that there is an increase in the rate of damage in the test group ($p < 0.001$), both in relation to the collection times and the groups – control and test. Note that there were no variations in the damage index between times 0 and 7 days. There is also evidence of an increase in the damage index, mainly at 21 days, when compared to 0, 7 and 28 days. It must also be noted the decrease in damage at 28 days compared to 21 days, since the animals were removed from the waters of the Caveiras River and returned to the control waters. This evidences the fact that the water from the Caveiras River is the likely cause of the damage found, and also the possible existence of a repair mechanism, when the offending agent is removed.

Still in relation to Table 2 and figure 2, it can be observed the existence of difference in the positive controls, Test and Control Groups, induced, only in the time of 21 days, a fact that corroborates the idea that the water from the Caveiras River is the cause of damage found.

Ferraro (2009), analyzing genotoxic damage in species of *Astyanax bimaculatuse, Cyprinus carpio* and *Rhamdia quelen,* through the Comet Assay, also found statistically significant responses in relation to their control groups in the bioassays, especially when the biomarker was: *Astyanax bimaculatuse* and *Rhamdia quelen*, showing the relevance of using these animals in experiments of this kind.

Cestari et al (2004) also evaluated genotoxic effects, by the Comet Assay, of fish exposed to lead-contaminated water, finding great genetic damage caused to fish distributed throughout South America, mainly on traíra, the species used in their study. The authors concluded, among other things, the fact that there is a need for more studies related to possible environmental damage in native fish.

Grisolia et al (2009), in studies with exposure of fish to the waters of Lake Paranoá, found much higher rates of DNA damage in detritive fish species, which feed at the bottom of the lake, as is the case of Jundiá, than in in relation to the other species, observations also reported by Piaia, Townsend and Baldisserotto (1999). These studies reinforce the importance of using biological parameters, as well as the use of Jundiá as a bioindicator of environmental

Damages index[1]				
Days:	0	7	21	28
Groups:				
Test	1.413aA	1.423aA	2.234cA	1.848bA
Control	1.469aA	1.369aA	1.649bB	1.588bB
Induced Test	3.171aB	3.188aB	3.231aC	3.216aC
Induced Control	3.043aB	3.118aB	3.047aD	3.106aC

[1]Lowercase letters mean significant difference in lines. Capital letters mean significant differences in columns, for the Kruskal-Wallis test ($p < 0,05$).

Table 2: Overall DNA damage index by comet assay.

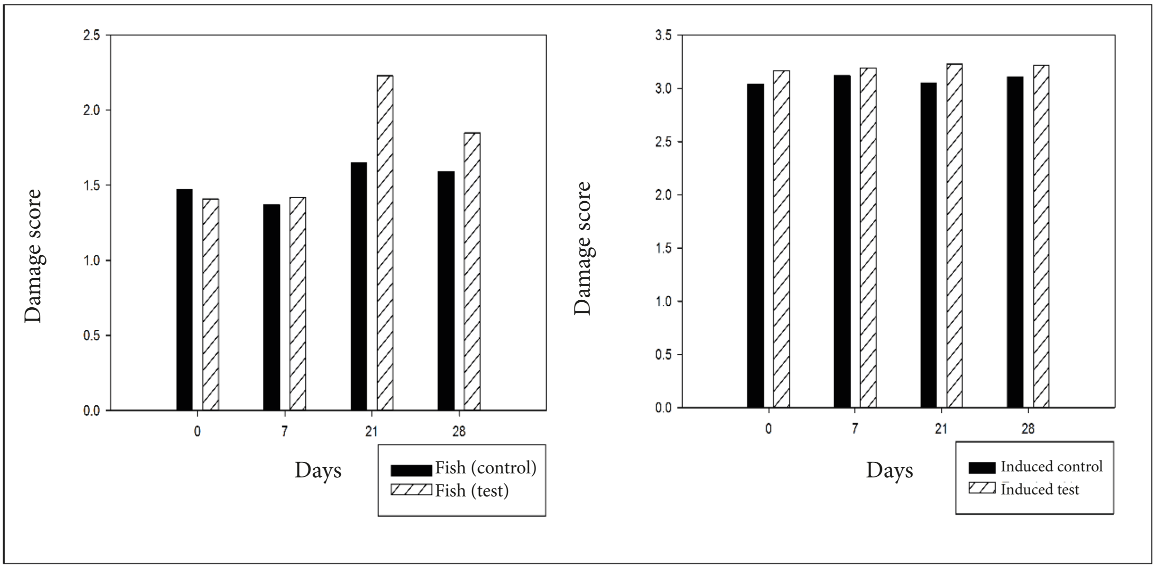

Figure 2: General damage index comparing control group with test group (left) and induced control group with induced test group (right).

Micronucleus Frequency [1]				
Days:	0	7	21	28
Groups:				
Test Group	0,067	0,067	0,100	0,100
Control Group	0,100	0,100	0,200	0,133

[1]Percentage (%) of Micronuclei found, in a universe of 3000 cells analyzed per animal.

Table 3: Frequency of Micronuclei found per group and per day of analysis.

contamination, in the experiment, and also the damage found in the present study.

The results of the MN Test (Table 3) in *Rhamdia quelen*, exposed to the waters of the Caveiras River showed a very low frequency of MN found. The greatest frequency meets on time 21 days. Hayashi et al. 1998, when evaluating aquatic organisms for genotoxic detection, suggested that the ability of the MN Test to detect low levels of contamination must be improved. However, Al-Sabti (2000) found significant results when analyzing Carp species, which may suggest species-specific differences for the detection of genotoxic damage in different species of organisms. This fact was also reported by Grisolia and Cordeiro (2000), when they analyzed three different species of fish exposed to the same treatment with mutagenic agents.

Ramsdorf et al (2009) also found no significant differences between the control and treated groups for pisceous micronuclei in lead ecotoxicity tests. Ferraro et al (2004), also observed the genotoxicity of this same xenobiotic, through the comet and micronucleus assay in native fish (traíra). In the comet assay, the authors, as well as the present study, observed a significant difference between the control and contaminated groups, reinforcing the fact that the comet assay is more sensitive and, therefore, generates more significant results, thus being a good test for environmental biomonitoring. Bücker, Carvalho and Alves-Gomes, (2006) also highlight the simplicity and sensitivity of the Comet Assay, which makes it an adequate test system for exposure biomonitoring.

CONCLUSION

The results show that the Caveiras River has physicochemical and microbiological characteristics that do not comply with CONAMA Resolution 357/05 for a class II and III river, which may, at points 2, 3 and 4, be classified as class IV.

The points located after the influence of the urban region are the most degraded, with the city of Lages being the main responsible for the pollution evidenced in the Caveiras river. The parameters of BOD5, total and thermotolerant coliforms are the ones that presented the greatest alterations.

The quality of the water available for recharge, in the areas of contact between the Caveiras River and the Botucatu sandstone, indicates a high risk of contamination of the Guarani Aquifer.

The waters of the Caveiras River, at the analyzed point, caused important and significant genotoxic damage in the analyzed species.

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