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Artigo Original

Micronucleus test in fish for *in situ* evaluation of the Sinos River water quality, in Brazil

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Abstract

The Sinos River basin is impacted by industrial and agricultural activities, as well as by low rates of urban wastewater treatment. The purpose of this study was to monitor de Sinos River water quality using the micronucleus test in fish and the analysis of water physicochemical parameters. Bryconamericus iheringii specimens were captured in December 2013 (summer) and July 2014 (winter) at two sites located in the Sinos River: Caraá, in the upper section of the basin, and Parobé, in the middle section. After capture, animals were immediately killed and blood samples were collected for the micronucleus test. No significant differences were observed in micronucleus frequencies between sites and sampling periods. However, in the summer, nuclear abnormalities frequencies observed in Parobé were significantly higher than in Caraá. A higher frequency of nuclear abnormalities was also found in fish captured in Caraá during winter, in comparison with frequencies found in the summer. The results for the water physicochemical analysis showed values of total phosphorous, aluminum, lead, copper and iron above the allowed limits established by the Brazilian legislation. The nuclear abnormalities induction found in the present study may be associated to the presence of cytogenotoxic substances in the water.

Keywords: Water pollution; Genotoxicity; Bryconamericus iheringii

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1 Introduction

The Sinos River basin, located in the northeast region of the state of Rio Grande do Sul, Brazil (FIGUEIREDO et al., 2010), extends over 3.800 km^2, corresponding to 1.5% of the total state area. The Sinos River constitutes its main water course, with 190 km of extension (FEPAM, 1999), and despite it is heavily impacted, it provides drinking water for 1.2 million people (FIGUEIREDO et al., 2010).

Aquatic environments receive a great variety of organic and inorganic substances from natural of anthropogenic sources, which can induce damages in the aquatic biota at molecular, biochemical and physiological levels (RAND, 1995). Environmental biomonitoring studies, especially using biomarker analysis in organisms exposed *in situ* to pollutants, provide tools for the identification of conditions capable of causing damages to human health and the aquatic biota (SILVA et al., 2003).

Biological assays are able to characterize the effects caused by contaminants through chronic and/or acute exposure, without the previous knowledge of the chemical substances in the water (OHE et al., 2004). Several studies have used genotoxicity tests as tools for investigating the quality of underground and surface waters (HOSHINA et al., 2008; DALZOCHIO et al., 2017; LIMA et al., 2018). The micronucleus test is considered an advantageous technique with a relatively simple analysis (UDROIU, 2006) and is widely used for the monitoring of genotoxic damages in populations exposed to mutagenic and carcinogenic substances. Micronuclei may be formed by chromosome fragments or whole chromosomes that are not incorporated by the main nucleus during cell division (AL-SABTI and METCALFE, 1995). Therefore, the micronucleus test detects clastogenic and aneugenic agents (BRUNETTI et al., 1988). Several mechanisms are involved in micronuclei formation, such as chromosome breaks and errors in the mitotic spindle. In addition, the concomitant analysis of the occurrence of erythrocyte nuclear anomalies allows the evaluation of another toxicity biomarker (PACHECO and SANTOS, 1998; VIEIRA et al., 2016).

Fish are considered adequate for the monitoring of genotoxins present in water because they are able to metabolize and accumulate xenobiotics (FLORA et al., 1993). The *Bryconamericus* genus (Characidae: Teleostei) includes small-sized fish, with no more than 10 cm of length, that live in different environments and are omnivorous, constituting an important food source for other fish (BRITSKI et al., 1988). The species *Bryconamericus iheringii* (Boulenger, 1887) is widely distributed in the Sinos River basin and is one of the most abundant species in this water resource (COSTA and SCHULZ, 2010).

Therefore, the purpose of this study was to monitor the water quality of two sites with different rates of anthropic pressure in the Sinos River, using the micronucleus test in *B. iheringii* and the analysis of water physicochemical parameters.

2 Material and Methods

Samplings were conducted in December 2013 (summer) and July 2014 (winter). Fish were captured at two sites in the Sinos River: the first is located in Caraá municipality (29º 43' 26" S 50º 16' 46" W), in the upper section of the basin, in a region with small agricultural properties near the river spring, whereas the second site is located in Parobé municipality, approximately 4 km downstream the mouth of Paranhana River (29º 41' 05" S 50º 50' 52" W), in the middle section of the basin, in an urbanized and industrialized region.

Ten specimens of *B. iheringii* were captured at each site in both sampling periods weighing approximately 4.9 g and measuring 7.2 cm. After capture, fish were immediately killed and blood samples were obtained from the caudal vein to perform the smear. The material was transported to the laboratory, where samples were fixed in absolute ethanol and stained with 5% Giemsa. All slides were coded and analyzed in an optical microscope, considering the analysis of 2000 erythrocytes for each animal.

Surface water samples were simultaneously collected for the evaluation of the following physicochemical parameters: biochemical oxygen demand (BOD_5), chemical oxygen demand (COD) (mgO_2/L), total phosphorous (mg/L), ammoniacal nitrogen (mg/L), suspended solids (mg/L), aluminum (mg/L), lead (mg/L), copper (mg/L), total chromium (mg/L), iron (mg/L), nickel (mg/L) and zinc (mg/L). Water analysis was performed according to the methodology described in Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The results were compared to the reference values described in the Resolution 357/2005 by CONAMA (Environment National Council) for Class 1 waters (BRASIL, 2005).

Precipitation data from the 30 days previous to each sampling were obtained using a mobile meteorological station (Davis Vantage PRO 2 VP USB NS) for Caraá. For Parobé, data from the closest meteorological station (50 km), located in Taquara, were obtained, using the WS2812 station in the *Faculdades Integradas de Taquara* (FACCAT, 2014).

For the comparison of cytogenetic damages between sampling sites and periods, the Mann-Whitney test was used. All analyses were performed using the Statistical Package for the Social Sciences – SPSS 22 considering a significance level of $p \leq 0.05$.

3 Results and Discussions

Among the techniques used to evaluate mutagenic effects *in situ*, the micronucleus test is frequently applied due to its easy methodology and low cost, particularly in studies considering fish as bioindicators (UDROIU, 2006; GOLDONI et al., 2014). The evaluation of micronuclei frequencies in freshwater fish allows the detection of DNA damage due to urban, industrial, heavy metals and pesticide discharges (BOLOGNESI and HAYASHI, 2011). In the present study, no significant differences were observed in micronucleus frequencies between sampling periods at the same site, as well as between sites in the same sampling period (Table 1). It is important to highlight that the spontaneous baseline frequency for micronuclei in fish is generally low and presents great interspecific variability, with mean values ranging from 0 to 1 micronuclei per 1000 cells (BOLOGNESI and HAYASHI, 2011). Therefore, the frequencies found in the present study (ranging from 0.00 to 0.05) may be considered as a baseline frequency variation for these populations of *B. iheringii*. Similarly, Bühler et al. (2012) have also observed low micronuclei frequencies (0.29‰) in the same fish species collected at a non-polluted site. The absence of significant differences between sampling sites and sampling periods suggests that the analyzed specimens are not exposed to mutagenic agents.

Table 1 – Micronuclei frequencies per 1000 cells (mean ± standard deviation) observerd in *B. iheringii* captured in December 2013 (summer) and July 2014 (winter) in two sampling sites in the Sinos River.

Site	Summer	Winter	p
Caraá	0.05 ± 0.15	0.00 ± 0.00	0.34
Parobé	0.05 ± 0.15	0.04 ± 0.14	0.94
p	1.00	0.38	

Data are expressed as mean ± standard deviation.

The assessment of nuclear abnormalities frequencies is considered complementary to the micronuclei analysis, considering that these anomalies are induced by cytogenotoxic agents (AYLLON and GARCIA-VAZQUEZ, 2000; GRAVATO and SANTOS, 2002; VIEIRA et al., 2016). Results for nuclear abnormalities observed in *B. iheringii* are shown in table 2. In Caraá, significantly higher frequencies were observed in the winter in comparison with the summer, which could be related to the higher levels of lead and copper verified in the water in this sampling period. Comparing the two sampling sites in the same period, a significant higher level of abnormalities was observed in the summer in Parobé in comparison with Caraá, possibly due to the higher level of urbanization and industrial activities in the area. Accordingly, water samples collected in Parobé showed higher concentrations of metals (aluminum, iron and lead) when compared to Caraá, which could explain the higher frequency of damages observed in that local. Heavy metals are known to be toxic agents, capable of inducing DNA damage and cancer (VALKO et al., 2006; MATOS et al., 2017). Furthermore, Parobé is used for recreational purposes in the summer. Fish collected in Caraá in the winter exhibited a significant increase in nuclear abnormalities frequencies, whereas no significant differences were found in fish collected in Parobé.

Table 2 – Nuclear abnormalities frequencies per 1000 cells (mean ± standard deviation) observerd in *B. iheringii* captured in December 2013 (summer) and July 2014 (winter) in two sampling sites in the Sinos River.

Site	Summer	Winter	p
Caraá	0.80 ± 0.53	2.14 ± 1.57	0.01*
Parobé	2.40 ± 1.82	2.5 ± 1.62	0.94
p	0.009	0.77	

Data are expressed as mean ± standard deviation.
*Mean frequencies statistically different when <0.05.

Considering the comparison between sampling periods, the higher frequency of nuclear abnormalities observed in Caraá during winter, when compared to the frequencies found in summer, can also be related to the higher levels of lead and copper found in that sampling period. However, it should be pointed that fish demonstrate exposure effects for a long period of time. Therefore, for a more concrete relation between cause and effect, the continuous monitoring of metal concentrations, as well as other substances, in both sampling sites is of extreme importance, considering that these contaminants, when released in aquatic ecosystems, become a major threat to the organisms due to their toxicity and genotoxicity. Our data are in accordance to laboratory studies performed by other authors who have also reported cytogenotoxic damages induced by contaminants present in waters from urbanized regions (SCALON et al., 2010). Conversely, Bergamaschi et al. (2015) and Bianchi et al. (2015) have found no genotoxic effect in *Leporinus obtusidens* and *Astyanax jacuhiensis*, respectively, exposed under laboratory conditions to water samples collected at sites in the upper and middle sections of the Sinos River. Additionally, Lima et al. (2018) have also observed low frequencies of micronuclei in fish collected in areas impacted by agricultural activities.

Water physicochemical analyses evidenced some parameters above the reference values established by the CONAMA resolution 357/2005 for class I waters (Table 3). In the summer, Caraá presented BOD levels above the limits. Total phosphorous and lead were above the limits in Parobé in both sampling periods, whereas aluminum was detected above the limits only in the summer in this same site. BOD and total phosphorous are generally associated to the release of untreated sewage into water resources (BLUME et al., 2010). The sources of lead include disposal of urban solid waste that may contain batteries, electronics, material colored with lead paint, package labels, fuel additives and atmosphere (OLIVEIRA et al., 2008; BUENO-KRAWCZYK et al., 2015). Copper levels were above the reference levels in both sampling sites in the winter. Concentrations of iron exceeded the limits at both sampling sites and periods. Aluminum and iron may be present in water due to the release of untreated domestic sewage, solid waste disposal near the river and also to the typical soil composition of the

Table 3 – Physicochemical parameters of the Sinos River water sampled at Caraá and Parobé in the summer (December 2013) and in the winter (July 2014).

Parameters	Caraá		Parobé		
	Summer	Winter	Summer	Winter	Reference Values[a]
BOD (mg O_2/L)	7*	< 0.5	5	< 0.5	5
COD (mg O_2/L)	20.2	0.6	24.9	9.9	-
Total phosphorous (mg/L)	0.1	0.03	0.14*	0.16*	0.1
Ammoniacal nitrogen (mg/L)	n.d.	n.d.	n.d.	n.d.	3.7
Suspended solids (mg/L)	5.5	n.d.	12	12.2	-
Aluminum (mg/L)	n.d.	n.d.	0.758*	n.d.	0.1
Lead (mg/L)	n.d.	0.008	0.021*	0.017*	0.01
Copper (mg/L)	n.d.	0.018*	n.d.	0.012*	0.009
Total chromium (mg/L)	n.d.	n.d.	n.d.	n.d.	0.05
Iron (mg/L)	0.118	0.022	0.637*	2.179*	0.3
Nickel (mg/L)	0.007	0.007	0.011	0.007	0.025
Zinc (mg/L)	0.013	0.007	0.01	0.015	0.18

[a]Limits according to CONAMA 357/2005 for Class I waters.
*Values in disagreement with established limits.
n.d.: not detected by the method.

region (ALLOWAY, 2013; BERGAMASCHI et al., 2015; KONZEN et al., 2015). Nickel and zinc values were within the limits at both sampling sites and periods whereas total chromium and ammoniacal nitrogen were not detected. Similar findings were observed by Dalzochio et al. (2018), where levels of aluminum and iron were also detected above the limits in Parobé.

Precipitation data did not show a great variation considering the comparison between sampling periods and sites. Caraá presented 183.4 mm and 175 mm in the summer and winter, respectively, whereas Parobé presented 130.3 mm and 119.8 mm in the summer and winter, respectively. The variation observed in precipitation rates does not seem to have caused effects in the biomarkers analyzed in the present study. The seasonality influence in micronuclei frequencies has been reported in previous studies, possibly due to its effects in pollutant concentrations However, Souza and Fontanetti (2006) observed a significant increase in micronuclei frequencies in the same fish species in sampling periods presenting dry and cold weather conditions with water samples from Paraíba do Sul river, São Paulo, indicating influence of seasonality and precipitation. Conversely, some studies do not demonstrate this influence (WIRZINGER et al., 2007, BOLOGNESI and HAYASHI, 2011). Sampling periods with less rainfall favor the occurrence of high levels of chemicals in water resources, whereas periods with high pluviosity favor the dilution of pollutants. However, rainfall can worsen pollution effects through a greater leaching of chemicals retained in soil or sewer systems (DUARTE et al., 2012).

4 Conclusions

The present study evidenced higher DNA damage and levels of metals in Parobé, where higher urbanization and industrial activities are observed. However, precipitation rates did not influence in the frequency of damages. Moreover, the fish specie used was sensitive to the cytotoxicity test and could be used in further biomonitoring studies.

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