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Assessing the spatial pattern of a river water quality in southern Brazil by multivariate analysis of biological and chemical indicators

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(With 3 figures)

Abstract

This study assessed the genotoxicity and chemical quality of the Rio dos Sinos, southern Brazil. During two years, bimonthly, cuttings of *Tradescantia pallida* var. *purpurea* with flower buds were exposed to river water samples from Caraá, Santo Antônio da Patrulha, Taquara and Campo Bom, which are municipalities located in the upper, middle and lower stretches of the Rio dos Sinos basin. Simultaneously, chemical parameters were analyzed, rainfall data were surveyed and negative (distilled water) and positive (0.1% formaldehyde) controls were made. Micronuclei (MCN) frequencies were determined in tetrads of pollen grain mother cells. From the upper stretch toward the lower, there was an increase in the frequency of MCN and in concentrations of chemical parameters. Cadmium, lead, copper, total chromium and zinc were present at the four sites and a concentration gradient was not demonstrated along the river. The multivariate analysis revealed that two principal components exist, which accounted for 62.3% of the observed variances. Although genotoxicity was observed in Santo Antônio da Patrulha, the water presented higher mean values for most of the assessed parameters, in the lower stretch, where urbanization and industrialization are greater. The spatial and temporal pattern of water quality observed reinforces the importance of considering the environmental factors and their effects on organisms in an integrated way in watercourse monitoring programs.

Keywords: genotoxic risk, micronuclei, pollutants, *Tradescantia pallida* var. *purpurea*, watercourse.

Avaliação do padrão espacial da qualidade da água de um rio no Sul do Brasil por meio da análise multivariada de indicadores biológico e químicos

Resumo

Este estudo avaliou a genotoxicidade e a qualidade química do Rio dos Sinos, Sul do Brasil. Durante dois anos, com periodicidade bimensal, ramos de *Tradescantia pallida* var. *purpurea* com botões florais foram expostos a amostras de água do rio de Caraá, Santo Antônio da Patrulha, Taquara e Campo Bom, municípios localizados nos trechos superior, médio e inferior da Bacia do Rio dos Sinos. Simultaneamente, foram analisados parâmetros químicos, levantados dados de precipitação e realizados controles negativos (água destilada) e positivos (0,1% formaldeído). Frequências de micronúcleos (MCN) foram determinadas em tétrades de células-mãe de grão de pólen. Do trecho superior em direção ao inferior, foi observado aumento da frequência de MCN e na concentração de parâmetros químicos. Cádmio, chumbo, cobre, cromo total e zinco estiveram presentes nos quatro pontos amostrais, sem apresentar um gradiente de concentração ao longo do rio. A análise multivariada demonstrou a existência de dois componentes principais que explicaram 62,3% das variâncias observadas. Embora em Santo Antônio da Patrulha tenha sido observada genotoxicidade, a água do Rio dos Sinos apresentou valores médios superiores para a maioria dos parâmetros avaliados no trecho inferior, onde a urbanização e a industrialização são maiores. O padrão espacial e temporal de qualidade da água observado reforça a importância de considerar os fatores ambientais e seus efeitos nos organismos de forma integrada em programas de monitoramento de cursos hídricos.

Palavras-chave: risco genotóxico, micronúcleos, poluentes, *Tradescantia pallida* var. *purpurea*, curso hídrico.

1. Introduction

The Rio dos Sinos basin, located in the state of Rio Grande do Sul, southern Brazil, has in Rio dos Sinos its main watercourse, which is divided into three stretches. The upper stretch is 25 Km long, where the river has rapid water flow, low demographic density (Figueiredo et al., 2010) and economy based on agriculture, and cattle and swine rearing (Dalla Vecchia et al., 2015). The middle stretch is 125 Km long and exhibits an increase in demographic density (Figueiredo et al., 2010). Economy is based on footwear and metallurgical industries, as well as small agricultural activities (Dalla Vecchia et al., 2015). In the lower stretch, the river flows very slowly for 50 Km (Figueiredo et al., 2010). This stretch presents high population density living almost exclusively in urban areas, and there is a concentration of metal-mechanical, leather and footwear, as well as food and petrochemical industries (Dalla Vecchia et al., 2015).

Although the Rio dos Sinos supplies a population of about 1.5 million people (COMITESINOS, 2015), it displays a significant water quality degradation scenario, which results from several human impacts related primarily to the prevailing conditions of urbanization, industrialization, inadequate agricultural practices, and lack of sewage and industrial effluent treatment (Blume et al., 2010; Costa et al., 2014; Dalla Vecchia et al., 2015).

In watersheds of major urban centers, water bodies receive organic and inorganic waste of various natures (Blume et al., 2010), which makes them increasingly difficult to analyze and determine all the chemical components that could compromise water quality (Nunes et al., 2011). Rapid changes in the physical and chemical composition of water and interactions between substances can induce toxic and genotoxic effects on living organisms, since inland waters are the main resources for domestic, industrial and agricultural uses (Costa et al., 2014). Given this reality, bioindicators can be integrated to physico-chemical studies in order to evaluate the synergistic or additive effects of complex pollutant mixtures in water and the damage observed in biological indicators, which demonstrate the environmental risks to which organisms are exposed (Nunes et al., 2011). Multivariate analyzes integrating chemical and biological indicators have been applied in water quality assessment (Zhang et al., 2009; Panizzon et al., 2013), helping to explain the tendencies observed in the parameters studied in the environments. However, no environmental assessment studies using such methods have been performed in the Rio dos Sinos basin yet.

In this context, this study aimed to: (i) evaluate the genotoxic effect of river water on *Tradescantia pallida* var. *purpurea* and its variation over time and space, (ii) analyze chemical variables concerning water quality, and (iii) investigate the relation between biological and chemical parameters, in order to explain the spatial pattern of water quality along the Rio dos Sinos. Taking into account the fact that there is an urbanization and industrialization gradient along the watercourse reflecting an increase in human impacts toward the lower stretch of the basin, we hypothesize that the quality of the Rio dos Sinos water will be lower and that its genotoxicity will be higher in municipalities with greater urbanization.

2. Material and Methods

2.1. Study area and characteristics of sampling points

This study was conducted in the Rio dos Sinos that is located in the east of the state of Rio Grande do Sul (29° 20' to 30° 10' S and 50° 15' to 51° 20' W), in southern Brazil (Figure 1), for a period of 24 months.

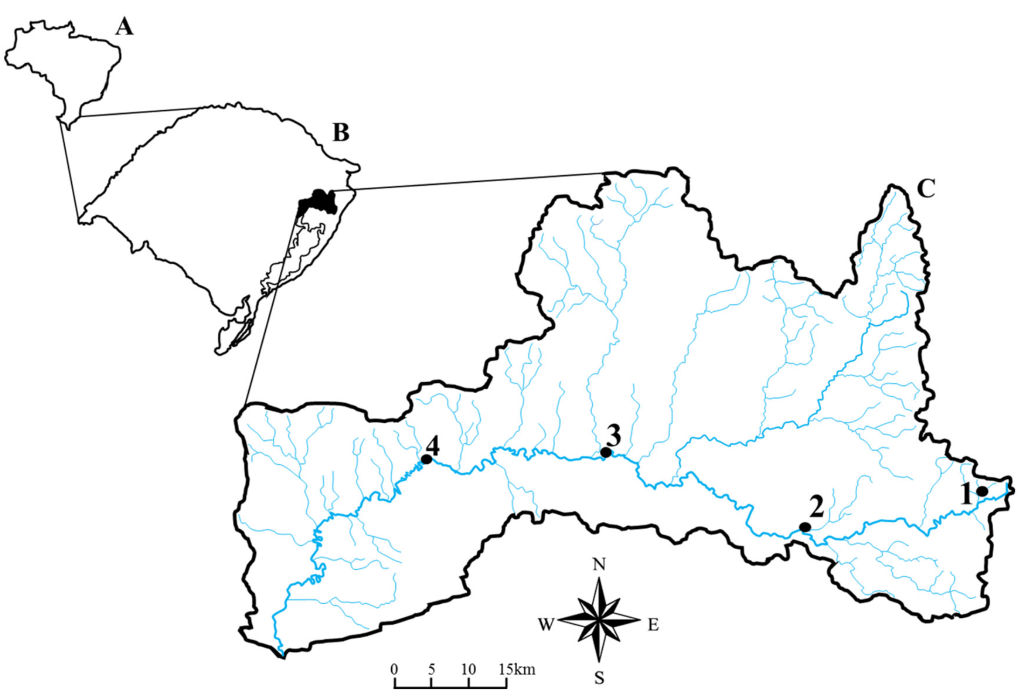

Figure 1. Location of water sampling points in the municipalities of Caraá (1), Santo Antônio da Patrulha (2), Taquara (3) and Campo Bom (4) in the Sinos River Basin (C), state of Rio Grande do Sul (B), Brazil (A).

The sampling points are located in the municipalities of Caraá (29° 44' 20.7" S and 50° 16' 18.3" W; Fraga district near the river source; alt. 298 m) and Santo Antônio da Patrulha (29° 46' 19.7" S and 50° 30' 57.3" W; Monjolo district; alt. 26 m) in the upper stretch, and in Taquara (29° 40' 38.9" S and 50° 46' 48.1" W; Empresa district; alt. 20 m) and Campo Bom (29° 41' 29.7" S and 51° 02' 11.1" W; Barrinha district; alt. 11 m) in the middle and lower stretches of the Rio dos Sinos, respectively (Figure 1). In Caraá, especially in the vicinity of the river springs, the riparian vegetation has a width exceeding 50 m with low human impact. In the other studied municipalities, the riparian vegetation consists of small forest fragments with width less than 50 m and varying degrees of human impacts (COMITESINOS, 2015).

Caraá presents a virtually rural population of 7,742 inhabitants and it is distributed over a 294.3 Km^2 area. Santo Antônio da Patrulha has a population of 41,579 inhabitants, which are distributed over a 1,049.8 Km^2 area. The area of Taquara is 457.8 Km^2 with population of 56,896 inhabitants. Campo Bom has an area of 60.5 Km^2 and a population of 63,339 inhabitants (IBGE, 2015). Whereas the economy of Caraá and Santo Antônio da Patrulha is predominantly agricultural, Taquara has an industrial and agricultural economy and in Campo Bom it basically consists of leather-footwear and metallurgical industries (Cassanego et al., 2014; Dalla Vecchia et al., 2015).

2.2. Water sampling

Water samples were collected on the surface of the Rio dos Sinos and transported to the laboratory according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Samples were collected from May 2012 to March 2014, bimonthly, totaling 12 samples of each point.

2.3. Chemical analyzes of water samples

Chemical analyzes of water samples were performed according to the methodology described in Standard Methods for the Examination of Water and Wastewater (APHA, 2012). The parameters analyzed were: a) biochemical oxygen demand (BOD_5) by respirometric (5210 D), b) total phosphorus (TP) by colorimetry (4500-P D), c) total Kjeldahl nitrogen (TKN) by titration (4500-N_{org} B), d) total suspended solids (TSS) by gravimetry (2540 D) and e) the metals cadmium (Cd), lead (Pb), copper (Cu), total chromium, (Cr) and zinc (Zn) by flame atomic absorption spectroscopy (FAAS) (3111 A). The classification of water samples was performed according to the values established by the Resolution 357/2005 from CONAMA for class 1 in fresh water bodies (Brasil, 2005).

2.4. Heavy metal evaluation index

The level of heavy metal pollution in each water sample from the Rio dos Sinos was estimated by the Heavy Metal Evaluation Index (HEI), based on the criteria used by Edet and Offiong (2002). This index was calculated by dividing the value found (V_e) for each metal by its maximum value (V_{max}) set in CONAMA Resolution 357/2005 for class 1 fresh water (Brasil, 2005). The sum (Σ) of the averages obtained for metals corresponds to the HEI value found in the water sample evaluated: $HEI = \Sigma(V_e/V_{max})$, where values under 10 indicate low pollution, values between 10 and 20 indicate medium pollution and values greater than 20 indicate a sample highly polluted by heavy metals.

2.5. Rainfall data

During monitoring, accumulated rainfall of three preceding days and of the day of water sample collection was recorded in each municipality; a procedure adopted in accordance with Endres Júnior et al. (2015). Rainfall data were obtained through a mobile weather station (Davis Vantage PRO 2 VP USB NS) located in Caraá (29° 44' 27" S and 50° 21' 58" W; alt. 364 m); from the Civil Defense in Santo Antônio da Patrulha and in Taquara (Rio Grande do Sul, 2014) and from the Weather Station no. 83961, located in Campo Bom (29° 41' S and 51° 03' W; alt. 25.8 m) (INMET, 2014).

2.6. Trad-MCN bioassay

Tradescantia pallida var. *purpurea* specimens were grown in pots (37 cm × 20 cm × 20 cm) containing 4 kg of commercial soil from the same batch and maintained on campus, according to Thewes et al. (2011). Plants were watered three times a week and fertilized monthly with 100 mL of an N:P:K solution (nitrogen:phosphorus:potassium, 10:10:10 w:w:w). All plants were obtained from vegetative propagation, with seedlings derived from the same population.

Trad-MCN bioassay followed the method described by Cassanego et al. (2014). From May 2012 to March 2014, bimonthly, 20 cuttings (10 to 15 cm long) of *Tradescantia pallida* var. *purpurea* with flower buds were collected and adapted in distilled water for 24 h to perform each genotoxicity test. Afterwards, these cuttings were exposed for 8 h to water samples from the Rio dos Sinos and then recovered in distilled water for 24 h. Both negative and positive controls were performed by the same methodology, only replacing the river water sample for distilled water in the negative control and for a formaldehyde solution 0.1% in the positive control (Thewes et al., 2011). During the bioassays, cuttings were kept under controlled conditions at 26 °C. After the recovery period, flower buds were fixed in absolute ethanol: acetic acid (3:1 v:v) for 24 h and then stored in 70% ethanol under refrigeration (4 °C). Flower buds were dissected and its anthers macerated with acetic carmine 1%. In each slide, 300 young tetrads of pollen grain mother cells were counted and the number of micronuclei (MCN) was recorded by optical microscopy (Olympus CX4), at 400x magnification, totaling 10 slides for each point and for each control by sampling. MCN frequencies were expressed in MCN/100 tetrads. In order to be considered as a MCN it should: (a) be smaller than one-third of the nucleus, (b) be separated from the nucleus and (c) have a similar color to the nucleus (Grisolia, 2002).

2.7. Statistical analyzes

Data from MCN, chemical parameters and rainfall were submitted to the Shapiro-Wilk normality test. Moreover, homogeneity of variances was assessed by the Levene test.

Subsequently, analysis of variance (ANOVA) was performed and differences between means were compared by the Tukey test, at 5% probability. Biological, chemical and rainfall variables (48 samplings, six variables) were assessed by principal component analysis (PCA). Correlation matrix was performed for PCA analysis, since the variables are estimated by different measurement units. Only eigenvalues higher than 1 were used as criteria for the extraction of principal components. Since the detection limit for DBO_5 was 5.0 mg O_2 L^{-1}, and the concentration of many samples was set as < 5.0 mg O_2 L^{-1}, this parameter was not tested by ANOVA and it was not included in the PCA. Statistical analyzes were performed using SPSS version 22 and PCA plot was created using the Paleontological Statistics Software Package for Education and Data Analysis (PAST) version 3.02 (Hammer et al., 2001).

3. Results

The lowest MCN frequencies were recorded in *Tradescantia pallida* var. *purpurea* flower buds exposed to Caraá water samples (1.57 to 2.47), which were significantly equal to those of their respective negative controls, with the exception of March 2013. The samples of Santo Antônio da Patrulha presented MCN frequencies significantly higher than those of the negative control in eight months. For the two sampling points located in the middle and lower stretches, Taquara and Campo Bom, flower buds demonstrated significantly higher MCN frequencies than those of negative controls in all months (Table 1). An overall mean comparison of MCN frequencies showed that Santo Antônio da Patrulha, Taquara and Campo Bom had significant genotoxicity in relation to Caraá and the negative control (Figure 2).

Throughout the study period, there was significant variation in MCN frequencies recorded in the flower buds exposed to water samples in each sampling point (Table 1). Along the 24 months, there was no statistical difference between the MCN frequencies, both for the negative control (Table 1) and the positive control (MCN frequencies from 4.07 to 5.63; $F = 2.429$; $p = 0.140$).

Chemical characteristics and heavy metal evaluation index (HEI) of water samples used for genotoxicity bioassays are in Table 2 and Figure 2. In 48% of water samples from Rio dos Sinos, BOD_5 showed values higher than 5.0 mg O_2 L^{-1}. All samples of Caraá presented concentrations lower than 5.0 mg O_2 L^{-1} and the highest mean values were observed in Campo Bom. The TP concentrations detected were higher than the value established by CONAMA Resolution 357/2005 for the freshwater class 1 (0.1 mg L^{-1}) in 25% of water samples, The lowest mean values were recorded in Caraá, which differed significantly from those observed in Campo Bom ($F = 3.787$; $p = 0.017$). A gradient of TKN was observed, with the lowest mean concentration in Caraá and Santo Antônio da Patrulha, an intermediate

Table 1. Micronuclei (MCN) frequency in *Tradescantia pallida* var. *purpurea* exposed to Rio dos Sinos water samples of Caraá, Santo Antônio da Patrulha, Taquara and Campo Bom, from May 2012 to March 2014.

Exposures		MCN frequency (mean ± standard deviation)					F	p
		Caraá	Santo Antônio da Patrulha	Taquara	Campo Bom	Negative control		
2012	May	1.57 ± 0.80 a	2.27 ± 0.87 ab	4.67 ± 1.04 c**	5.00 ± 1.05 c**	1.33 ± 0.38 a	40.481	<0.001
	July	2.30 ± 0.64 ab	3.47 ± 0.76 c**	3.70 ± 1.08 bc**	4.70 ± 0.79 bc**	1.93 ± 0.66 a	19.355	<0.001
	September	1.73 ± 0.34 ab	2.53 ± 0.42 abc*	3.57 ± 0.97 ab**	3.70 ± 0.53 ab**	1.60 ± 0.26 a	30.678	<0.001
	November	1.77 ± 0.52 ab	2.43 ± 0.32 abc*	3.27 ± 0.58 ab**	3.47 ± 0.63 ab**	1.57 ± 0.22 a	31.519	<0.001
2013	January	1.57 ± 0.44 a	3.00 ± 1.17 abc*	3.23 ± 0.89 ab**	3.63 ± 1.14 ab**	1.53 ± 0.48 a	12.438	<0.001
	March	2.47 ± 0.42 b**	3.20 ± 0.74 bc**	3.23 ± 0.50 ab**	3.33 ± 0.77 a**	1.33 ± 0.35 a	21.129	<0.001
	May	1.97 ± 0.40 ab	2.56 ± 0.74 abc*	3.20 ± 0.52 ab**	3.37 ± 0.79 a**	1.53 ± 0.52 a	16.317	<0.001
	July	1.87 ± 0.50 ab	2.17 ± 0.90 ab	3.30 ± 0.51 ab**	3.07 ± 1.17 a**	1.43 ± 0.32 a	11.225	<0.001
	September	1.77 ± 0.39 ab	2.30 ± 0.55 ab*	3.13 ± 0.63 ab**	3.17 ± 0.57 a**	1.53 ± 0.39 a	21.370	<0.001
	November	1.83 ± 0.48 ab	2.37 ± 0.51 ab	3.23 ± 0.45 ab**	3.33 ± 1.05 a**	1.70 ± 0.33 a	15.307	<0.001
2014	January	1.97 ± 0.40 ab	2.47 ± 0.48 abc*	3.00 ± 0.61 ab**	3.57 ± 0.54 ab**	1.70 ± 0.37 a	24.238	<0.001
	March	1.63 ± 0.40 a	2.07 ± 0.47 a	2.53 ± 0.53 a*	2.70 ± 0.48 a*	1.80 ± 0.74 a	7.370	<0.001
	F	3.137	3.822	4.823	6.158	1.614		
	p	0.001	<0.001	<0.001	<0.001	0.105		

Means followed by different letters in the same column differ significantly according to the Tukey test (p=0.05). Asterisks indicate significant (*) and highly significant (**) differences between the frequency of MCN recorded for each sample point and the negative control according to the Tukey test (p=0.05).

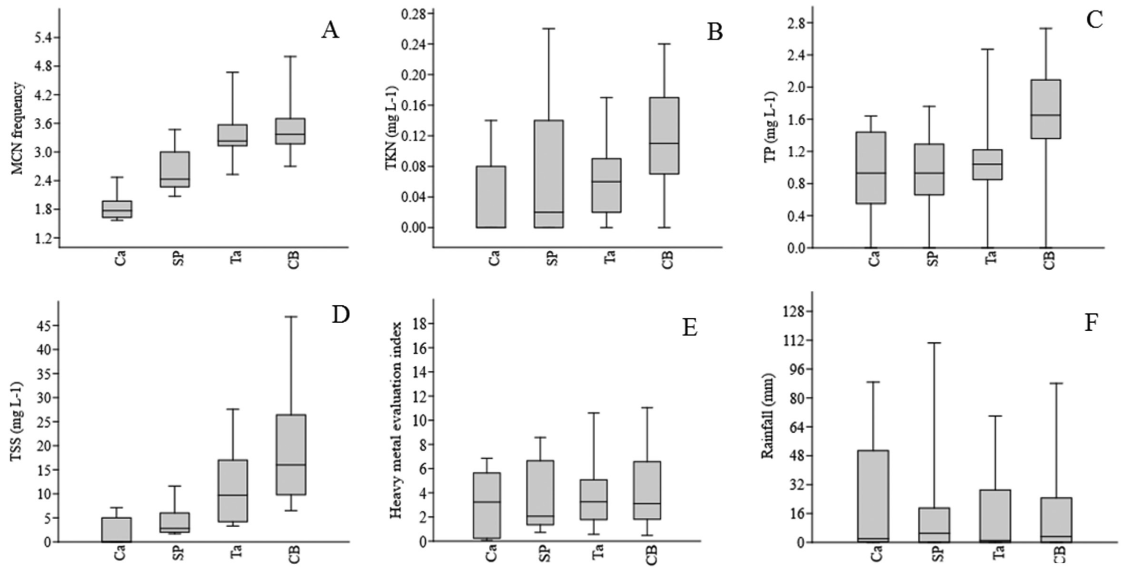

Figure 2. Box plots of MCN frequencies (A) in *Tradescantia pallida* var. *purpurea*, concentrations of total Kjeldahl nitrogen (B), total phosphorus (C) and total suspended solids (D), heavy metal evaluation index (E) of water samples and rainfall (F) at the sampling points of Caraá (Ca), Santo Antônio da Patrulha (SP), Taquara (Ta) and Campo Bom (CB) municipalities.

Table 2. Chemical characteristics and pollution index of Rio dos Sinos water samples and rainfall in the municipalities of Caraá, Santo Antônio da Patrulha, Taquara and Campo Bom, from May 2012 to March 2014.

Exposures		BOD_5 ($mg\ O_2\ L^{-1}$)	TP ($mg\ L^{-1}$)	TKN ($mg\ L^{-1}$)	TSS ($mg\ L^{-1}$)	HEI	Rainfall (mm)
Caraá							
2012	May	< 5.0	nd[1]	0.62	nd	5.64	0.0
	July	< 5.0	0.03	1.09	1.4	0.07	4.1
	September	< 5.0	nd	nd	nd	0.24	86.7
	November	< 5.0	nd	0.93	nd	5.06	1.4
2013	January	< 5.0	nd	1.44	7.1	2.97	88.8
	March	< 5.0	0.14*	1.50	3.9	3.71	50.8
	May	< 5.0	0.04	0.93	nd	6.85	0.3
	July	< 5.0	nd	0.55	5	2.57	0.8
	September	< 5.0	0.08	0.41	nd	3.23	2.0
	November	< 5.0	nd	1.01	5	3.57	7.6
2014	January	< 5.0	nd	1.43	2.1	5.75	0.0
	March	< 5.0	0.1	1.64	nd	0.12	12.5
Santo Antônio da Patrulha							
2012	May	8.0*	nd	0.70	1.7	3.82	0.0
	July	< 5.0	0.02	1.05	2.8	0.73	5.2
	September	< 5.0	0.26*	0.98	9.2	1.36	110.5
	November	< 5.0	nd	1.29	2.7	7.34	0.0
2013	January	< 5.0	0.04	1.76	11.6	1.82	32.0
	March	6.0*	0.14*	1.65	2.9	5.46	19.0
	May	5.0*	0.04	0.93	1.7	8.58	5.0
	July	5.0*	nd	0.66	6	2.02	0.0
	September	7.0*	0.22*	0.63	3	4.31	0.0
	November	5.0*	nd	nd	2.5	2.06	0.0
2014	January	5.0*	nd	1.13	2	6.65	0.0
	March	5.0*	0.05	0.92	3	1.13	0.0

[1]nd: not detected by the analytical method. [2]ni: not informed by Resolution 357/2005 (Brasil, 2005). *Indicates value higher than that established in CONAMA Resolution 357/2005 (Brasil, 2005), for class 1 fresh water.

Table 2. Continued...

Exposures		BOD_5 ($mg\ O_2\ L^{-1}$)	TP ($mg\ L^{-1}$)	TKN ($mg\ L^{-1}$)	TSS ($mg\ L^{-1}$)	HEI	Rainfall (mm)
Taquara							
2012	May	< 5.0	nd	0.85	4.2	4.71	0.0
	July	< 5.0	0.02	nd	9.7	1.78	2.0
	September	< 5.0	0.1	1.18	27.6	0.57	55.0
	November	< 5.0	0.03	1.22	3.5	4.53	0.0
2013	January	< 5.0	0.09	2.40*	19.2	1.74	70.0
	March	< 5.0	0.17*	1.05	13.6	5.07	29.0
	May	5.0*	0.06	0.94	3.3	10.61	1.0
	July	5.0*	nd	1.04	9.8	3.26	0.0
	September	5.0*	0.09	0.82	10.3	3.93	0.0
	November	6.0*	0.08	1.17	7.3	2.88	3.0
2014	January	5.0*	0.02	2.47*	9.5	6.35	0.0
	March	5.0*	0.06	1.01	17	2.10	1.8
Campo Bom							
2012	May	< 5.0	0.17*	2.01	8.7	4.48	0.0
	July	7.0*	0.07	nd	9.8	1.81	1.6
	September	< 5.0	0.11*	1.37	26.4	0.64	88.1
	November	6.0*	0.1	1.36	12	7.68	0.0
2013	January	< 5.0	0.14*	2.73*	46.8	3.11	61.1
	March	10.0*	0.24*	2.10	19.8	5.15	24.6
	May	9.0*	0.21*	2.08	6.5	11.04	3.2
	July	5.0*	nd	1.44	13.3	0.48	0.0
	September	7.0*	0.07	1.26	31	4.48	4.2
	November	6.0*	0.09	1.65	19	2.79	3.9
2014	January	9.0*	0.12*	2.02	25	6.57	0.0
	March	5.0*	0.13*	2.09	16	2.24	4.8
Reference values for class 1 fresh water - CONAMA 357/2005							
		≤ 3.0	0.1	2.18	ni[2]		

[1]nd: not detected by the analytical method. [2]ni: not informed by Resolution 357/2005 (Brasil, 2005). *Indicates value higher than that established in CONAMA Resolution 357/2005 (Brasil, 2005), for class 1 fresh water.

concentration in Taquara, and the highest concentration in Campo Bom ($F = 3.871$; $p = 0.015$). In only 6.2% of the water samples, concentrations were higher than the reference value (2.18 $mg\ L^{-1}$). For TSS, the highest mean values were recorded in Campo Bom and the lowest in Caraá and Santo Antônio da Patrulha, while in Taquara the concentration was intermediate ($F = 15.104$; $p < 0.001$). No legal limit is set for this parameter in CONAMA Resolution 357/2005 (Table 2).

Among the five metals evaluated in the river water samples, only Zn was detected in all samplings, although its concentration was in accordance with the standards established by CONAMA Resolution 357/2005 for class 1 fresh water. Cd concentrations were higher than the reference value (0.001 $mg\ L^{-1}$) established by the CONAMA Resolution 357/2005 for class 1 fresh water in 50% of the samples, with the highest concentration (0.008 $mg\ L^{-1}$) recorded in Taquara and Campo Bom. Lead (Pb) detected concentrations were above the value established by legislation (0.01 $mg\ L^{-1}$) for class 1 fresh water in 58% of the water samples, with the highest concentration (0.039 $mg\ L^{-1}$) recorded in Taquara. Copper (Cu) was over the limit established for class 1 (0.009 $mg\ L^{-1}$) in accordance with the legislation in only 6.2% of the samples, with the highest concentration (0.017 $mg\ L^{-1}$) in Caraá. Total Cr was detected in only two samples of each point, being within the legal limit.

The values for the HEI recorded for the Rio dos Sinos water samples ranged from 0.07 to 6.85 in Caraá, 0.73 to 8.58 in Santo Antônio da Patrulha, from 0.57 to 10.61 in Taquara and 0.48 to 11.04 in Campo Bom (Table 2, Figure 2). Among the 48 water samples evaluated, 95.8% had low degree of heavy metal pollution (HEI < 10) and 4.2% had a medium pollution index (HEI = 10-20). None of the samples demonstrated high degree of heavy metal pollution (HEI > 20). No difference was found for the overall HEI means among the sampling points ($F = 0.235$; $p = 0.872$).

Accumulated rainfall data in the three preceding days and on the day of collection of water samples are shown in Table 2 and Figure 2. Since this variable showed large variances (alterations, discrepancies), no significant differences among municipalities were observed ($F = 0.145$; $p = 0.932$).

PCA analysis indicated the existence of two sets of variables with correlation within each one. These two components explained 62.3% of the total variance. The first principal component explained 38.304% of the variance. The variables that showed greater correlation with this component were MCN, TP, TKN and TSS. These variables correlated positively with each other. The second principal component explained 24.006% of the variance, and the two variables that composed this component, rainfall and HEI, correlated negatively with each other. The biplot graph (Figure 3) based on sampling points and variables assessed showed a spatial separation of the four studied municipalities.

4. Discussion

Water from all monitored sampling points caused significant genetic damage in the meiotic cells of *Tradescantia pallida* var. *purpurea*. A gradual increase was observed in MCN frequencies, from the sampling point near the river source in Caraá, to the lowest stretch point in Campo Bom, which indicates a potentially genotoxic pollution gradient along the river.

MCN frequency of the flower buds exposed to water from Caraá were statistically equivalent to those of the respective negative controls in most samples, suggesting the absence of genotoxic agents. MCN frequencies of up to 2.0 are considered as a basal rate, since they may occur by spontaneous mutations even when the plants are kept in non-polluted environment (Pereira et al., 2013). High frequencies of MCN recorded in the positive control showed the genotoxic potential of formaldehyde in *Tradescantia pallida* var. *purpurea*, which demonstrated the response of plants to the agent and the efficiency of the method used, as previously observed in Trad-MCN bioassays for domestic effluents (Thewes et al., 2011).

As observed in this study, genetic damage in *Tradescantia pallida* var. *purpurea* was also reported by Umbuzeiro et al. (2007) for Cristais River water in the metropolitan region of São Paulo (São Paulo, Brazil). A frequency of 1.87 MCN was recorded in a tributary of the river, considered as a reference point, and 6.22 MCN were recorded at the river point downstream from a textile industry. In the Rio dos Sinos basin, Endres Júnior et al. (2015) observed MCN frequencies between 1.30 and 6.48 in flower buds exposed to water samples from Vila Kunz stream, located in the municipality of Novo Hamburgo and MCN ranging from 1.19 to 1.62 in the negative control. In a study integrating the Trad-MCN bioassay and physicochemical parameters,

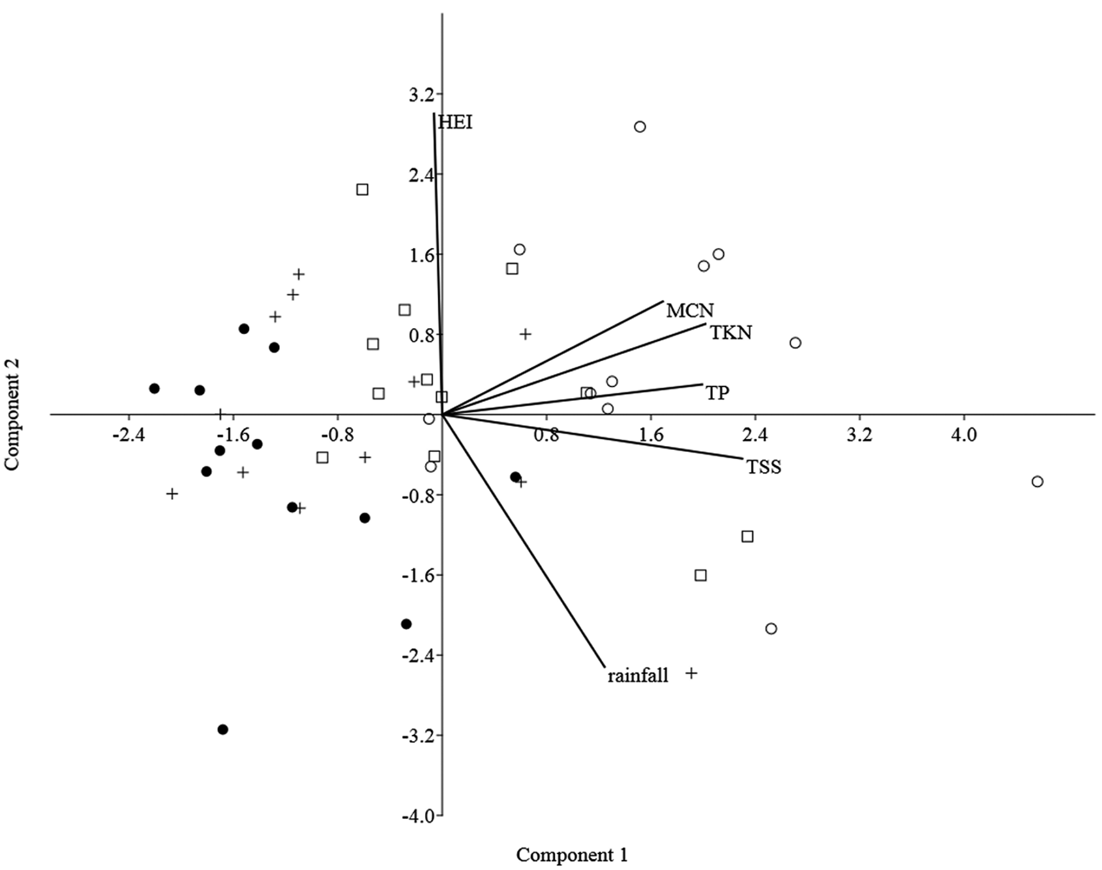

Figure 3. Principal analysis component biplot graph for the two components' scores of the studied sampling points (Caraá: dot, Santo Antônio da Patrulha: plus, Taquara: square, Campo Bom: circle).

Kieling-Rubio et al. (2015) reported MCN frequencies between 1.71 and 3.85 in inflorescences exposed to water samples from the sources of the Estancia Velha/Portão, Pampa and Schmidt streams, located in the lower stretch of the Rio dos Sinos basin, and between 1.50 and 2.00 in the negative control.

The gradient observed for genetic damage in *Tradescantia pallida* var. *purpurea* was also demonstrated for the chemical parameters and heavy metal index evaluated. In general, lower mean concentration of chemicals and therefore, smaller values for HEI, were recorded in Caraá water samples and the highest concentrations were observed for Campo Bom, where most of the chemical parameters values were higher than the standards established in CONAMA Resolution 357/2005 for class 1 fresh water (Brasil, 2005). The data of chemical analyzes corroborate with the water quality evaluation of the Rio dos Sinos performed by Blume et al. (2010) and Dalla Vecchia et al. (2015), which evidenced the highest pollutant concentration at points located in the lower stretch.

In the upper stretch of the Rio dos Sinos the largest pollutant contribution probably occurs by diffuse sources at the points assessed, since there is a low population density in Caraá and in Santo Antônio da Patrulha, of 24.8 and 37.8 inhabitants/Km^2, respectively, and virtually an agricultural economy (IBGE, 2015) that is mainly characterized by irrigated rice cultivation (Panizzon et al., 2013). In the 2012/2013 season, 13,503 metric tons of rice were produced in Santo Antônio da Patrulha, 9.5 times the amount produced in Caraá (IRGA, 2015), which implies the use of agricultural inputs (Panizzon et al., 2013). In turn, in Taquara, with 119.35 inhabitants/Km^2, and in Campo Bom, with 992.79 inhabitants/Km^2 and intense industrialization (IBGE, 2015), pollution of river water may be mostly related to punctual sources, mainly from the discharge of domestic and industrial effluents (Blume et al., 2010; Nunes et al., 2011). In the Rio dos Sinos basin, the contribution of domestic sewage to the degradation of the main watercourse is significant; since only about 5% of the sewage is treated (COMITESINOS, 2015) and no data are available for the four studied municipalities. Further, several tributaries of the Rio dos Sinos in the lower stretch contribute with organic pollution (Costa et al., 2014; Kieling-Rubio et al., 2015). Especially in Campo Bom, the data indicated that water quality was poor, since high concentrations of DBO_5, NTK and TP parameters were found. NTK is the predominant nitrogen form existent in untreated domestic sewage. The presence of PT in water is also mainly due to sewage discharges, with fecal organic matter and powder detergents domestically used in large-scale being the main source (Sperling, 2005). The DBO_5 was not a representative parameter, since the detection limit was higher than the maximum concentration allowed by law for freshwater class 1, and comparison among samples was not viable.

Previous studies evaluating the Rio dos Sinos water quality have shown high concentrations of pollutants and toxic or genotoxic effects in indicator organisms (Nunes et al., 2011; Costa et al., 2014), though the association between the data obtained from the bioassays and the chemical factors has not been proven. In the present study, by applying the principal component analysis we obtained more information about the grouping of the evaluated parameters and their importance, as this method permits the explanation of the variance of a large number of variables that contribute to water quality diagnosis (Zhang et al., 2009). Variables that correlate with their respective main components at levels of 0.75 or higher contribute significantly to the variation in water quality (Pejman et al., 2009). Considering this, all variables included in the PCA demonstrate importance for the difference among the sampling points.

As regards the two principal components, the distribution interpretation was based on Keho (2012) and indicated the grouping of samples of each municipality. Thus, Caraá samples, except for one, showed lower values than the overall mean for MCN, TP, TKN and TSS, which composed the first component. For Santo Antônio da Patrulha, some samples presented mean values, but for Taquara this tendency was more evident. A significant number of Campo Bom samples showed higher values than the mean for these parameters.

5. Conclusion

The spatial pattern of water quality observed in this study support the hypothesis that there is a reduction in the quality and an increase in the genotoxicity of river water from the upper to the lower stretch of the Rio dos Sinos. The MCN frequency, total Kjeldahl nitrogen, total phosphorus and total suspended solids were parameters able to point out the peculiar conditions of each assessed environment. Consider systemically the factors which affect the environmental scenario in an integrated way is relevant for the efficient management of water resources.

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