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Environment

Temporal analysis of genotoxicity and environmental factors related to water quality of a watershed in South Brazil

Análise temporal da genotoxicidade e fatores ambientais relacionados à qualidade da água de uma bacia hidrográfica no Sul do Brasil

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ABSTRACT

Environmental pollution is one of the main problems underlying the current scenario of degradation of aquatic ecosystems. This study aimed to determine the water quality of the Rio dos Sinos watershed in South Brazil by analyzing genotoxicity and environmental factors of four sub-basins in two periods (P1 = 2012 – 2013 and P2 = 2018 – 2019). Water genotoxicity was analyzed using the micronucleus test with Tradescantia pallida var. purpurea. In parallel, a Rapid Habitat Assessment Protocol (RHAP) was employed and data on physico-chemical parameters, land use and occupation and the number of inhabitants were collected and examined for possible relationships with genotoxicity. Genotoxicity did not differ significantly over time for three of the four studied sub-basins. There was a significant relationship between water genotoxicity and number of inhabitants. Furthermore, environments with high genotoxicity, low water quality and high environmental degradation in the first monitoring period remained in the same condition in the second monitoring period; the most preserved areas of the basin may come to resemble these environments in the future

Keywords: Water pollution; Environmental monitoring; Land use

RESUMO

A poluição ambiental é um dos principais problemas relacionados ao atual cenário de degradação dos ecossistemas aquáticos. O objetivo deste estudo foi determinar a qualidade da água da Bacia Hidrográfica do Rio dos Sinos, no sul do Brasil, por meio da análise da genotoxicidade e de fatores ambientais integrados de quatro sub-bacias, em dois períodos bianuais de monitoramento (P1 = 2012 - 2013 e P2 = 2018-2019). A genotoxicidade da água foi avaliada por meio do teste de micronúcleos em Tradescantia pallida var. purpurea. Em paralelo, um protocolo de avaliação rápida de habitats (PARH) foi realizado, análises físico-químicas, dados de uso e ocupação do solo e do número de habitantes também foram coletados e examinados para possíveis relações com a genotoxicidade. Não houve diferença significativa na genotoxicidade ao longo do tempo para três das quatro sub-bacias estudadas. Houve relação significativa entre a genotoxicidade da água e o número de habitantes. Ambientes que apresentavam alta genotoxicidade, baixa qualidade hídrica e alta degradação ambiental no primeiro período, se mantiveram na mesma condição no segundo período e futuramente as áreas mais preservadas da bacia podem vir a se assemelhar com estes ambientes.

Palavras-chave: Poluição hídrica; Monitoramento ambiental; Uso do solo

1 INTRODUCTION

Anthropogenic activities, such as the disorderly growth of urban centers, industrialization and the inadequate disposal of domestic and industrial effluents into watercourses, are the main factors responsible for poor water quality (FOLLMAN *et al* 2018; MENDES *et al* 2020; VISCARDI *et al* 2020). In addition, changes in land use and occupation, such as riparian forest fragmentation, wetland alteration and agricultural expansion with large areas of monoculture and pastures, associated with the indiscriminate use of agrochemicals, also have great impacts on water systems (MERLO *et al* 2011; KIELING-RUBIO *et al* 2015; BIANCHI *et al* 2017). These environmental losses compromise the health of ecosystems and all organisms at various levels of biological organization, from molecular to community, including humans (ALVES *et al* 2014; PAUL *et al* 2021).

In Brazil, water quality monitoring is carried out by conventional analyses determined by Resolution 357/2005 of the National Council for the Environment (Conselho Nacional de Meio Ambiente, CONAMA), which classifies water based on physico-chemical and microbiological parameters (BRASIL, 2005). However, this guideline does not consider toxic and genotoxic effects as criteria in assessing the quality of freshwater, and so these factors remain little explored for the assessment of environmental risk within the country (MENDES *et al* 2020). Biomonitoring is an important tool for evaluating the environmental impacts caused by toxic substances released into the environment as it allows an integrated analysis of ecosystem quality (MERLO *et al* 2011; GUAN *et al* 2017; IYER; BHOLAY, 2015). Watersheds are considered environmental management and planning units in Brazil (BRASIL, 1997; SILVA; VIEIRA; THALÊS, 2021).

The Rio dos Sinos Watershed, located in South Brazil and represented here by its main water resource, the Rio dos Sinos, presents a scenario of water quality degradation due to the urban and economic model prevailing in the municipalities that comprise its sub-basins (FIGUEIREDO *et al* 2010; DALLA VECCHIA *et al* 2015; CASSANEGO; DROSTE, 2017; SIMÕES *et al* 2019). Although the Rio dos Sinos serves as a source of supply for around 1,375,000 inhabitants (IBGE, 2020), only 4.5% of all sewage generated in the basin is treated, resulting in low water quality in stretches exposed to high anthropization (RODRIGUES et al 2015).

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Although there is information on the physico-chemical and biotic components of Brazilian watersheds (ALVES *et al* 2018; CURADO *et al* 2017; NOBREGA; SOUZA; MEDEIROS, 2019), there remains a lack of long-term data that could enable an understanding of the gradual changes that take place in these important ecosystems. The present study provides biomonitoring data for the Rio dos Sinos watershed to perform a temporal analysis of water genotoxicity in relation to land use and occupation, number of inhabitants, and habitation quality, as determined by a rapid assessment protocol, for four sub-basins in two monitoring periods.

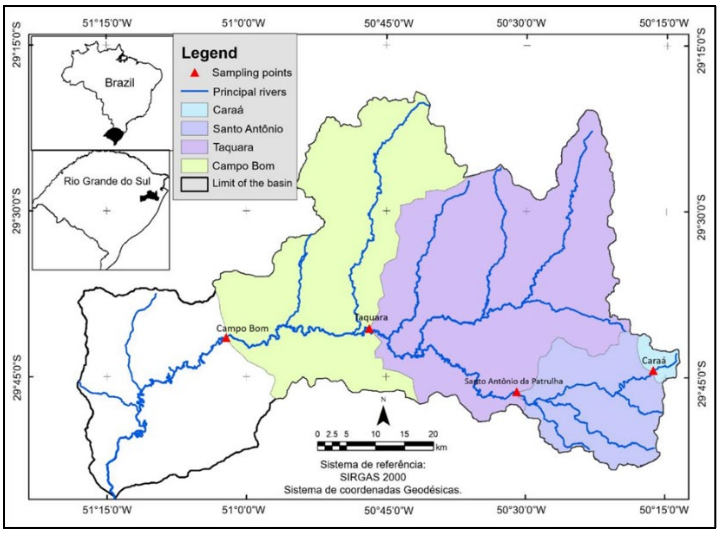
2 MATERIAL AND METHODS

2.1 Study area and sampling points

The study was carried out in the Rio dos Sinos watershed (RSHB), located in the state of Rio Grande do Sul, South Brazil (29º20' to 30º10'S, 50º15' to 51º15'W) (Fig. 1). RSHB encompasses approximately 3,693 km², with around 94% of the population living in urban areas, and is distributed in 30 municipalities, where developed economic activities range from agriculture and livestock to intense industrialization (COMITESINOS, 2017; FEPAM, 2020; SEMA, 2020). Biomonitoring covered a distance of approximately 77 km along the Rio dos Sinos and occurred at four sampling points, one in each incremental sub-basin.

(Fig.1). The first point is located close to the river's source (29°44'20.7" S, 50°16'18.3" W) in the Caraá sub-basin, which has an estimated population of 8,270 inhabitants distributed among 295 km² with a predominantly agricultural economy (IBGE, 2020; FEE, 2019).

Figure 1 Location of the sampling points in the incremental sub-basins of the Rio dos Sinos Watershed



Source: Authors (2021)

The second point is located in the Santo Antônio da Patrulha sub-basin (29º46'19.7" S, 50º30'57.3" W), which covers an area of 1,879 km² and has an estimated population of 77,572 and an agriculture-based economy (IBGE, 2020; FEE, 2019). The third point is located in the Taquara sub-basin (29º40'38.9" S, 50º46'48.1" W), which encompasses an area of 1,515 km² and has about 64,560 inhabitants and a diversified economy, based on footwear metallurgical industries and small-scale agricultural activities (IBGE, 2020; FEE, 2019). The fourth point is located in the Campo Bom sub-basin (29º41'29.7" S, 51º02'11.1" W), which encompasses an area of 706 km² and is predominantly urbanized, with an estimated population of 296,920 inhabitants and a diversified economy, based on shoe leather and small-scale agricultural industries (IBGE, 2020; FEE, 2019).

2.2 Water collection

Water samples were collected at the four sampling points of the RSHB on the same day (Fig. 1) and immediately sent to the laboratory for genotoxicity bioassays and physico-chemical analyses. Two monitoring campaigns were carried out, one from May 2012 to March 2013 (P1), (CASSANEGO; DROSTE, 2017) and the other from September 2018 to September 2019 (P2). The physico-chemical analyses followed Standard Methods for the Examination of Water and Wastewater (APHA; AWWA; WPCF, 2012).

2.3 Water genotoxicity

Water genotoxicity was determined by micronucleus test with Tradescantia tetrads (Trad-MCN). Twenty inflorescences of Tradescantia pallida var. purpurea (10 to 15 cm long) containing pre-anthesis flower buds were used for each treatment (river water and negative control). The inflorescences remained partially submerged in distilled water for an adaptation period for 24 h. The inflorescences were then exposed for 8 h to water samples, from the four sampling points of RSHB and for each monitoring period, simultaneously with a negative control of only distilled water. After a recovery period of 24 h (CASSANEGO *et al* 2014), the flower buds were fixed in a solution of absolute ethanol: acetic acid (3:1 v:v) for 24 h and then stored in 70% ethyl alcohol under refrigeration (4ºC). Slides for the analysis of tetrads were prepared by dissecting the flower buds and macerating the anthers with 1% acetic carmine.

Three hundred tetrads per each of ten slides were analyzed for each sampling point, for a total of 75,000 tetrads per sampling campaign and 150,000 throughout the entire study. Micronuclei (MCN) were observed under optical microscopy at 400x (Olympus CX4) and frequencies expressed as MCN/100 tetrads (THEWES *et al* 2011).

2.4 Water physico-chemical analyses

The analyzed physico-chemical variables were: biochemical oxygen demand (BOD^5), total phosphorus (TP), total nitrogen (TN), total suspended solids (TSS) and the metals cadmium (Cd), lead (Pb), copper (Cu), total chromium (Cr) and zinc (Zn). Table 1. Values for the physico-chemical variables were compared with limits established by Resolution 357 of the Conselho Nacional de Meio Ambiente (CONAMA) (BRAZIL, 2005).

Table 1 Analytical methods used to assess water physico-chemical parameters and the respective detection limits

Parameter	**LD (mg L^{-1})**	**Method [1]**	**Principle of method**
BOD_5	1.000	SM 5210 D	Respirometry
Total Phosphorus	0.016	SM 4500 P D	UV
Total Nitrogen	1.390	SM 4500 Norg	Acid digestion followed by titration
Suspended Solids	0.900	SM 2540 D	Gravimetry
Cadmium	0.001	SM 3110 B	Atomic absorption
Lead	0.032	SM 3110 B	Atomic absorption
Copper	0.004	SM 3110 B	Atomic absorption
Chromium	0.057	SM 3110 D	Atomic absorption
Zinc	0.003	SM 3110 B	Atomic absorption

Source: Authors (2021)
Legend: 1SM = Standard Methods for the Examination of Water and Wastewater (APHA, 2012)

The analytical methods used to assess the water physico-chemical characteristics and the detection limit considered for each variable are listed in Table 1. Values for the physico-chemical variables were compared with limits established by Resolution 357 of the Conselho Nacional de Meio Ambiente (CONAMA) (BRAZIL, 2005).

2.5 Land use and occupation

The delimitation and definition of land use classes was done using the database provided by the Projeto Mapbiomas - Projeto Brasileiro de Mapeamento Anual de Uso e Cobertura do Solo, based on Landsat satellite images (MAPBIOMAS, 2020). Satellite images were transformed into percentage area, for each monitoring period, for each of the following land use classes: mosaic agriculture, monoculture agriculture, wetlands, native forest or forest, native grassland, pasture, forestry, exposed soil and urban sprawl. The QGIS® program (DEVELOPMENT TEAM, 2016) was used to delimit the RHB, as well as its incremental sub-basins.

2.6 Rapid Habitat Assessment Protocol (RHAP) and inhabitation

A Rapid Habitat Assessment Protocol (RHAP), adapted from Callisto *et al* (2002), was applied. Twenty variables were analyzed for the characterization of environments according to: type of bank occupation; bank erosion and bed siltation; anthropic changes; vegetation cover over the river bed; water odor; water oiliness; water transparency; sediment odor; bottom oiliness; bottom type; habitat diversity; substrate types; mud deposit; sedimentary deposits; river channel alteration; water flow characteristics; riparian forest presence; stability of margins; riparian forest extension; and presence of aquatic plants. Each sampling point received a value from 0 to 100 and the results were converted based on the scale proposed by Callisto *et al* (2002), where a sum of 0 to 40 points indicates an impacted environment; 41 to 60 points an altered environment; and 61 to 100 points a natural environment. The absolute number of inhabitants of each municipality in the four analyzed sub-basins was acquired from the IBGE (2020) and based on studies by Moraes *et al* (2018).

2.7 Statistical analysis

Data of MCN frequencies met the assumptions of normality (KolmogorovSmirnov test), and so data for P1 and P2 were compared using Student's t-test. The relationship between mean MCN frequency and number of inhabitants was evaluated by transforming the values into natural logarithms [(ln (x + 1)] and applying the Pearson correlation coefficient. These statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 25 (SPSS Inc., Chicago, IL, USA), with a significance level set at 5%. A cluster analysis was performed to evaluate the dissimilarity among the sampling points and between P1 and P2, recorded as a function of physico-chemical and genotoxicity variables. Grouping was performed by hierarchical cluster analysis, considering Euclidean distance and the standardization of variables by the Ward method, in the BioEstat version 5.3 program (AYRES *et al* 2007).

3 RESULTS AND DISCUSSION

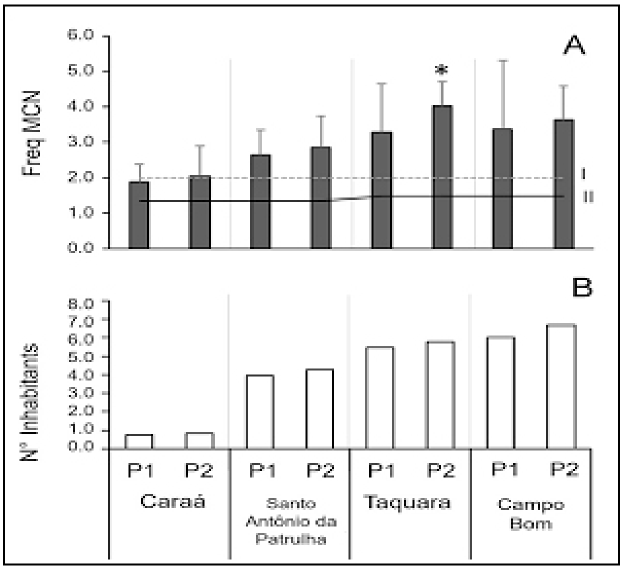
3.1 Water Genotoxicity

Genotoxicity of the water samples from the RSHB did not differ between monitoring periods for Caraá (t=-1.069; p=0.287), Santo Antônio da Patrulha (t=-0.903; p=0.369) and Campo Bom (t=-1.243; p=0.287), while P2 had greater toxicity than P1 (t=-3.144; p=0.002) at Taquara (Fig. 2A). The negative control did not differ significantly between monitoring periods (t=-1.134; p=0.261). These results give credibility to the Trad-MCN bioassay and show that the water of the RSHB remained genotoxic over the two monitoring periods for sampling points with different environmental characteristics. Except for Caraá, the genotoxicity for the sampling points of the RSHB were significantly higher than that of the negative control, with the highest being for Taquara (P1: t=13.20; p<0.001, P2: t=11.632; p<0.001), followed by Campo Bom (P1: t=12.401; p<0.001, P2: t=12.671; p<0.001) (Fig. 2A, II).

The results obtained in P2 corroborate those of Cassanego and Droste (2017), who observed changes related to water quality along the longitudinal gradient of the RSHB, from the source of the Rio dos Sinos in the municipality of Caraá to Campo Bom located in the lower section of the river. The environmental toxicity of freshwater can be observed in plant and even animal species at different trophic levels, from aquatic macroinvertebrates to fish, when exposed to contaminated water from a given watershed (OLIVEIRA *et al* 2012; SIMÕES *et al* 2019; DOMINGUES *et al* 2021). From campaigns carried out between 2007 and 2008, Bieger *et al* (2010) evaluated the water quality of tributaries of the RSHB through biotic indices and aquatic macroinvertebrates and observed changes related to water quality along the longitudinal gradient of the basin, corroborating Cassanego and Droste (2017) and the data of the present study. This gradient towards the mouth of the river corresponds to a series of environmental characteristics that cause the dynamics of degradation related to toxicity to change in space but not over time.

There was a population increase between monitoring periods of approximately 10.53% in the municipalities that make up the studied sub-basins, and a positive relationship was found between genotoxicity and number of inhabitants in P1 ($r = 0.806$; $p<0.001$) and in P2 ($r = 0.598$; $p = 0.005$). These results highlight the negative impact that urban occupation has on water quality (ROCHA *et al* 2018; DOMINGUES *et al* 2021) and may reflect observed changes in land use and occupation.

Figure 2 Frequency of micronuclei (MCN) in T. pallida var. purpurea (A) and number of inhabitants (x 104) (B)



Source: Authors (2021); 2Pereira et al (2012)

Legend: Freq MCN: Frequency of micronuclei; *indicates a significant difference between monitoring periods; I = basal rate of MCN accepted for species2 and II = negative control; No. inhabitants = number of inhabitants (x 104)

The lower genotoxicity observed in flower buds exposed to water samples collected in Caraá was related to local environmental conditions. This section of the RSHB has the lowest population density and a more conserved environment, without apparent urban sprawl, as evidenced by the maintenance or permanence of native forest areas, when compared to the other sampling points. The HARPH results corroborate the findings of Rocha-Uriartt *et al* (2015), who also observed an environment with low environmental degradation near the sources of the Rio dos Sinos. Thus, even though the Caraá sub-basin had an increase in population, there were no differences in the structure of the municipalities regarding the types of activities developed there, (IBGE, 2020) so that the region does not have industries and other activities that decrease not only the quantity, but also the quality, of effluents discarded in the river waters.

3.2 Physico-chemical parameters

The analysis of the water quality parameters revealed that 52% of them were above the limit established by legislation in the first monitoring period P1 (2012 – 2013) and 36% were still high and above the limit in the second monitoring period P2 (2018 – 2019) (Table 2). We highlight the presence of toxic metals, such as cadmium and lead, which were detected in at least one sampling at each sampling point. These substances have low biodegradability and biosolubility in lipid tissue and, thus, accumulate in animals as they advance in the food chain (MENDES et al 2020). However, toxic metals have been present in RSHB for many years. Vargas et al (2001) had already identified metals in RSHB sediments during campaigns between 1992 and 1994, including chromium, lead, copper, zinc and nickel, many of which are still detected today (BIANCHI et al 2019; SIMÕES et al 2019; VISCARDI et al 2020).

Table 2 (A) Minimum and maximum values for the water quality parameters of four sampling points in the Rio dos Sinos watershed in the monitoring periods P1 (2012 – 2013) and P2 (2018 – 2019)

Sampling points					
		Caraá		Santo Antônio da Patrulha	
Parameter	**Unit**	**P1**	**P2**	**P1**	**P2**
BOD_5	mg O_2 L^{-1}	<1.000/<5.0	*nd/<1.0	<5.000/8.000	nd/1.0
TP	mg L^{-1}	nd/0.14	0.016/0.085	nd/0.260	0.052/0.083
NTK	mg N in NH3 L-1	nd/1.64	nd/1.39	0.660/1.760	nd/1.390
TSS	mg L^{-1}	nd/7.1	0.9/5.2	1.700/11.600	3.500/7.900
Cd	mg L^{-1}	nd/0.005	0.001/0.006	nd/0.005	0.001/0.005
Pb	mg L^{-1}	nd/0.005	0.001/0.006	nd/0.031	nd/0.032
Cu	mg L^{-1}	nd/0.017	nd/0.004	nd/0.006	nd/0.004
Cr	mg L^{-1}	nd/0.006	nd/0.057	nd/0.080	nd/0.057
Zn	mg L^{-1}	0.003/0.043	nd/0.003	0.010/0.024	0.003/0.012

Table 2 (B) Minimum and maximum values for the water quality parameters of four sampling points in the Rio dos Sinos watershed in the monitoring periods P1 (2012 – 2013) and P2 (2018 – 2019)

Sampling points					
	Taquara		Campo Bom		CONAMA
Parameter	**P1**	**P2**	**P1**	**P2**	**357/2005**
BOD_5	<5.000/6.000	nd/1.000	<5.000/10.000	nd/1.000	5
TP	nd/0.170	0.047/0.105	nd/0.240	0.035/0.150	0.1
NTK	nd/2.400	nd/1.390	nd/2.730	nd/1.390	2.18
TSS	3.300/27.600	4.000/18.900	6.500/46.800	0.900/51.700	ni
Cd	nd/0.008	0.001/0.006	nd/0.008	0.001/0.000	0.001
Pb	nd/0.039	nd/0.032	nd/0.034	nd/0.030	0.01
Cu	nd/0.0014	nd/0.004	nd/0.009	nd/0.004	0.009
Cr	0.007/0.025	nd/0.057	nd/0.008	nd/0.050	0.05
Zn	0.003/0.028	0.003/0.010	0.007/0.038	nd/0.003	0.18

Source: Authors (2021)

Legend: *nd = not detected by the analytical method; P1 = monitoring period 1; P2 = monitoring period 2; Bold = values above the limit established by CONAMA 357/2005 (BRAZIL, 2005) for freshwater classes I and II

In addition, RSHB is also impacted by a high organic load dumped into the river, which reflects the lack of sewage treatment (SEMA, 2020; SNIS, 2019), and contamination by a series of other potentially toxic and genotoxic pollutants (OLIVEIRA *et al* 2012). In a study carried out in RSHB during the same period as P1 (2012 – 2013), Bianchi *et al* (2017) detected the presence of 24 chemicals, including pesticides and hydrocarbons.

Although the concentrations of the detected agrochemicals were below the limit established by Brazilian legislation, the bioassays of the aforementioned study indicated the presence of toxic and genotoxic substances in the water at all monitored points. These results emphasize the importance of biomonitoring water resources in a manner that includes biological tools that can provide answers regarding the effects that these pollutants can have on exposed individuals, including humans (GUAN *et al* 2017).

3.3 Land use and occupation

Data on land use and occupation show that the RSHB underwent a series of changes during biomonitoring because of the degree of anthropogenic transformation to which it was subjected. We highlight the expansion of

agricultural frontiers, with the replacement of areas planted with temporary crops, called mosaic agriculture, with monoculture (rice cultivation) and pastures. Furthermore, the Taquara sub-basin experienced a disappearance of wetlands that were present in P1 and no longer detected in P2 (Table 3). This loss was probably linked to increased urban occupation, which in turn can also explain the increase in water genotoxicity observed in P2, thus reflecting deterioration of the environmental quality of this sub-basin over time.

The RSHB is located in an area of transition between the Atlantic Forest and Pampa biomes, and studies have indicated that the main changes in land use and occupation, due to the disorderly process of occupation of the basin, that most contribute to the loss of natural vegetation and other environmental losses are expansion of urban areas and agricultural and forestry activities (COSTA *et al* 2017; SOUZA JUNIOR *et al* 2020).

Table 3 (A) Changes in land use and occupation in the incremental area of four subbasins of RSHB (%) in the monitoring periods P1 (2012 – 2013) and P2 (2018 – 2019)

Classes	Caraá			Santo Antônio da Patrulha		
	2012	2019	Variation	2012	2019	Variation
Agricultural Mosaic	9.32	4.55	-3.62	9.32	14.87	-4.94
Monoculture	0.09	0.18	0.12	0.09	2.50	0.42
Water	NA	NA	NA	NA	0.02	0.00
Wet areas	NA	NA	NA	NA	NA	NA
Forest	80.60	81.33	0.69	80.6	61.81	-0.18
Native grassland	NA	NA	NA	NA	NA	NA
Pasture	9.77	13.70	4.07	9.77	17.01	3.70
Silviculture	0.19	0.25	0.04	0.19	3.58	0.85
Exposed soil	NA	NA	NA	0.09	0.11	0.06
Urban area	NA	NA	NA	0.02	0.04	0.02

Table 3 (B) Changes in land use and occupation in the incremental area of four subbasins of RSHB (%) in the monitoring periods P1 (2012 – 2013) and P2 (2018 – 2019)

Classes	Taquara			Campo Bom		
	2012	2019	Variation	2012	2019	Variation
Agricultural Mosaic	17.80	11.94	-4.28	18.60	13.00	-4.15
Monoculture	2.51	3.34	0.62	1.70	2.40	0.54
Water	0.06	0.06	0.00	0.11	0.10	0.00
Wet areas	1.68	NA	NA	0.06	0.00	-0.02
Forest	62.80	62.00	-0.52	61.8	61.00	-0.41
Native grassland	NA	1.37	-0.18	1.15	0.90	-0.15
Pasture	8.83	13.70	3.43	9.06	13.70	3.15
Silviculture	5.80	7.00	0.85	5.20	6.10	0.65
Exposed soil	0.08	0.12	0.04	0.16	0.20	0.06
Urban area	0.41	0.50	0.07	2.19	2.50	0.26

Source: Authors (2021)
Legend: NA: not applicable

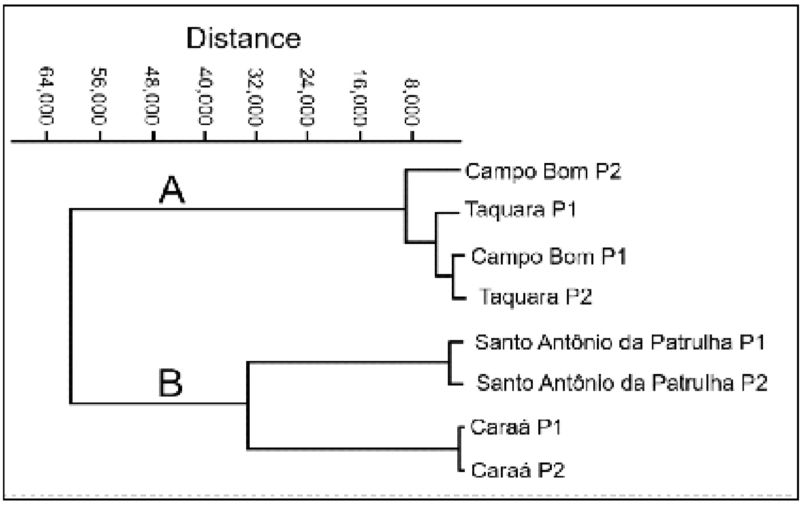
3.4 Cluster analysis

The replacement of areas of vegetation with impermeable spaces causes an increase in the volume of water that flows during the rainy season, which generates overflowing and flooding in stretches with high urbanization. In addition, the suppression of riparian forest contributes to the loss of water quality because increased erosion favors the carrying of disaggregated soil particles and, consequently, siltation downstream of the watercourse, as well as leaching of a great variety of substances (REIS *et al* 2015; DOMINGUES *et al* 2021; SILVA; VIEIRA; THALÊS, 2021). In addition, the cultivation of irrigated rice, as is the case in the upper section of the basin where the economy is basically agricultural (IBGE, 2020), makes use of inputs such as fertilizers, pesticides and herbicides, which in turn reach watercourses by runoff or percolation and generate a contamination gradient throughout the basin (PANIZZON *et al* 2012; PAUL *et al* 2021).

The dendrogram generated based on genotoxicity and physico-chemical water-quality variables for RSHB revealed two groups (A and B) with about 60% dissimilarity. Group A contains the smpling points of Taquara and Campo Bom subbasins for the two monitoring periods, respectively. The data from these environments intertwine, with the water quality of Taquara in P2 being closer to the water quality of Campo Bom in P1 than to that of Taquara in P1. Thus, these two environments/monitoring periods are distant from Taquara P1, the three of which form a group distant from Campo Bom P2 (Fig. 4). Cluster B is formed by the sampling points that make up the upper section of the basin, which form two subgroups, one of Caraá P1 and P2 and one of Santo Antônio da Patrulha P1 and P2.

The findings presented here are consistent with studies that indicate a gradual deterioration of the RSHB, including in the most preserved areas, so that in a few years the water quality in the Caraá sub-basin may be more similar to that of Santo Antônio da Patrulha, which will possibly tend to approximate the characteristics of the water of Taquara (BLUME *et al* 2010; BARBOSA *et al* 2020).

Figure 4 Dendrogram resulting from cluster analysis based on the mean values of physico-chemical parameters and genotoxicity of water of the sampling points of RSHB (Caraá, Santo Antônio da Patrulha, Taquara and Campo Bom), in the monitoring periods of P1 (2012 –2013) and P2 (2018 – 2019)



Source: Authors (2021)

In a study of *Astyanax jacuhiensis* (COPE, 1894) (Characiformes: Characidae) in the same watershed, Bianchi *et al* (2019) found the same spatial pattern of environmental degradation according to differences in water quality influenced by the characteristics of each section of the basin. These spatial differences seem, therefore, to be repeated for other indicator organisms and/or assessment methods. However, long-term monitoring that performs temporal comparisons has been little discussed, which reinforces the importance of the data presented here.

The data presented here corroborate the cited studies in the sense that, despite the observed temporal variation, the water of the RSHB has remained toxic over many years. Nonetheless, this water resource continues to be used for public purposes, such as supplying about 1,375,000 people and serving as a source of recreation and fishing (SEMA, 2020; FEPAM, 2020). Thus, the results confirm the need for urban planning and good agricultural and land use practices in RSHB, in association with technological solutions and environmental education, to confront environmental problems and ensure the multiple uses of the water of this important source (CALLISTO *et al* 2012; COSTA *et al* 2014; BIANCHI *et al* 2019).

4 CONCLUSION

The division of Rio dos Sinos watershed into sub-basins and the understanding of their characteristics can help to determine actions for environmental recovery and effluent treatment in the involved municipalities. The integrated assessment methodology presented here is reliable and representative and facilitates knowing the adverse effects that anthropization has on natural resources. Furthermore, it can be easily applied in integrated environmental diagnoses of other watersheds. In this way, the need for the management of water resources and the contribution of this study, aiming to gradually improve the watershed, is evidenced.

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