Water Air Soil Pollut (2020) 231: 318
https://doi.org/10.1007/s11270-020-04698-5

Environmental Quality and Cytogenotoxic Impact of the Waters of a Stream Receiving Effluents from Tannery Industry

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Received: 28 February 2020 / Accepted: 4 June 2020 / Published online: 15 June 2020

Abstract The Estância Velha stream integrates the Sinos River Basin that provides drinking water in one of the most important Brazilian centers, the "Capital of tanneries," and it receives effluents from tannery industries. In Brazil, water quality is monitored only through physicochemical and microbiological parameters, and considering the biomarkers' importance in complementing the analysis, the present study was aimed at evaluating the environmental quality of the Estância Velha stream also through cytogenotoxic criteria, at the stream source (site 1), as well as upstream (site 2) and downstream (site 3) of the demographically most dense area. The results for dissolved oxygen, color, total phosphorus, silver, and thermotolerant coliforms classified the Estância Velha stream as class 4 in general; that is, the water is suitable only for navigation and to landscape harmony. Overall, the water was classified as poor by Water Quality Index (WQI) and as hypereutrophic by Trophic State Index (TSI). The main genotoxic alterations (micronuclei and nuclear buds) were observed in site 2, in which were obtained the highest levels of aluminum, silver, iron, and manganese. Despite this, most of the effluents released from the region are not treated prior to being discharged into the stream, suggesting the requirement for effluent treatment to ensure the quality of the water source available.

Keywords Estância Velha stream · Sinos River Basin · Physicochemical · Thermotolerant coliforms · *Allium cepa* · CONAMA Resolution no. 357/2005

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1 Introduction

According to the Ministry of Environment (Brasil 2005), the Brazilian territory comprises approximately 12% of all the fresh water on the planet, which amounts to 200,000 micro-basins scattered in twelve hydrographic regions. However, much of the freshwater in the country is polluted, and therefore, proper management of the available water resources has become a necessity with an increase in population and water scarcity. In order to achieve proper management of the resources, integrated and sequential environmental monitoring of the water is essential to understand their status and quality (Naime and Fagundes 2005).

The huge amount of sanitary sewage released in the water bodies may significantly alter the quality of the latter, increasing the discharge of organic load, and consequently, that of pathogenic microorganisms, resulting in severe eutrophic impacts on the fauna, flora, and humans (Smith and Schindler 2009). In addition, the current stage of development of the society has contributed to the generation of agricultural and industrial wastes which possess great potential for environmental contamination (Conceição et al. 2007). An increase in the amount of untreated sewage dump, combined with agricultural runoff and inadequately treated wastewater from the industries, has resulted in a decline in the water quality worldwide (Voulvoulis and Georges 2016). One of the ways in which the impacts exerted by human interference in the aquatic systems could be assessed is the characterization of the water quality, which would allow its correct management and even remediation. Monitoring the state of water resources is of fundamental importance because it is through the pollution that harmful, toxic, or pathogenic substances are introduced in the water resources, modifying the physical, chemical, and biological characteristics of the aquatic environment (Schwarzenbach et al. 2010).

The Estância Velha stream is located in the metropolitan region of Porto Alegre, Rio Grande do Sul (RS) State, South Brazil. The stream originates in the municipality with the same name, which is also known as the "Brazilian capital of tanneries." This stream corresponds to a stretch of 8 km, and its water directly receives effluents from the tannery and leather-footwear industries. In addition, only 2.8% of the sanitary sewage in the municipality of Estância Velha is collected for treatment, and out of this amount, only 17.6% is actually treated, according to the National Sanitation Information System (SNIS) data (Brasil 2015). The Estância Velha stream integrates the Sinos River Basin which supplies water to approximately 1.6 million people (Benvenuti et al. 2015). The tannery and leather-footwear sectors are considered among the ten industrial sectors that cause most of the environmental damage, as they are responsible for the huge consumption of water resources and the generation of pollutants (Júnior et al. 2007). In the process of leather tanning, there is the generation of sludges with high polluting potential, mainly due to chromium which is highly toxic. Contamination in the Sinos River Basin was first identified in 2001 (Vargas et al. 2001), through its mutagenic activity and the presence of metals in its sediments, including iron, manganese, chromium, lead, copper, zinc, and nickel.

In addition to the traditional methodologies to monitor water quality, ecotoxicological studies serve as excellent alternatives for verifying the quality of water and the effects that the pollutants may exert on the ecosystem (Morais et al. 2016). One of the ways to assess the toxic potential of a water body is to employ *Allium cepa* for the detection of mutagenic activities. The application of *A. cepa* in the evaluation of the cytotoxic potential and in determining the germination rate, the mitotic index, and the development of chromosomal abnormalities as a result of industrial effluents such as those originating from tanneries has been described in previous studies. The test is routinely used throughout the world and is considered a valuable tool in determining the environmental contamination, with an extensive database of already-tested chemicals (Júnior et al. 2007; Silveira et al. 2017).

In Brazil, surface water quality is monitored only in terms of physicochemical and microbiological parameters, in accordance with the limits established by the Brazilian National Environment Council (CONAMA) Resolution no. 357 (Brasil 2005), which does not provide any specific information regarding the use of cytogenotoxic tests for the assessment of water quality. In this context, considering the interest of assessing the impact of pollutants dispersed in water, as well as the importance of the use of biomarkers in complementing the physicochemical analysis of water, the present study was aimed at evaluating the environmental quality of the water from the Estância Velha stream, located in the region of tannery and leather-footwear industries in RS/Brazil, through physicochemical, microbiological, and cytogenotoxic criteria.

2 Materials and Methods

2.1 Water Sampling

All the sample points were defined on the basis of their geographical characteristics (Fig. 1): site 1 (S1), the source (29° 38′ 32.5″ latitude south and 51° 09′ 29.1″ longitude west) was located in a region little urbanized and having rural properties in the surroundings

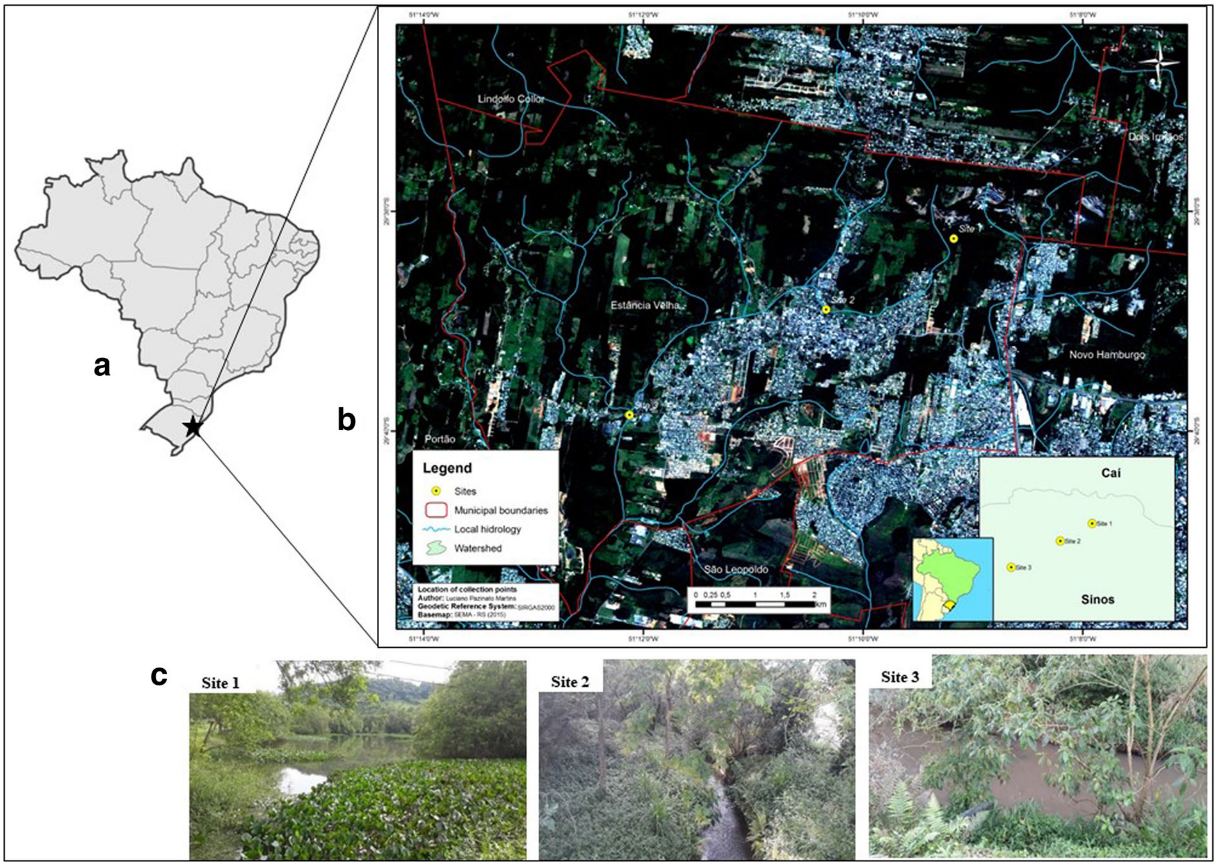

Fig. 1 The Estância Velha stream. **a** Map of Brazil showing the location of Estância Velha municipality. **b** Map of the area. **c** Water sampling points of the Estância Velha stream: site 1 source, site 2 upstream, and site 3 downstream of the area of greatest demography

(Estância Velha 2012) (Fig. 1 b and c); considering the anthropogenic and industrial occupations of the region (29° 38′ 53.6″ latitude south and 51° 10′ 20.0″ longitude west), it was established as site 2 (S2), the location upstream of the area with greatest demographic density, a region that allowed the use of residential, commercial, and industrial environments (Estância Velha 2012) (Fig. 1b and c); and site 3 (S3) was a region (29°39′ 51.2″ latitude south and 51°12′ 07.5″ longitude west) downstream and close to the encounter with the Portão stream within the industrial zone of Estância Velha, which allowed the installation of large industries with medium or high polluting potential (Estância Velha 2012) (Fig. 1 b and c). Samples were collected in October 2017 and analyses were performed in triplicate.

The collected water samples were stored in 1000-mL and 500-mL sterilized glass vials: (1) glass vials were first submerged in waters of the sample sites for a water wash of the point to be collected, an (2) glass vials were again submerged in waters of the respective sample points and immediately sealed and refrigerated at 4 ± 2 °C to protect from light (Júnior et al. 2007).

2.2 Physicochemical and Microbiological Analyses

In order to perform the analysis of the presence of metals in the water, the samples were sent to SGS Geosol Laboratories (Vespasiano/Minas Gerais, Brazil), which utilized the inductively coupled plasma spectroscopy of optical emission for the analysis (APHA 2005). The analysis of temperature, pH, dissolved oxygen, and nitrate was conducted at La Salle University (Canoas/RS), in accordance with the *Standard Methods for the Examination of Water and Wastewater* (APHA 2005).

In order to perform the microbiological analysis, serial dilutions to the order of 10^{-5} were made for each water sample. In order to quantify the thermotolerant coliforms, most probable number (MPN) technique was utilized, in which the dilutions were inoculated on a chromogenic substrate (Colilert®, Idexx) in 5 sets with 5 tubes each, followed by incubation at 35 °C for 24 h. The confirmation of the thermotolerant coliforms was achieved when the positive tubes submitted to ultraviolet light exhibited emission of fluorescence (Edberg et al. 1988).

The physicochemical and microbiological parameters analyzed in the present study allowed the classification of the sampled points according to the classification criteria described in the CONAMA Resolution no 357 (Brasil 2005).

2.3 Water Quality Indices

The parameters that were used to calculate the Water Quality Index (WQI) (CETESB 2003) were mostly the indicators of contamination caused by the discharge of domestic sewage, i.e., water temperature, pH, dissolved oxygen, biochemical oxygen demand, thermotolerant coliforms, total nitrogen, total phosphorus, total residue, and turbidity; each parameter had its respective weight (w) and quality value (q), which were fixed according to their importance in determining the overall conformation of the water quality (CETESB 2003). Based on this approach, different levels of water quality were classified as follows: excellent (90–100); good (70–90); average (50–70); poor (25–50); and very poor (0–25), according to CETESB (2003).

The Trophic State Index (TSI) indicates the quality of water based on its enrichment with nutrients and the effect this water exerts in terms of excessive growth of algae or increase in the infestation with aquatic macrophytes (Kieling-Rubio et al. 2015). This index is calculated from the values of phosphorus, which is a measure of potential river eutrophy according to Carlson (1977), by utilizing the equation $TSI = 10[6 - ((0.42–0.36(\ln TP))/\ln 2)] - 20$, where the value of total phosphorus (TP) is expressed in terms of microgram per liter ($\mu g\ L^{-1}$). The categories of the trophic state are as follows: ultra-oligotrophic ($TSI \leq 47$), oligotrophic (low productivity) ($47 < TSI \leq 52$), mesotrophic (moderate productivity) ($52 < TSI \leq 59$), eutrophic (high productivity) ($59 < TSI \leq 63$), supereutrophic ($63 < TSI \leq 67$), and hypereutrophic (extremely high productivity) ($TSI > 67$) (Kieling-Rubio et al. 2015).

2.4 *Allium cepa* Test

The *Allium cepa* assays were performed using pesticide-free seeds (Baia Periforme variety, Isla Sementes Ltda., Brazil). The onion seeds were exposed to water samples in two Petri dishes for each point. A total of fifty seeds of *A. cepa* were placed in each plate over a filter paper, and 8 mL of each water sample to be tested was added for the germination of the seeds. Control tests were performed using distilled water (negative control) and copper sulfate (0.0002 g/L) (positive control) (Yildiz et al. 2009). When the roots reached a length of 1.5 cm exposed to five days, they were fixed in acetic acid and ethanol (1:3 (v/v)) for 24 h. Two fixed root tips from each plate were rinsed with distilled water, hydrolyzed in 1 N HCl at 60 °C for 8 min, and rinsed again to prepare the slides. Giemsa staining method was used to stain the slides (Guerra and Souza 2002). The slides were prepared by squashing the root meristems with one drop of 45% acetic acid (Fiskesjo 1993). Subsequently, the slides were mounted in synthetic resin for further analysis. Ten slides were prepared for each treatment, and 500 cells were analyzed per slide, which amounted to 5000 cells per treatment. The mitotic index (MI) [(number of dividing cells/total number of observed cells) × 100] was estimated using these cells. In a similar manner, nuclear alterations were identified and determined as micronucleus (MN), nuclear buds (NB), and anaphase with nucleoplasmic bridge (NPB) frequencies. Additionally, toxicity was determined based on the seed germination index (GI), which was calculated as the ratio of the number of seeds that germinated to the total number of seeds that were allowed to germinate.

2.5 Statistical Analysis

Data normality was assessed using the Kolmogorov–Smirnov test. The cytogenotoxic variables exhibited a non-parametric distribution when assessed using the Kruskal–Wallis test in association with Dunn's test for multiple comparisons. The values of $p < 0.05$ were considered significant. All statistical analyses were done by the GraphPad Prism version 5.0 program (GraphPad) for the comparisons.

In order to perform an integrated analysis of the physicochemical and microbiological parameters, principal component analysis (PCA) was performed in Past 3.14 software.

3 Results

3.1 Physicochemical and Microbiological Analyses

For most of the physicochemical parameters analyzed (Table 1), the water samples were classified within class 1, according to the criteria established by CONAMA Resolution no. 357 (Brasil 2005), i.e., water destined to be supplied for human consumption post-simple treatment, for protection of aquatic communities, and for irrigation of raw vegetable crops. As presented in Table 1, the concentration of silver (Ag) at the three sampling points was 0.113 mg/L (S1), 0.082 mg/L (S2), and 0.062 mg/L (S3). The color obtained alteration resulted in the classification of water in class 4; i.e., the water may only be used for navigation and to landscape harmony. Dissolved oxygen (DO) value of 2.6 mg/L at S1 (source) and the total phosphorus content at S2 (0.24 mg/L) also classified the waters in class 4. Within the limits of class 2, which comprises waters that may be destined for human consumption post-conventional treatment methods, for protection of aquatic communities, and for aquaculture and fishing activities, at S2, the DO parameter value was 5 mg O_2/L, while the biochemical oxygen demand (BOD) values at S2 and S3 were 3.3 mg/L and 3.95 mg/L, respectively. The iron (Fe) frames at the three sites presented values of 0.94 mg/L (S1), 2.61 mg/L (S2), and 2.27 mg/L (S3), while manganese (Mn) attained the values of 0.29 mg/L (S1), 0.24 mg/L (S2), and 0.29 mg/L (S3), classifying the waters in class 3, i.e., the water intended for human consumption post-conventional or advanced treatment methods; for the irrigation of arboreal, cereal, and forage crops; and for amateur fishing. The value of Mn remained stable between the points, while Fe exhibited a concentration increase beyond S1, which could be a natural increase along the course of the stream or may imply a point of discharge of this component in the stream path between S1 and S2. The aluminum (Al) parameter exhibited variation between the points, classifying S2 in class 3 with 0.22 mg/L and then decreasing again at S3 (0.05 mg/L). When the results were analyzed, S1 and S3 points of the Estância Velha stream presented the classification of their waters in a similar way, i.e., class 3 only for the Fe and Mn parameters and class 4 for Ag and the DO parameter. On the other hand, S2, in addition to the similarity to the other points, was also classified in class 3 for Al, with a peak of anomalous concentration of 0.22 mg/L relative to the other points sampled. No significant levels of the metals commonly found in tannery effluents such as chromium, copper, and cadmium were detected in the present study. Therefore, the results revealed the presence of certain heavy metals in the Estância Velha stream waters in amounts beyond the standards acceptable, according to the CONAMA Resolution no. 357 (Brasil 2005), and therefore, the waters of the stream were classified as class 4 waters in general, based on the DO levels, color, and the amounts of total phosphorus and silver present.

Microbiological analyses of thermotolerant coliforms (TC) performed with the water samples indicated the presence of these organisms at all the sampling sites (Table 1). S1 (source) exhibited a higher value of TC (7×10^3 MPN/100 mL) in comparison with S3 (downstream), which was compatible with the low DO index value attained for this point (2.6 mg O_2/L). S2 exhibited a high index of TC (5.4×10^4 MPN/100 mL), presenting the lowest microbiological quality among the waters. The analysis of water sampled from S3, where the highest coliform index was expected as it was located in an area with a high concentration of residences and industries, presented the lowest value for TC among all the sampled points (3.1×10^3 MNP/100 mL), as well as the highest DO concentration among all the samples (6.3 mg O_2/L). According to the CONAMA Resolution no. 357 (Brasil 2005), the uses other than bathing should not utilize water with a TC value exceeding 2.0×10^2 MPN/100 mL. Therefore, the microbiological analyses also classified the waters of the Estância Velha stream in class 4.

3.2 Water Quality Indices

According to the Water Quality Index (WQI), the classifications identified for each point are presented in Table 2; the waters of the stream were classified as poor in S2 and S3 and reasonable in S1 (source). Overall, the water in the Estância Velha stream was classified as poor according to the WQI. The TSI classified S1 as ultra-oligotrophic, while S2 and S3 were classified as hypereutrophic, corroborating with the high concentration of phosphorus obtained in S2 and S3.

3.3 Integrated Analysis of Physicochemical and Microbiological Factors

As depicted in Fig. 2, all the sampled points exhibited low environmental quality, which could be attributed to

Table 1 Physical-chemical and microbiological analyses of the Estância Velha stream water samples: site 1 (S1), site 2 (S2), and site 3 (S3). Classification according to the CONAMA Resolution no. 357 (Brasil 2005): class 1 (green), class 2 (yellow), class 3 (orange), and class 4 (red)

Parameters	S1	S2	S3	Standard (Class 1) CONAMA nº 357
Temperature (ºC)	20	20	19	NI
pH	7	7.2	7.1	6,0–9,0
Dissolved Oxygen (mg O_2/L)	**2.6**	**5.0**	6.3	>6
Aluminum (mg/L)	<0.05	**0.22**	0.05	<0.1
Barium (mg/L)	0.03	0.09	0.07	<0,7
Beryllium (mg/L)	<0.004	<0.004	<0.004	<0,04
Boron (mg/L)	<0.2	<0.2	<0.2	<0,5
Cadmium (mg/L)	<0.001	<0.001	<0.001	<0,001
Lead (mg/L)	<0.01	<0.01	<0.01	<0,01
Cobalt (mg/L)	<0.01	<0.01	<0.01	<0,05
Copper (mg/L)	<0.009	<0.009	<0.009	<0,009
Total Chrome (mg/L)	<0.01	<0.01	<0.01	<0,05
Hexavalent Chrome (mg/L)	<0.01	<0.01	<0.01	NI
Total Phosphorus (mg/L)	<0.02	**0.24**	**0.14**	<0.1
Iron (mg/L)	**0.94**	**2.61**	**2.27**	<0.3
Lithium (mg/L)	<0.1	<0.1	<0.1	<2,5
Manganese (mg/L)	**0.29**	**0.24**	**0.29**	<0.1
Nickel (mg/L)	<0.01	<0.01	<0.01	<0,025
Silver (mg/L)	**0,113**	**0.082**	**0.062**	<0.01
Vanadium (mg/L)	<0.02	<0.02	<0.02	<0,1
Zinc (mg/L)	<0.1	<0.1	<0.1	<0,18
Nitrate (mg/L)	ND	0.29	ND	<10,0
Nitrite (mg/L)	ND	ND	ND	<1.0
Biochemical Oxygen Demand$_5$ (mg/L)	0.05	**3.3**	**3.95**	<3.0
Chemical Oxygen Demand (mg/L)	3.0	58.7	80.7	NI
Chloride (mg/L)	4.0	12.8	26.0	<250
Phosphate (mg/L)	ND	0.7	0.4	NI
Bromide (mg/L)	ND	0.29	0.29	NI
Fluoride (mg/L)	0.02	0.15	0.12	<1.4
Sulfate (mg/L)	8.6	8.2	24.4	<250
Total solids (mg/L)	111	135	175	<500
Turbidity (NTU)	7.5	10.6	13.9	<40
Color (mg Pt/L)	**103**	**95**	**112.5**	0
Thermotolerant coliforms (MPN/100ml)	**7.0×10^3**	**5.4×10^4**	**3.1×10^3**	$<2.0 \times 10^2$

ND not detected, *NI* not in the resolution

Table 2 Water Quality Index (WQI) and Trophic State Index (TSI) in sites 1 (S1), 2 (S2), and 3 (S3) in water samples from the Estância Velha stream

Sample	WQI	Classification	TSI	Classification
S1	52	Average	< 47	Ultra-oligotrophic
S2	31	Poor	240	Hypereutrophic
S3	39	Poor	140	Hypereutrophic

the increase in the concentrations of certain physico-chemical and microbiological factors analyzed. In PCA, principal component 2 separated S1 and S2 from S3. It also separated the increase in the thermotolerant coliforms from the DO levels and total coliforms, while component 1 separated S1 from the other points.

3.4 Cytogenotoxic Analyses

The results revealed alterations in the germination index (GI) and genotoxic parameters (Table 3) when compared with the negative control group, indicating cytotoxic and mutagenic activities of the water samples from S3 and S2, respectively, in the Estância Velha stream.

The cytotoxic activities were evaluated using GI and mitotic index (MI). In terms of GI, a lower frequency of germinated seeds was observed in the stream samples when compared with the negative control, although the difference was significant only in S3 (Table 3). No

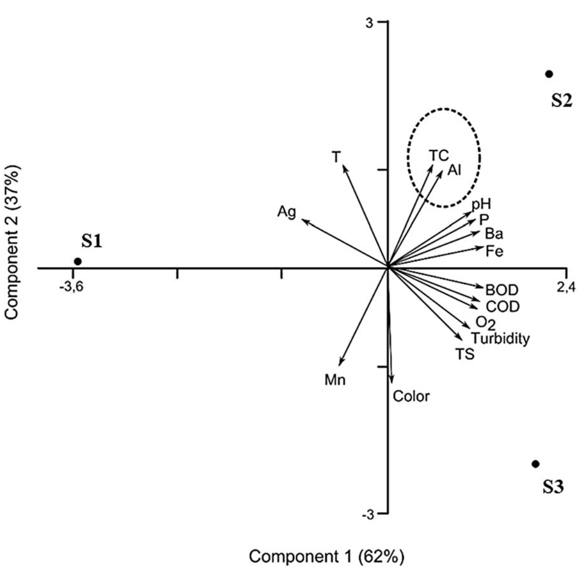

Fig. 2 Principal component analysis (PCA) of the physical-chemical and microbiological characteristics analyzed for the water samples collected from the Estância Velha stream

significant difference was detected among the points in terms of MI. The genotoxic analysis of the water samples of the Estância Velha stream indicated that the micronucleus (MN) frequency increased in S2 compared with the negative control. No significant differences in terms of the nuclear bud (NB) and nucleoplasmic bridges NPB frequencies were evidenced (Table 3).

4 Discussion

The Estância Velha stream is a part of the Sinos River Basin which provides drinking water to 1.6 million inhabitants in one of the most important industrial centers of Brazil (Petry and Schulz 2006). This stream runs through densely populated regions, serving as a receiving water body for tanneries as well as for domestic sewage. Tannery effluents are among the most hazardous pollutants released by industries, as these are highly complex and are characterized by high contents of nitrogenous compounds, chromium, copper, cadmium, and sulfides, among others (Durai and Rajasimman 2011). All the tanneries in the Rio Grande do Sul (RS) State (Brazil) have effluent treatment systems installed in them since 1980, as it was a requirement of FEPAM (State Foundation for the Protection of the Environment, RS/Brazil). In the period between 1990 and 2002, only 4% of the total chromium analyses exceeded the limit established by the CONAMA Resolution no. 357 (Brasil 2005), demonstrating the efficiency of the chromium removal systems installed in the tanneries of the region (FEPAM 1997). It was probably due to this reason that high levels of chromium, hexavalent chromium, cadmium, lead, mercury, nickel, and copper were not detected in the stream waters in the present study.

Despite this, frequent reports of small fish mortalities in the Estância Velha stream are available, which may be due to high organic load of effluents thrown into the stream leading to a higher consumption of oxygen, along with the generation of bad odors and damage to aquatic life, establishing dissolved oxygen (DO) as an efficient indicator of pollution (Naime and Fagundes 2005). Naime and Nascimento (2009), in a study with the water samples from Pampa stream, which is another tributary of the Sinos River Basin (RS, Brazil), obtained lower values for DO (< 2 mg O_2/L) in comparison with those sampled at S1 (source; class 4 for DO) of the Estância Velha stream, classifying these waters compatible with the "out of class" condition (Brasil 2005).

Table 3 Cytogenotoxic parameters observed in meristematic cells of *Allium cepa* seeds exposed to water samples from sites 1 (S1), 2 (S2), and 3 (S3) at the Estância Velha stream. Data are means ± standard error of two replicates

Group	Germination index (%)	Mitotic index	Micronucleus frequency	Nuclear bud frequency	Nucleoplasmic bridges frequency
Negative control[a]	100	1.82 ± 0.78	0.90 ± 0.41	0.20 ± 0.13	0.30 ± 0.30
S1	72	1.20 ± 0.51	0.20 ± 0.13	0.10 ± 0.10	0.00 ± 0.00
S2	84	0.50 ± 0.19	4.40 ± 1.32*	1.00 ± 0.37	0.40 ± 0.22
S3	56*	1.56 ± 0.47	2.60 ± 0.43	0.00 ± 0.00	0.30 ± 0.21
Positive control[b]	84	1.28 ± 0.28	1.70 ± 0.63	0.10 ± 0.10	0.20 ± 0.20

[a] Distilled water; [b] Copper sulfate (0.0002 g/L); significant difference in relation to the negative control group (*$p < 0.05$). Kruskal-Wallis test

The results of the physicochemical analyses indicated high concentrations of Fe, Mn, and Al (with Al exhibiting specifically high values in S2), classifying the Estância Velha stream in class 3 at all the sampled points, which according to the CONAMA Resolution no. 357 (Brasil 2005) comprises water intended for human consumption post-conventional or advanced treatments; for the irrigation of arboreal, cereal, and forage crops; and for amateur fishing. Metals are among the most common contaminants of water bodies (Peláez-Rodriguez et al. 2000), and their origin may be associated with the edaphic profile of the region (Streck et al. 2008) or it may be related to the release of industrial effluents and the leaching of agricultural products into the water body. The soils of the region have been classified as red-yellowish argisols (Streck et al. 2008), rich in Fe, which could also have been the cause of the color change in water that resulted in classifying the Estância Velha stream in class 4 (Brasil 2005). This soil is formed mainly of basalt, with high contents of iron and magnesium and low silica content (Muradás et al. 2018), thereby exhibiting low natural fertility, strong acidity, high saturation of Al, and susceptibility to erosion and degradation (Rio Grande do Sul 2019). According to a report by the National Department of Mineral Production (Brasil 2017), there are two basalt extraction companies in the municipality of Estância Velha, and these companies may have influenced the concentrations of Fe observed in the stream waters in the present study. The case of high Al concentration in neutral pH water may be associated with the discharge of high loads of effluents in this region (Blume et al. 2010) since the resulting concentrations of dissolved Al are usually much less than 0.1 mg/L (Flaten 2001). Lima and dos Santos (2012) analyzed potentially toxic metals in Claro River (São Paulo, Brazil) and obtained similar results for Al and Fe, which was attributed to the soil of the region, and the high Mn content obtained was attributed to contamination of agrochemicals. The reported concentrations of manganese were above the established limit (Brasil 2005) in all the sampled sites of the Estância Velha stream. Deepali and Gangwar (2010) reported a value of 0.988 mg/L for manganese in a tannery effluent around Haridwar (India). Manganese may be among the least toxic trace elements if ingested, although if inhaled, it takes a direct path to the brain tissue, and the lung uptake could provide a source of continuing exposure (Weiss 2006).

The high concentration of Ag obtained in the present study, as well as the parameter of color, classified the Estância Velha stream into the lowest class, class 4 (Brasil 2005), and corroborated with the study reported by COMITESINOS (2014) for this stream. Silver ion is one of the most toxic heavy metals, among others, such as cadmium, chromium (VI), copper, and mercury (Ratte 1999). Nordberg et al. (2007) reported that silver is not much abundant in waters (> 5 μg L^{-1}), as several of its salts are little soluble in water, except for the use in the industrial processes and for the disinfection of drinking water (Quadros and Marr 2010). Manufacturers have begun incorporating silver nanoparticles as a biocide in soaps, toothpaste, shampoo, cosmetics, water purifiers, etc. (Quadros and Marr 2010). Ag is classified as a low potential human health risk metal; however, it is bioaccumulative and Ag poisoning through chronic exposure causes argyria (Liu et al. 2008).

In the present study, it could be verified that the levels of thermotolerant coliforms (TC) in the samples analyzed were above the limit (Brasil 2005), classifying the Estância Velha stream as class 4, indicating the discharge of sanitary effluents without proper treatment (CETESB 2009). S1 (source) presented a high density of TC and the lowest dissolved oxygen value between the sampled points. This result was unexpected, as it is presumed that the closer the watercourse is to the forming agent, the less it would be affected by contaminations (Marmo and Joly 1962). However, since the source of the stream was in a rural area with animal husbandry in practice, its waste may have mixed with the water of the region, and there may also have been the use of organic fertilization in the agricultural practices of this region. Despite this, according to the data provided by SNIS (Brasil 2015), only 2.84% of the sanitary sewage produced in the municipality of Estância Velha is collected for treatment, and out of this amount, only 17.57% is treated. At S3, the highest index of coliforms was expected, as the point was in an industrial and urbanized area, the lowest values for the index among all the points sampled, as well as the highest concentration of DO, were obtained. The decrease in TC at this site may be because S3 received a low amount of untreated effluents. This fact may also be related to the Municipal Sanitation Basic Plan (Estância Velha 2014) for sanitary sewage, which aims for an expansion plan that would provide 78% coverage with a separating network and treatment from 2019. In the PCA plot, component 1 separated the S1 from the other points as well as from most of the analyzed parameters and also revealed a close relationship between the Al concentration and the TC population, in relation to S2, implying that the low quality of the water samples from this point could have an anthropogenic origin.

Total phosphorus values obtained for points S2 and S3 in the Estância Velha stream were higher, classifying the S2 in class 4 and the S3 in class 3 (Brasil 2005). The amount of phosphorus was directly related to TSI, which indicated extremely high values, classifying the waters of this stream as hypereutrophic. The TSI indices describe the effect of human activities on the waters, in addition to assisting in the development of the handling and management plans for the aquatic ecosystem using strategies to promote the sustainability of the water resources (Kieling-Rubio et al. 2015). In a study reported by Kieling-Rubio et al. (2015) for the Estância-Velha/Portão stream, a TSI value of 54.96 was obtained, classifying the waters as mesotrophic, while the WQI has been used by the Environmental Company of the State of São Paulo (CETESB) since 1975, and today it is the main water quality index used in Brazil. Since this index requires simple measurement, it has been widely used for monitoring the water quality in the Sinos River Basin (COMITESINOS 2014). However, the WQI does not analyze several important parameters, such as toxic substances, pathogenic protozoa, and the substances that interfere with the organoleptic properties of water (ANA 2018). The WQI of the Estância Velha stream was classified as poor in general, in the present study. The factors that contributed most negatively to the index were DO, BOD, and TC values, the latter being the one that causes the maximum decreases in the WQI of the water bodies (CETESB 2003). These parameters could be associated with the organic matter originating from the discharge of domestic sewage without treatment, as was the case of the Estância Velha stream in which 99.5% of the domestic and industrial sewage is not treated (Brasil 2015). Analyzing the water quality assessment of Sinos River, Blume et al. (2010) reported that the source exhibited a WQI value that existed between good and average, while the WQI of other sites scored between average and poor. These results suggested that the environmental impact observed in the Sinos River was caused by the untreated sewage from the tannery, metallurgical, and timber industries located in the region, creating a severe environmental hazard in this area (Blume et al. 2010).

In relation to cytotoxic analysis, S3 exhibited a lower germination index (GI) which could be related to reduced organic matter load at this point, as observed in terms of higher values of DO (6.3 mg O_2/L). This could cause low stimulation to root elongation, in order to reduce the test sensitivity and/or to mask a possible toxic effect (Rodrigues and Bianchini 2007). Júnior et al. (2007) evaluated the water and sediment samples collected from three different points in the Estância Velha stream, and a root growth reduction was observed, evidencing the toxic effect of water post-spring of the stream. In the case of mitotic index (MI), high values were observed at the end of the stream, near to the municipality boundary during summers and in the stream spring during winters. The lowest MI values were obtained at the intermediate point, located at 4 km from the stream spring, during summers, as well as at the end of the stream during winters. According to the authors, a reduction in the stream water quality was observed post the stream spring, as this region receives contributions from industrial and domestic wastes until the Portão stream formation (geographical limit of the municipality), indicating an increase in the chromium level in the stream since the location of the spring. These results differ from the findings of the present study, in which no significant difference was detected in the MI values among the points.

Similar to the results of the present study, Santos et al. (2016) evaluated the three water samples collected from the São Francisco River (Petrolina, Brazil), near to a tannery effluent discharge, and authors observed a GI reduction in the waters from all the three points in comparison with the negative control. These data led the authors to assume that phytotoxic substances might be influencing the ecosystem quality and interfering in the growth of *A. cepa* roots. At the same time, MI value exhibited no evidence of cytotoxicity in the analyzed samples, as no significant differences were observed in relation to the negative control.

The main genotoxic alterations were observed in the water from S2, in which the highest levels of Al, Ag, Fe, and Mn were observed. These results corroborated with previous studies that indicated toxicity related to high concentrations of these metals (Peláez-Rodriguez et al. 2000; Achary et al. 2008; Duarte et al. 2012). In the Estância Velha stream, Júnior et al. (2007) observed higher micronucleus (MN) frequency at the intermediate point of the stream, although no significant results were revealed. The absence of significant mutagenic activity could be justified, according to the authors, by the absence of mercury and hexavalent chromium in the water, suggesting that chromium was present in its trivalent form in the water. The authors did not perform analyses for aluminum and silver. Previous studies have associated the increased toxicity in the test organisms with exposure to heavy metals; for example, in a study conducted by Othman et al. (2015), chironomid larvae (*Chironomus javanus*) were exposed to various heavy metals, and the bioconcentration of these elements was measured, verifying that the metals Fe, Al, and Mn, among others, caused toxicity. Water samples collected in Sinos River (Parobé city, Brazil) showed higher concentrations of metals (aluminum, iron, and lead) when compared with Caraá city, which could explain the higher frequency of nuclear abnormalities observed in *Bryconamericus iheringii*.

In the present study, the levels of Mn, Fe, and Ag could be associated with the mutagenicity observed in the cells exposed to the S2 water sample. According to Beyersmann and Hartwig (2008) in general, metal genotoxicity is caused by indirect mechanisms, and in spite of the diverse physicochemical properties of metal compounds, the interference with cellular redox regulation and induction of oxidative stress may cause oxidative DNA damage. Besides, aluminum was found only in the S2. This metal is one of the major pollutants of soil and water and may be transferred via trophic animals and cause serious hazards to ecosystems and human health, as this element interferes with several processes, such as cell division kinetics; it may cause the inhibition of microtubule polymerization, promotion of chromosomal adhesion, and nuclear fragmentation. Micronucleus induction could be related to the chromosomal delays during anaphase due to spindle malfunction (Duarte et al. 2012). According to Pereira et al. (2013), Al exposure causes toxic effects in humans, and most of the studies related to genotoxic and cytotoxic potentials of aluminum have demonstrated that this element induces micronuclei and chromosomal changes in the human cells. Studies conducted by Fiskesjo (1993), Achary et al. (2008), and Qin et al. (2013), which utilized *A. cepa* as a test organism, identified that cytotoxicity increased in response to aluminum exposure. Duarte et al. (2012) also observed in their studies conducted with *Oreochromis niloticus* that aluminum exerted a genotoxic and mutagenic effect.

5 Conclusions

The monitoring of the Estância Velha stream by utilizing various bioindicators is essential for the evaluation of its water quality, considering the preponderant use of this stream in rainwater management; industrial effluents, particularly from the leather industry; and sewage effluents. Our work is the first one to determine the physicochemical, microbiological characteristics with possible cytotoxic and genotoxic changes in this important tributary of the Sinos River Basin.

The results obtained in the present study demonstrated that according to the CONAMA Resolution no. 357 (Brasil 2015), the water from the Estância Velha stream is classified in class 4 which comprises waters that may only be used for navigation and to landscape harmony, mainly due to the color, and the concentrations of DO, total phosphorus, Ag, and thermotolerant coliforms. The stream was also classified as poor according to the WQI and hypereutrophic according to TSI. With the use of cytogenotoxic tests performed for the assessment of water quality, the main genotoxic alterations were observed in the water from point S2, downstream of the demographically densest area, which also exhibited the highest concentration of Al in addition to high concentrations of Ag, Fe, and Mn; these high metal concentrations might be related to the alterations obtained in the water from S2. No significant levels of toxic metals usually present in the tannery effluents, such as chromium, copper, and cadmium, were detected in the water samples analyzed in the present study. This could be attributed to the efficiency of the effluent treatment systems required by FEPAM for the operation of industries in the Sinos River Basin region. Despite this, most of the domestic effluents from the city of Estância Velha are not treated prior to being discharged into the stream, suggesting that the requirement of effluent treatment should be reinforced to ensure the health of the ecosystems and consequently, the quality of the source water available for humans.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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