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Environmental assessment of Luiz Rau stream (Brazil) utilizing Allium cepa TEST

Avaliação ambiental do córrego Luiz Raul (Brasil) utilizando Allium cepa TEST

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ABSTRACT

The aim of this study was to monitor the water quality of Luiz Rau stream, through physicochemical parameters, environmental characteristics of the site, and genotoxicity test in Allium cepa. Four water samples from Luiz Rau stream were collected once a week for one month and some physicochemical parameters were verified. The environmental characteristics were performed by the Quick Assessment Protocol (QAP) and evaluated at the sampling site. For the genotoxicity assay, the Allium cepa test was utilized. In relation to the water physicochemical parameters, low levels of dissolved oxygen (DO) and high levels of ammonia, and phosphorus were obtained, which demonstrates the poor quality of the stream. The QAP results identified the stream as impacted. The mitotic index parameter was altered in the seed exposed to raw water samples in the third and fourth sample collection, demonstrating cell cycle reduction and proliferation respectively; witch can be associated to the presence of contaminated effluents in the water. In relation to the genotoxicity, the micronuclei were increased in seeds exposed to four raw samples in comparison to the control group. Therefore, the results obtained can characterize the Luiz Rau stream as impacted, mostly by anthropic actions, corroborating with other publications.

Keywords: Citotoxicity; Genotoxicity; Quick Assessment Protocol; Rainfall

RESUMO

O objetivo deste estudo foi monitorar a qualidade da água do arroio Luiz Rau através de parâmetros físico-químicos, características ambientais do local e teste de genotoxicidade em Allium cepa. Quatro amostras de água do arroio Luiz Rau foram coletadas semanalmente durante um mês e alguns parâmetros físico-químicos foram verificados. As características ambientais foram realizadas pelo Protocolo de Avaliação Rápida (PAR) e avaliadas no local de amostragem. Para o ensaio de genotoxicidade, foi utilizado o ensaio com Allium cepa. Em relação aos parâmetros físico-químicos da água, foram obtidos baixos níveis de oxigênio dissolvido (OD) e altos níveis de amônia e fósforo, o que demonstra a má qualidade do córrego. Os resultados do PAR identificaram o arroio como impactado. O parâmetro do índice mitótico esteve alterado nas sementes expostas às amostras de água bruta na terceira e quarta semanas de coleta, demonstrando redução e proliferação do ciclo celular, respectivamente; o que pode estar associado à presença de efluentes contaminados na água. Em relação à genotoxicidade, os micronúcleos estiveram aumentados em sementes expostas às quatro

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amostras brutas em comparação ao grupo controle. Portanto, os resultados obtidos permitem caracterizar o córrego Luiz Rau como impactado, principalmente por ações antrópicas, corroborando com outras publicações.
Keywords: Citotoxicidade; Genotoxicidade; Protocolo de Avaliação Rápida; Pluviosidade

1 INTRODUCTION

The aquatic ecosystem is currently characterized as one of the most impacted, containing several types of micropollutants responsible for affecting even the available drinking water sources (MANZANO; ROBERTO; HOSHINA; MENEGÁRIO; MARIN-MORALES, 2015; ZEGURA; HEATH; ERNSA; FILIPI, 2009). Such pollutants indicate a risk factor for aquatic biota and consequently for human health (DALZOCHIO et al. 2017; GURGEL; NAVONJA; AMARAL, 2016).

The Sinos River is located in the eastern region of the state of Rio Grande do Sul in southern Brazil, supplying 32 municipalities (about 1.6 million inhabitants), and it is considered as one of the most polluted rivers in the country (IBGE, 2010). Due to its long extension it ends up receiving rural and industrial pollutant sources (BENVENUTI; KILIELIN-RUBIO; KLAUCK; RODRIGUES, 2015), fact demonstrated in several studies with alternative organisms such as plants (CASSANEGRO; DROSTE, 2017), cell cultures (BIANCHI et al. 2017), physicochemical and microbiological evaluations (NASCIMENTO et al. 2015), and even with native fish (DALZOCHIO et al. 2018).

The poor quality of this water resource, besides being reported in the literature as mentioned previously, is also reported in the media (EXTRA CLASSE, 2011; JORNAL NH, 2014), and it is directly related to the water quality of its respective tributaries, such as the Luiz Rau stream. This stream is characterized by the excessive dumping of domestic and industrial effluents along its 14 km long, and for these reasons, it is known as “black stream”' in the region (PMNH, 2016).

As an environmental problem that is reported throughout the country, many studies investigate the impacts of these waters, including the genotoxicity of Luiz Rau stream water in Tradescantia pallida cells (PETRY; COSTA; BENVENUTTI; RODRIGUES; DROSTE, 2016). However, this study differs from the other studies because it is characterized as a monitoring of the genotoxicity of a collection point, located in the center of the municipality of Novo Hamburgo, where the anthropic impact is extreme, and it also considered whether there is the influence of rainfall in the results.

Therefore, the aim of this study was to monitor, in an integrated way, the water quality of Luiz Rau stream, through physicochemical parameters of the water, environmental characteristics of the site, and genotoxicity test in *Allium cepa*.

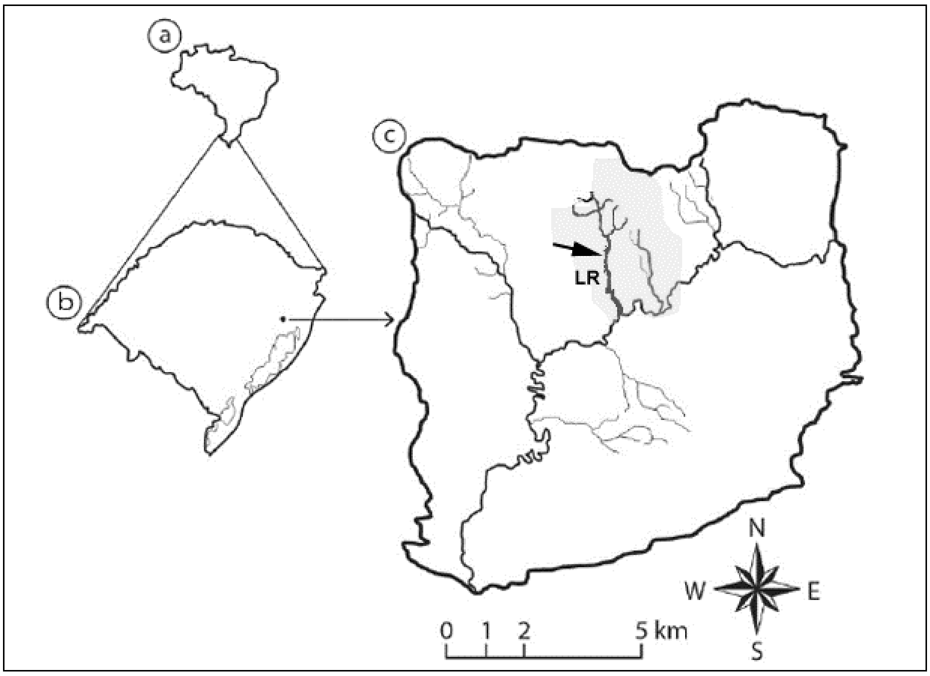
2 MATERIAL AND METHODS

2.1 Water collection and analysis

Water samples were collected weekly in sterile flasks for one month (one sample per week) in the central stretch of the Luiz Rau stream in Novo Hamburgo (Fig. 1) (29 ° 40 '53.27 " S and 51 ° 07' 49.13" W), on the dates: May 12, 2018 (S1); May 19, 2018 (S2); May 26, 2018 (S3); June 03, 2018 (S4). The temperature and the dissolved oxygen content were measured on site by means of a Hanna multiparameter portable probe (HI 98194). After that, the samples were immediately transported to the Laboratory of Comparative Histology, in which the parameters pH, chemical oxygen demand (COD), phosphorus, phosphate, ammonia, nitrite, and nitrate were measured by means of a multiparameter bench photometer (HANNA 83213 and 83214).

The sampling point was chosen due to the large number of vehicles and pedestrians, and also because it is one of the most easily accessible places in the stream. The collections were always carried out in the afternoon (between 1 pm and 5 pm) and during autumn, a time when the amount of rain is not as high as in winter, nor as low as in summer, thus preventing the effects from being totally masked by the dilution of pollutants, or enhanced by the temperature or rarity of water. Even so, the rainfall record was performed as described below.

Figure 1- Map delimiting the site of the collection, through the black arrow, in Luiz Rau stream (LR) in the lower stretch of Sinos River basin (C), in the municipality of Novo Hamburgo (B), Rio Grande do Sul (A). Adapted from Petry et al., 2016



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2.2 Quick Assessment Protocol (QAP)

In the four water sampling dates, the QAP model provided by Calisto et al., (2002) was also performed at the sampling point, aiming to evaluate the effects caused by the anthropogenic action at the site.

2.3 Genotoxicity assay in *Allium cepa*

Seeds of *A. cepa* (Periform Bay variety) were previously germinated in distilled water at 22 °C in Petri dishes. The samples were then exposed for 24 hours at different dilutions of water samples from the Luiz Rau stream (100%, 50%, and 25%), while a control group was maintained in distilled water. After the exposure, the roots were collected, fixed in Carnoy's solution, composed by ethanol: glacial acetic acid, in proportions 3:1 (v:v), overnight and stored in 70% ethanol at 4 °C. Five slides were prepared for each group/dilution, the meristematic regions of two roots were used for each slide. For this, the roots were washed in distilled water, subjected to hydrolysis in

1N HCl at 60 °C and then washed again. The roots were stained with 1% acetic orcein and mounted on a slide by manual crushing.

The slides were analyzed under an optical microscope (Olympus DSC2000) where the mitotic index was evaluated considering the number of dividing cells into 1000 cells - used as a microscopic parameter for cytotoxicity. To assess genotoxicity, the number of cells with chromosomal aberrations (CA) and micronuclei (MN) in 100 cells was recorded (BIANCHI; MANTOVANI; MARIN-MORALES, 2015).

2.4 Rainfall data

Rainfall data for the sample period and seven previous days were obtained through the website of the National Institute of Meteorology (INMET) and was expressed as accumulated rainfall.

2.5 Data analysis

The data obtained by the *A. cepa* assay were tested for their normality by the Kolmogorov-Smirnov test. Due to non-normality, nonparametric tests (Kruskal-Wallis) were applied. In order to identify a possible correlation between the amount of accumulated rainfall and genotoxicity data, the Spearman correlation was tested. The software used for all analysis was GraphPad Prism 6, and the results are expressed in graphs or tables with mean and standard deviation.

3 RESULTS AND DISCUSSION

The physicochemical parameters of the water samples can be verified in Table 1, we highlight in bold and underlined values that surpass Brazilian legislation. The pH of the samples was stable, always being around 7. However, we highlight the low values of dissolved oxygen (DO) and high values of ammonia, which cause concern for aquatic biota as well as high levels of phosphorus.

Table 1. Physicochemical parameters of the water samples

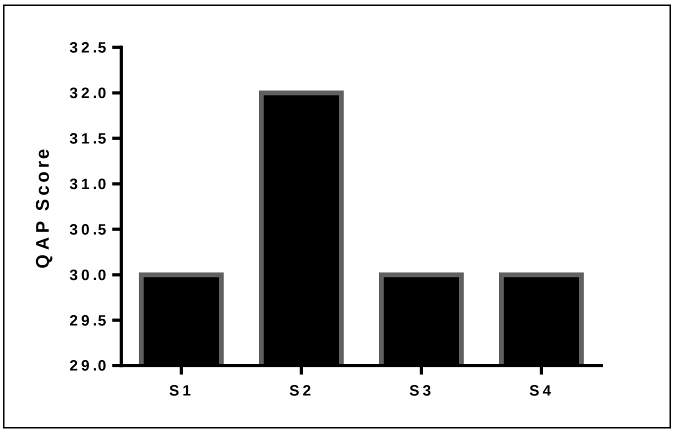
	Samples			
Parameter	S1	S2	S3	S4
pH	7.0	7.4	7.3	7.3
COD (mgO_2 L^{-1})	44	2	28	27
DO (mgO_2 L^{-1})	5.0	0.71	1.66	5.3
Phosphate (mg L^{-1})	2.49	1.65	4.4	2.31
Phosphorus (mg L^{-1})	4.2	1.0	1.7	1.7
Ammonia (mg L^{-1})	18.21	16	17	15
Nitrite (mg L^{-1})	0.46	0.06	0.03	0.29
Nitrate (mg L^{-1})	0.9	0.7	0.0	9.2

"COD" represents chemical oxygen demand and "DO" represents dissolved oxygen. Legislation refers to Resolution nº 357/2005 of the National Environmental Council (CONAMA) for Class III waters, which is, intended for human consumption after conventional or advanced treatment.
Source: authors

Knowing that normal levels of DO result from the balance between the processes that produce and consume oxygen, reduced levels, which is the case of the present study, can derive from the discharge of sewage without previous or adequate treatment, especially when associated with high levels of ammonia, which is also the case. It is also worth noting that in the collection of S2 the DO levels obtained (0.71) would not allow the classification of water even in Class IV (Resolution n° 357/05 of CONAMA), water intended only for navigation and landscape harmony. In addition, the reported high ammonia values, although they are in excess of that allowed by the legislation, may be attenuated due to the low water temperature at the time of collection, whereas the sampling period is autumn. These findings demonstrate the worsening of the water quality of this studied point when compared to previous studies carried out in the same stream (PETRY et al. 2016; HECK et al. 2017; MACHADO; RODRIGUES; SOUZA; LINDEN; OSÓRIO, 2018).

About the QAP score, which can be observed in Figure 2, the same is used as a tool to evaluate the effects caused by anthropic activity. It can be observed that, except for the second sample, the sampling point was 30 points. The average water temperature was around 16 ºC, and the depth ranged from 24.5 cm to 75 cm, due to the presence or absence of rain at the time or previously to the collection. The authors' score at the fourth collection classifies the respective water body as impacted, according to the authors responsible for the elaboration of the methodology (CALLISTO; FERRERIA; MORENO; GOULART; PETRUCIO, 2002). In addition, observations revealed moderate erosion at brooks, presence of domestic sewage, as well as rotten egg odor and moderate oiliness in both water and sediment. These characteristics reflect in the total absence of aquatic plants, native forest and ciliary forest around and along the extension of the stream, derived mainly from anthropogenic interventions such as tubing, rectifications, sidewalks and streets and corroborating with other authors that also had characterized the place as impacted, according to the same protocol (MACHADO et al. 2018).

Figure 2- QAP score for the four samples



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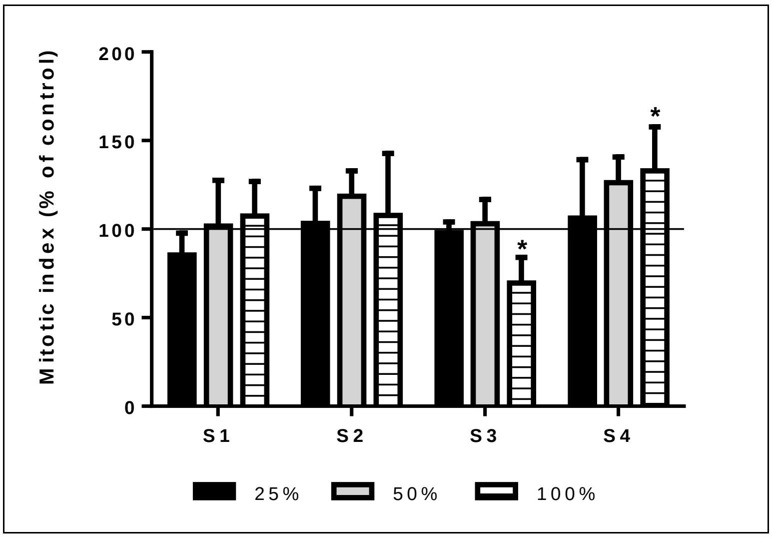
Regarding the mitotic index, the raw samples (100%) of the S3 and S4 collections caused changes in the number of cells in division, as can be observed in Figure 3.

Figure 3. Mitotic index obtained in samples. Data are expressed as mean ± standard deviation, and one asterisk represents a statistical difference in relation to the control (line) horizontal ($p < 0.05$).



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The mitotic index is a parameter used to evaluate the cytotoxicity in cells of *A. cepa*, therefore, changes in this index represent cell problems (LEME; MARIN-MORALES, 2009). A reduction in the number of dividing cells, as occurred in S3, may be associated with the mitodepressive effects of substances present in the water at the time of collection, that is, it may interfere with the normal development of mitosis, preventing some cells from entering prophase and performing the total cell cycle (DEBNATH; MONDAL; HAJRA; MONDAL, 2018). This type of effect is usually associated with exposures to pesticides, fungicides, tannery effluents and industrial effluents rich in magnesium, sodium, and chlorides associated with the manufacture of paper and cellulose (DATTA; SINGH; SINGH; SINGH, 2018; OZAKCA; SILAH, 2013; YADAV; RAJ; PURCHASE; FERRERIRA; SARATALE; BHARAGAV, 2019; HAQ; KUMAR; LOHANI; SATYNARAYANA, 2017).

In contrast, increases in the number of dividing cells, as observed in S4, may characterize disordered and uncontrolled cell proliferation and result in the induction of carcinomas (DÜSMAN; ALMEIDA; PINTO; LUCCHETTA; VICENTINI, 2014), this effect has already been reported in exposures to domestic effluents, surface waters, which consequently also have domestic effluent, and sewage sludge (STAPULIONYTÉ et al.

2019; RODRIGUES; DALZOCHIO; GEHLEN, 2016; DÜSMAN et al. 2014; MARTINS; SOUZA; SOUZA, 2016). Therefore, both changes in the mitotic index observed cause concern and may be associated with the disposal of domestic effluents and the leather and footwear sector, which characterize the collection site.

As the crude samples were the only ones interfering with the mitotic index, when we observed the number of micronuclei in the three concentrations of the samples, only the crude samples increased the incidence of *A. cepa* cells with micronuclei (MN) in the four collections, as can be observed in Figure 4. The same pattern of response can be observed for the quantification of chromosomal aberrations (CA) (Figure 5), but in addition, in the S2 collection, the concentration of 50% also resulted in the increase of chromosomal aberrations. In order to verify if these genotoxic damages were related to the amount of rain, the Spearman correlation was applied between the micronucleus/chromosomal aberrations index and the accumulated amount of rainfall (Figure 6). However, there was no correlation between the data (p= 0.33 and 0.75 respectively).

Figure 4. Number of micronuclei in *A. cepa* cells resulting from the four samples and three concentrations (horizontal line represents the control group). Data are expressed as mean ± standard deviation, two asterisks represents a statistical difference in relation to the control (p < 0.01).

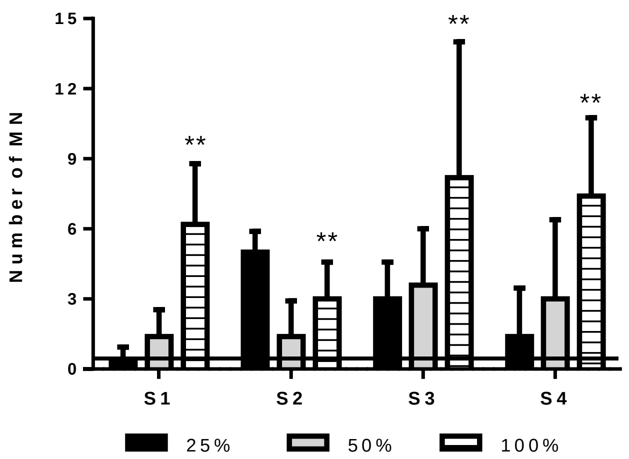

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Figure 5. Number of chromosomal aberrations in *A. cepa* cells resulting from the four samples and three concentrations (horizontal line represents the control group). Data are expressed as mean ± standard deviation, one asterisk represents a statistical difference in relation to the control ($p < 0.05$) and two asterisks represent ($p < 0.01$)

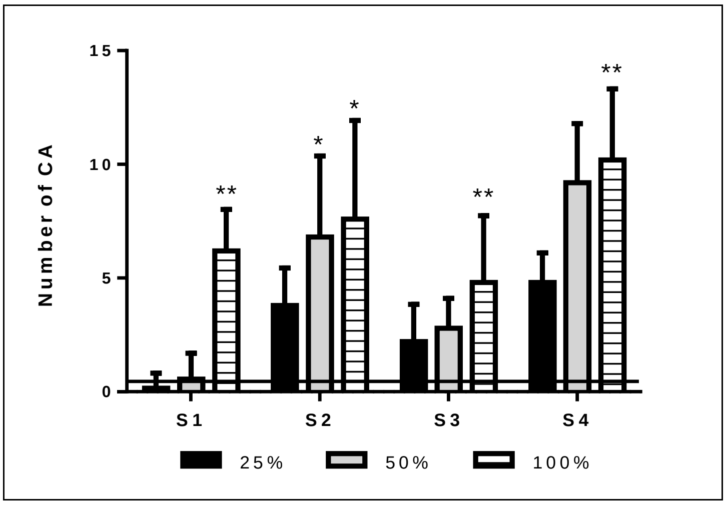

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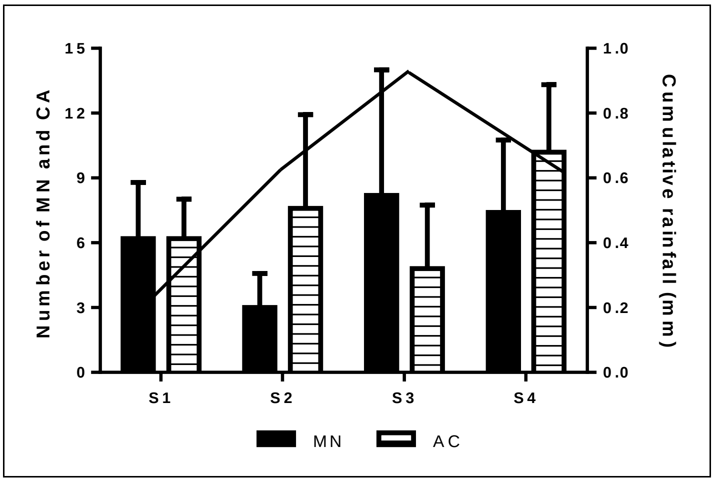
Figure 6. Spearman correlation was applied between genotoxic damages in crude samples (MN and CA) and accumulated rainfall



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Chromosomal aberrations and micronuclei are considered markers of genotoxicity, since they represent damage to the genetic material of the cell, that is, to DNA. These structural chromosomal changes can be induced by a number of factors, such as DNA breaks, inhibition of DNA synthesis, and altered DNA replication (ALBERTINI et al. 2000). The quantification of micronuclei have been considered by many authors the most efficient and simple endpoint to analyze the mutagenic effect promoted by chemical products. Micronuclei are derived from damages that are not repaired erroneously in the parental cells, and they are easily observed in the daughter cells as similar structure to the main nucleus, but in smaller size (RIBEIRO, 2003; LEME; MARIN-MORALES, 2009).

In addition, MN may arise from the development of some CAs, such as chromosomal breaks and losses (FERNANDES; MAZZEO; MARIN-MORALES, 2007), and therefore both techniques are mostly used as complements. These damages are associated and already described in the literature as a result of exposures to metals, pesticides, hydrocarbons, and complex mixtures, which is the case of environmental samples (SETH; MIRSA; CHAUHAN; SINGH, 2008; DATTA et al. 2018; LEME; ANGILIS; MARIN-MORALES, 2008; MARTINS et al., 2016).

Although the results obtained through the bioassay with *A. cepa* did not show a significant correlation with the accumulated rainfall data, a higher incidence of MN and CA was observed in the periods of higher rainfall, such as S2, S3, and even S4. This fact may be associated to the soil leaching process by surface water runoff occurring in the municipality during rainy seasons since the stream is located in a totally urban area where there is a deficiency of vegetation cover. Kienzler et al., (2015) report that compounds used in parking lots and sidewalks in North America, for example, are rich in hydrocarbons and other related compounds. The same authors affirm that the runoff of these pollutants is associated with the development of genotoxicity and incapacity of repairs in the DNA in the RTL-W1 fish liver cell line. Other authors (COSTA et al., 2012) have evaluated the ability of potential environmental problems, previously identified (pentachlorophenol, creosote and hydrosalt CCA) in Taquari River (Brazil), to cause genotoxicity through the runoff of such compounds. Positive results for mutagenicity, genotoxicity and cytotoxicity were observed in *Salmonella* and *Allium cepa*. Therefore, we can supposed the fact that the parameters evaluated in the water have undergone through changes during the four weeks of evaluation, it may be directly related to the previous rainfall volume and at the time of collection, and consequently the concentration and availability of nutrients and other aquatic contaminants that were not verified, as nutrients, hormones, drugs and other substances from domestic sewage.

4 CONCLUSIONS

Knowing that the water quality of the tributaries directly influences the quality of the receiving water courses, the genotoxic and cytotoxic potential reported here from the water samples from the Luiz Rau stream interferes directly in the water quality of the Sinos River, contributing to its current classification as the fourth most polluted river in the country.

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