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Original Article

Water quality assessment of the Demetrio stream: an affluent of the Gravataí River in the South of Brazil

Qualidade da água do arroio Demetrio: um afluente do rio Gravataí no sul do Brasil

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Abstract

The Gravataí River basin, one of the main water sources of the metropolitan region of Porto Alegre, is among the ten most polluted rivers in Brazil. Water quality is monitored only through physico-chemical and microbiological parameters in Brazil, and in this context, considering the importance of the use of biomarkers in complementing the analysis of water, the present study aimed to evaluate the environmental quality of the main affluent of the Gravataí River, Demetrio stream, through physico-chemical, microbiological, and cytogenotoxic criteria, at the stream source (P1), whereas samples P2 and P3 were obtained from the upstream near the area with the highest urban density and the downstream near the meeting point with the Gravataí River, respectively. The results for copper concentration and color classified the Demetrio stream as Class 4 in general, that is, the water is suitable only for navigation and to landscape harmony. The main genotoxic alterations (micronuclei and nuclear buds) were observed in P2, in which were obtained the highest levels of copper, in addition to iron and manganese. Anthropic interventions were observed in P1 and P2; however, due to its low metal concentration, P3, near the Gravataí River, manifested an improvement in environmental quality.

Keywords: environmental monitoring, cytogenotoxicity, *Allium cepa*, CONAMA resolution nº 357/2005.

Resumo

A bacia do rio Gravataí, uma das principais fontes de água da região metropolitana de Porto Alegre, está entre os dez rios mais poluídos do Brasil. No Brasil a qualidade da água é monitorada apenas através de parâmetros físico-químicos e microbiológicos e, nesse contexto, considerando a importância do uso de biomarcadores para complementar a análise da água, o presente estudo teve como objetivo avaliar a qualidade ambiental do principal afluente do Rio Gravataí, o arroio Demétrio, através de critérios físico-químicos, microbiológicos e citogenotóxicos, na nascente do arroio (P1), a montante e próximo à área com maior densidade urbana (P2) e a jusante e próximo ao ponto de encontro com o rio Gravataí (P3). Os resultados para a cor da água e para a concentração de cobre classificaram o arroio Demétrio como Classe 4 em geral, ou seja, esta água é adequada apenas para navegação e harmonia da paisagem. As principais alterações genotóxicas (micronúcleos e brotos nucleares) foram observadas no P2, no qual foram obtidos os maiores teores de cobre, além de ferro e manganês. Intervenções antrópicas foram observadas em P1 e P2; no entanto, devido à sua baixa concentração de metais, o P3, próximo ao rio Gravataí, manifestou uma melhoria na qualidade ambiental.

Palavras-chave: monitoramento ambiental, citogenotoxicidade, *Allium cepa*, resolução CONAMA nº 357/2005.

1. Introduction

In recent years, human activities have posed serious threats to water resources (Kaliberda et al., 2008). The quality of water for domestic supply is affected by pollution caused by different sources, such as domestic, industrial, urban, and agricultural effluents (Merten and Minella, 2002). The release of sanitary sewage into water bodies severely affects fauna, flora, and humans (Carvalho and Orsine, 2011). According to the National Sanitation Information System - SNIS (Brasil, 2015), although the treatment rate increased by 11% between 2005 and 2015, only 42.7% of all Brazilian sanitary sewage is treated, and in some regions the rate is still less than 10%. The average rate of sewage collection ranged from 20% to 40% in the State of Rio Grande do Sul (RS), Brazil (Brasil, 2016).

In Brazil, polluted rivers water when monitored, its quality can be determined according to the standards established in the Resolution of the National Environmental

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Council (CONAMA) nº 357 (Brasil, 2005). This "water quality" does not refer to the state of purity, it denotes the physicochemical characteristics of water (Merten and Minella, 2002). According to Sperling (2005), water quality of a region is determined by its natural conditions as well as by the use of soil in the river basin. However, the most proper way to understand the health of the ecosystem is to biomonitor the environment because physicochemical measures of water quality can determine contamination sources, but they do not evaluate at biological responses to pollution. One biological monitor that has been an important tool for environmental monitoring studies is the *Allium cepa*. The *A. cepa* test has been used to detect a great variety of environmental pollutants as heavy metals, pesticides, aromatic hydrocarbons, textile dyes, complex mixtures, and the results obtained have been satisfactory in the different studies (Leme and Marin-Morales, 2009; Dalzochio et al., 2016).

Gravataí River is among the ten most polluted rivers in Brazil (Pessoa, 2017), and is located in the metropolitan region of Porto Alegre (RS, Brazil) with an area of approximately 2.020 km^2, providing public water supply to about 500.000 inhabitants in five municipalities (Salomoni et al., 2011). Demetrio stream is one of the main affluent of the Gravataí River basin, covering 12.51% of the total area (Volpi and Jungblut, 1994). Throughout its course, the Demetrio stream runs through rural and urban areas, flowing directly into the river Gravataí, on its right bank (Quevedo et al., 2017). The hydrographical basin of Gravataí river has a relevant social, economic, and cultural importance. The industrial cluster has grown considerably over the past years as has the urban population as well and this generated an increase of pollutants dumped in the river, as a result of farming irrigation, water supply for animals, industrial and domestic sewage, solid residue, urban draining, rural diffusing sources, and atmospheric pollutants precipitation (Salomoni et al., 2011).

In this context, considering the interest to assess the impact of pollutants dispersed in water, as well as the importance of the use of biomarkers to complement the water physicochemical analysis, this study aims to evaluate the environmental quality of the Demetrio stream water, main affluent of Gravataí River, through physical-chemical, microbiological and cytogenotoxic criteria.

2. Materials and Methods

2.1. Demetrio stream water samples

Water samples were collected in December/2016, and all sample points were defined according to their geographical characteristics (Figure 1). Sample point 1 (P1) was collected from near the source (latitude –29.77029 and longitude –50.872087), whereas samples 2 (P2) and 3 (P3) were obtained from the upstream near the area with the highest urban density (latitude –29.919287 and longitude –50.935974) and from the downstream near the meeting point of the Demetrio stream with the Gravataí River (latitude –29.945642 and longitude –50.981018).

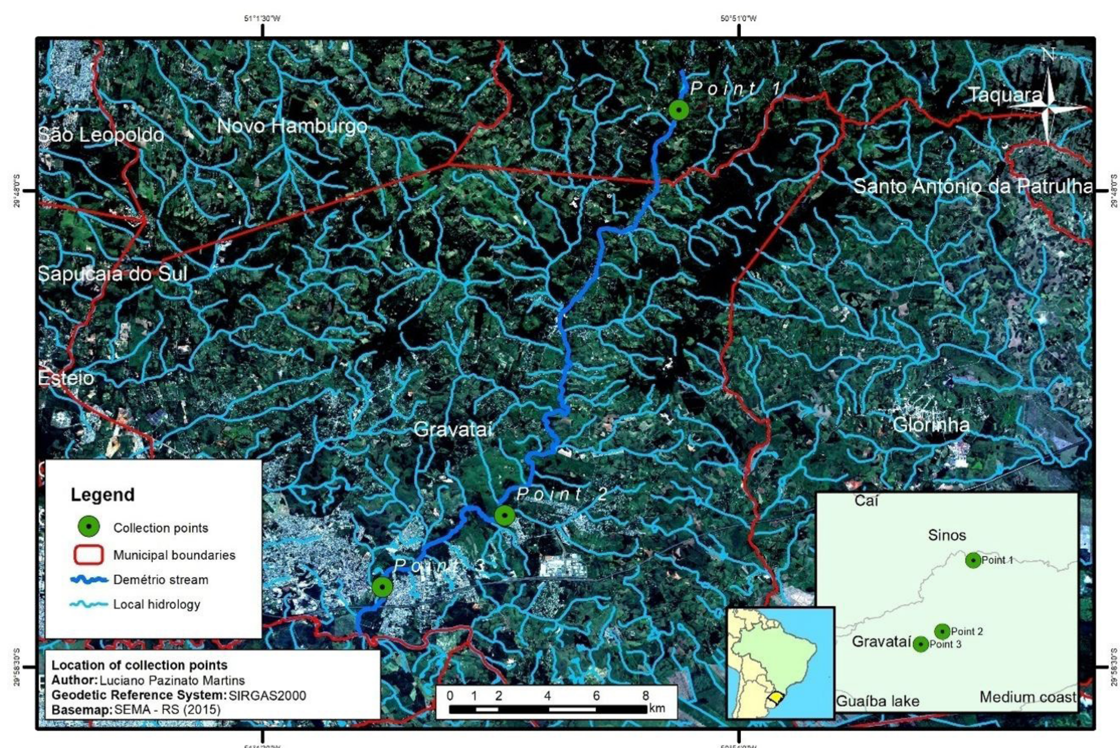

Figure 1. Satellite view of the area and water sampling points of Demétrio stream: point 1 source, point 2 and point 3, upstream near the area with the highest urban density and from the downstream near the meeting point of the Demétrio stream with the Gravataí River.

The collected water samples were stored in 1,000 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, (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.

2.2. Physicochemical and microbiological analysis

The collected water samples were sent to the Laboratory of Environmental Studies and Nanotechnology Development of the University La Salle (Canoas/RS) and analyzed for the physicochemical parameters: chloride, fluoride, nitrate, sulfate, turbidity, and color (APHA, 2005). Temperatures and pH values of the samples were measured at the time of collection (HI 8424 pH/ORP meter- Hanna Instruments). The identification and quantification of dissolved aluminum (Al), cadmium (Cd), lead (Pb), cobalt (Co), dissolved copper (Cu), total chromium (Cr), dissolved iron (Fe), manganese (Mn), mercury (Hg), nickel (Ni), and zinc (Zn) were performed in the Green Lab Laboratory (Porto Alegre/RS). The adopted analytical method was based in Standard Methods for the Examination of Water and Wastewater (APHA, 2005) and detection limits (mg/L) for Al, Cu, Fe and Zn: 0.006, Cd: 0.0006, Pb: 0.004, Co: 0.001, total Cr: 0.003, Mn and Ni: 0.001 and Hg: 0.0002.

From each sample, serial dilutions were made up to the order of 10^{-5} dilution. For the quantification of total and thermotolerant coliforms, the Most Probable Number (MPN) technique was used, and the dilutions were inoculated on a chromogenic substrate Colilert (Idexx) in five sets of five tubes and then incubated for 24 h at 35 ºC. The tubes that changed their colors to yellow were considered as positive for total coliforms. Further, the positive tubes were submitted to ultraviolet light, and the tubes with the emission of fluorescence were considered as positive for thermotolerant coliforms (Edberg et al., 1988).

2.3. Allium cepa test

All assays were carried out only with one kind of *A. cepa* seed (variety Baia Periforme; Isla® Sementes) to avoid different responses in different stages of the process (Caritá and Marin-Morales, 2008). *A. cepa* seeds were exposed to water samples in a Petri dish for each sample point. The control tests were carried out with distilled water (for negative control) and copper sulfate (0.0002 g/L) (for positive control) (Carvalho et al., 2011). After five days of exposure, seed roots were immediately immersed in acetic acid and ethanol (1:3; v/v) for 24 h. Root meristems were washed with distilled water and hydrolyzed in 1 N HCl at 60 °C for 10 min, then washed in distilled water again, dried in filter paper, and finally, exposed to Schiff's reagent in the dark for 15 min (Mello and Vidal, 1978). After staining, root meristems were rinsed with distilled water until the complete removal of reagents. Slides were first prepared from the squashing of root meristems with one drop of 45% acetic acid (Fiskesjo, 1993), then frozen at −20 °C, and finally, fixed with 70% ethanol by removing the coverslip. The prepared slides were contra stained in a fast-green solution for 2 s and then dried at room temperature overnight (Matsumoto et al., 2006). The contra-stained slides were mounted in synthetic resin for further analysis. According to Leme and Marin-Morales (2008), for each treatment were analyzed by counting 5000 cells, being 500 cells per slide, comprising a total of 10 slides, and then the value of mitotic index (MI) [(number of dividing cells/total number of observed cells) × 100] was estimated. Similarly, nuclear alterations due to micronucleus, nuclear buds, and other chromosomal alterations (sum of interphase, anaphase, and telophase with nucleoplasmatic bridge, micronucleus in telophase, anaphase, and metaphase with a chromosomal loss) were also identified and measured. In addition, toxicity was evaluated based on seed germination index (GI) [the ratio of the number of germinated seeds to the total number of seeds allowed to germinate].

2.4. Statistical analysis

Data normality was assessed by the Kolmogorov-Smirnov test. The cytogenotoxic variables were measured by the Kruskal-Wallis test associated with Dunn's test and indicated a non-parametric distribution. The p-values <0.05 were considered significant. All statistical analyses were performed using GraphPad Prism version 5.0. For correlation analysis, the Microsoft Excel RQUAD function was used. For the integrated analysis of physicochemical and microbiological parameters, Principal Component Analysis (PCA) was performed in Past 3.14 software.

3. Results

3.1. Physicochemical and microbiological analysis

For most of the physicochemical parameters analyzed (Table 1), such as cadmium, lead, chloride, cobalt, total chromium, fluoride, mercury, nickel, nitrate, sulfate, zinc, turbidity, and pH, 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 irrigation of raw vegetable crops. All samples, based on their color patterns, were classified as Class 4 (Brasil, 2005) that is, waters that can only be used for navigation and landscape harmony. Iron concentrations in all samples were classified as Class 3 of water intended for human consumption, after conventional or advanced treatment, to the irrigation of arboreal, cereal and forage crops, and amateur fishing. P3 had the highest iron (Fe) concentration of 0.495 mg/L, whereas the lowest value was obtained for P1 (0.137 mg/L). According to the physicochemical pattern of copper (Cu), P1, P2, and P3 were classified as Class 1 (<0.006 mg/L), Class 4 (0.021 mg/L), and Class 3 (0.012 mg/L), respectively. Moreover, manganese (Mn) was also a metal that was classified according to their concentrations, samples P2 (0.172 mg/L) and P3 (0.146 mg/L) were classified as Class 3 (Brasil, 2005). In addition, aluminum (Al) was only found in P3 (Class 3).

Table 1. Physicochemical parameters of the sampled points of the Demétrio stream: point 1 (P1), point 2 (P2) and point 3 (P3).

Parameters	P1	P2	P3	Standard (Class 1) CONAMA nº 357 (Brasil, 2005)
Aluminum (mg/L)	<0.006*	0.089*	0.127#	<0.1
Cadmium (mg/L)	<0.0006*	<0.0006*	<0.0006*	<0,001
Lead (mg/L)	<0.004*	<0.004*	<0.004*	<0,01
Chloride (mg/L)	4.938*	7.977*	6.837*	<250
Cobalt (mg/L)	<0.001*	<0.001*	<0.001*	<0,05
Copper (mg/L)	<0.006*	0.021†	0.012#	<0,009
Total Chromium (mg/L)	<0.003*	<0.003*	<0.003*	<0,05
Iron (mg/L)	0.137#	0.445#	0.495#	<0.3
Fluoride (mg/L)	0.086*	0.118*	0.125*	<1.4
Manganese (mg/L)	0.052*	0.172#	0.146#	<0.1
Mercury (mg/L)	<0.0002*	<0.0002*	<0.0002*	<0.0002
Nickel (mg/L)	<0.001*	<0.001*	<0.001*	<0,025
Nitrate (mg/L)	2.433*	2.142*	3.186*	<10,0
Sulfate (mg/L)	1.868*	3.455*	3.041*	<250
Zinc (mg/L)	<0.006*	<0.006*	<0.006*	<0,18
Turbidity (UNT)	3.35*	6.14*	7.48*	<40
Color (MG Pt/L)	92.35†	178.61†	188.39†	0
pH	6.5*	6.7*	6.8*	6,0 – 9,0
Temperature (°C)	21.7	22.5	23.6	NI
Thermotolerant coliforms (MPN/100ml)	7.0 x 10^2§	4,9 x 10^2§	1.7 x 10^3#	<2.0 x 10^2
Total coliforms (MPN/100ml)	5,4 x 10^4	5,4 x 10^4	5,4 x 10^4	NI

NI: not in the resolution. Classification according to the CONAMA Resolution nº 357 (except temperature) (Brasil, 2005): Class 1 (*), Class 2 (§), Class 3 (#) and Class 4 (†).

Therefore, the results revealed the presence of certain heavy metals in the Demetrio 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 in general, based on the Cu levels and color.

Considering the total analysis of the results, P1 (source), except for its color and Fe concentration, was classified as Class 1. The degree of color change in all samples (Class 4) manifested an increasing trend from P1 to P3 and was proportional to the increase in turbidity (Class 1), which is considered as one of the interference factors for the color criterion. Another important factor for color change was the presence of heavy metals, such as Fe (Class 3) as well as the relative proportionality of variation with color at each sampling point (Figure 2). In addition, Mn in P2 and P3 (Class 3) interfered with the color pattern.

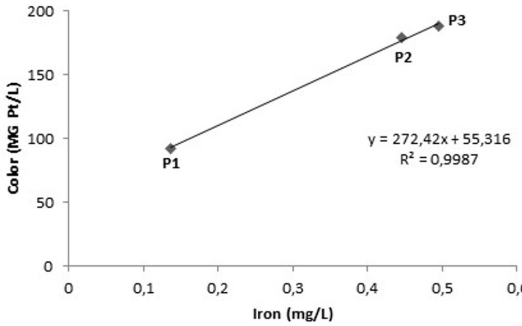

Figure 2. Color and Fe correlation patterns in relation of three water samples of Demétrio stream: point 1 (P1), point 2 (P2) and point 3 (P3).

Another factor that correlated Fe and Al to natural soil factors was the increasing distributions of the levels found in relation to the distances of the sample points (Figure 3). The analysis of correlation among Al, Fe, and the distance in which the Demetrio stream traversed was very intense, thus strengthening the hypothesis of the natural increase in concentrations of these two elements. However, Cu did not present any correlation with natural soil factors, although it manifested the characteristics of anthropic impacts. It was found that the degree of Cu concentration decreased from P2 (Class 4) to P3 (Class 3) (Figure 3), thus showing a point of discharge of this component in the path between P1 and P2. Furthermore, the presence of Mn also reduced the quality of the stream from Class 1 in P1 to Class 3 in P2 and P3 (Figure 3).

All sampled points had total and thermotolerant coliforms both at near the source (P1) as well as before and after the passage through the area of greater urban density (P2 and P3, respectively). The total coliform value at all sampling points was found to be 5,4 x 10^4/100 mL (Table 1). In contrast, for thermotolerant coliforms, a variation between the samples was observed. The highest value (1.7 x 10^3/100 mL) was found in P3 (Class 3), whereas P1 and P2 were classified as Class 2, with 7.0 x 10^2/100mL and 4.9 x 10^2/100mL, respectively (Table 1).

3.2. Integrated analysis of physicochemical and microbiological factors

PCA was used to investigate the relationships between microbiological and physicochemical parameters found in the sampling points of the Demetrio stream (Table A1 (Annex A) and Figure 4). The two principal components together explained 100% of the total variability, being that PC1 explained 78.9% and PC2 explained 21.1% of the variability. The obtained results allowed to constitute two distinct components of relations through the representation of vectors. Component 1 separated P1 from other sample points and all analyzed parameters. According to Figure 4, it was assumed that P1 had the best water quality. However, P2 and P3 were attributed to the decline in the environmental quality of the Demetrio stream due to the increases in concentrations of all the analyzed elements. In addition, component 2 separated P2 from P3 and grouped the presence of coliforms with nitrates.

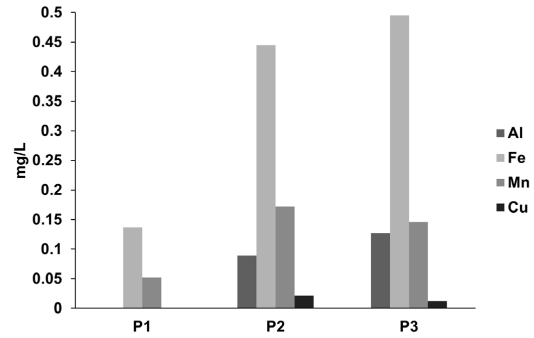

Figure 3. Al, Fe, Mn and Cu analysis of three water samples of Demétrio stream: point 1 (P1), point 2 (P2) and point 3 (P3).

3.3. Cytotoxic and genotoxic potential analysis

The values of germination index (GI) did not differ among the seeds exposed to the Demetrio stream water samples. Considering GI of the negative control group as 100%, the values varied from 86% to 91% of seeds germinated (Table 2). The values of mitotic index (MI), micronuclei (MN), nuclear bud (NB), and others chromosomal alterations observed in *A. cepa* cells are also presented in Table 2.

It was found that the percentages of mitotic cells (metaphase, anaphase, and telophase) did not differ between the groups. When compared to negative control, an increase in micronucleus for P1 ($P<0.01$) and P2 ($P<0.01$), in nuclear buds for P2 ($P<0.01$), and in other chromosomal alteration for P1 ($P<0.05$) and P2 ($P<0.01$) were observed (Table 2). Therefore, although P3 presented less cell division as compared to others sample points, the phytotoxic (GI)

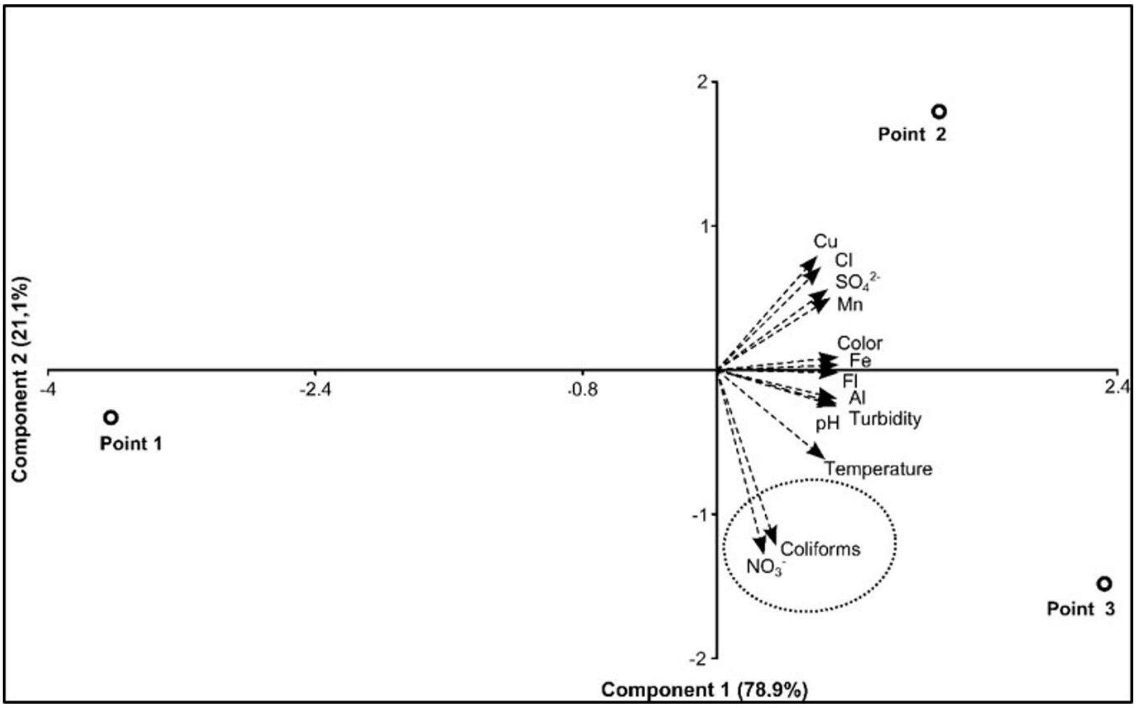

Figure 4. Integrated analysis of physicochemical and microbiological factors (PCA) of three water samples of Demétrio stream: point 1, point 2 and point 3. Component 1, with 78.9% affinity, separates component 2 with 21.1% affinity.

Table 2. Mean and standard deviation of germination index (GI), mitotic index (MI), micronuclei (MN), nuclear bud (NB) and some of others chromosomal alterations observed in meristematic cells of *A. cepa* exposed to water samples of Demétrio stream: point 1 (P1), point 2 (P2) and point 3 (P3).

Groups	GI (%)	MI	MN	NB	Chromosomal alterations[a]
Negative control[b]	100	0.60 ± 0.40	1.80 ± 3.49	0.00 ± 0.00	0.10 ± 0.32
P1	91.25 ± 26.52	1.32 ± 1.04	9.60 ± 5.89**	0.50 ± 0.71	1.50 ± 1.65*
P2	88.75 ± 15.91	1.10 ± 0.62	10.60 ± 7.75**	1.60 ± 1.70**	1.00 ± 0.82**
P3	86.25 ± 1.77	0.58 ± 0.46	5.10 ± 4.23	0.30 ± 0.67	0.70 ± 0.95
Positive control[c]	87.50 ± 3.54	1.36 ± 1.16	6.70 ± 5.60	0.70 ± 1.57	1.40 ± 1.35

[a]Sum of interphase, anaphase and telophase with nucleoplasmatic bridge, micronucleus in telophase and anaphase and metaphase with chromosomal loss; [b]Distilled water; [c]Copper sulfate (0,0002 g/L); Significant difference in relation to negative control group (*P<0,05 e **P<0,01). ANOVA/Kruskal-Wallis test.

and the cytotoxic (MI) effects of the Demetrio stream water samples were not statically revealed. In the case of genotoxic and mutagenic parameters, some sample points manifested difference in relation to negative control.

4. Discussion

The Demetrio stream is one of the main affluents of the Gravataí River, which is among the ten most polluted rivers in Brazil (Pessoa, 2017). Our work is the first one to determine the physicochemical, microbiological characteristics with possible cytotoxic and genotoxic changes in this important affluent of the Gravataí River. It should be noted that the results obtained represent a single point in time, and seasonal variations could not be considered in our study.

It is evident that the presence of heavy metals in the Demetrio stream was above acceptable standards according to CONAMA Resolution nº 357 (Brasil, 2005). In an earlier study (Rio Grande do Sul, 2012), it was expressed that Class 4 elements were found near the mouth of the Gravataí River. Moreover, Mn and Fe were classified as Class 3, as well as in the report by Bourscheid Engenharia e Meio Ambiente S.A. (2012), whereas other elements remained in Class 1 (Rio Grande do Sul, 2012). It is proved that these metals are associated with agricultural, domestic, or industrial contamination; hence, their dissolved forms tend to present a greater risk to living organisms. Frías-Espericueta et al. (2003) performed a toxicity study for Cu, Zn, Fe, and Mn with shrimp larvae (*Litopenaeus vannamel*) and concluded that the dissolved metals presented higher toxicity to the organisms because they were absorbed with greater ease in comparison to particulates. Ribeiro et al. (2012) surveyed the pollutants that interfered with the water quality of the São Francisco River (Brazil) and posited that Fe, Al, and Mn were related to domestic pollution; Cu, Cd, and Ni originated from urban-industrial pollution and agricultural use; barium (Ba), Al, Fe, Pb, and Cr with Zn in suspension were found in industrial areas.

According to the National Health Foundation (FUNASA, 2013), the color pattern classified as Class 4 could be due to Fe and Mn, and the high concentration of Fe could be attributed to the presence of iron, hematite, and goethite in Red-Yellow Alic Argis soil (IBGE, 2002). Rio Grande do Sul (2012) and Bourscheid Engenharia e Meio Ambiente S.A. (2012) expounded that the color change reduced the quality of the Demetrio stream to Class 4. The gradual increase in Al content of P3 could be associated with the type of soil in which the watercourse was found (Rio Grande do Sul, 2017). The results of PCA analysis corroborate that P2 presented a direct relationship between Cu and Mn, whereas the increase in concentration between P2 and P3 was related to the natural conditions of the Demetrio stream: color, Fe, Al, and pH.

Furthermore, Cu was found as Class 4 in P2, and according to Benites et al. (2014), Cu is the major agricultural pollutant. Rural properties and silviculture areas were close to the sample points of the Demetrio stream, thus facilitating the Cu contamination of water by agricultural pesticides and fertilizers (Menezes et al., 2009). Sampaio et al. (2013) advocated that industrial discharge could be another reason for the high concentration of Cu. The Economics and Statistics Foundation (FEE, 2012) classified the polluting potentials of industries in the municipalities of State of Rio Grande do Sul (Brazil) in relation to environmental risks and placed the city of Gravataí in the 4th place. This information highlights the possible risks of contamination of the Demetrio stream. According to Benites et al. (2014), Cu could be absorbed by living organisms, and the high concentration could even cause cancer (Barbosa et al., 2010).

The release of organic effluents into water results in the growth of pathogenic microorganisms, which in turn causes numerous diseases (Márquez et al., 1994). According to CONAMA Resolution nº 357 (Brasil, 2005), in water classified as Class 1, the limit of 200 thermotolerant coliforms per 100 mL should not be exceeded. In sample P1, it is possible to observe the use of water for recreation, thus even being near the source, it receives a load of thermotolerant coliforms. It was presumed that as P1 was closer to the forming agent, it would be less affected by possible anthropogenic contamination. In sample P2, which precedes the area with greater urban density, water is used for fishing activities. Due to the lack of basic sanitation in most of the population residing in the route between P2 and P3, sample P3 (Class 3) is used for bathing and fishing.

The Demetrio stream runs its widest stretch in the Gravataí city, which provides the highest amount of

Brazilian sanitation with 53.84% of the city sewage being collected for treatment (Brasil, 2016). However, due to the increase in urban density and irregular release of sewage, high coliform rates are often found in the Demetrio stream. In a previous study (Rio Grande do Sul, 2012), high levels of total coliforms, phosphorus, and chemical oxygen demand were observed, it could be associated with the lower dilution of domestic effluents from the Gravataí city. Alves et al. (2015) studied the "Canal de Passagem" located in the Vitória city (Espírito Santo, Brazil) and stated that the places most affected with fecal pollution are closer to contamination sources. Oliveira et al. (2012) also confirmed this trend along the Dilúvio stream in Viamão and Porto Alegre (Rio Grande do Sul, Brazil); however, in Lake Guaíba, a reduction in thermotolerant coliform concentration was noticed due to the interference resulting from the variation in water of the Lake. It is important to note that sample P3 of the Demetrio stream was selected at 2.8 km from the mouth of the Gravataí River in order to avoid any interference from the variation in river water. The level of coliforms found near the source (P1) may be related to the presence of rural residences and properties (nearly 20 meters from the stream) located at higher elevation, about 250 m above sea level, with its mouth on the Gravataí River, where it is located at an altitude of approximately 5 m (Rio Grande do Sul, 2012). This altimetric amplitude shows an important characteristic regarding the water's drainage in this basin. Vanzela et al. (2010) expressed that inhabited areas, mainly rural dwellings, tend to increase the thermotolerant coliform indexes in their waters due to the raising of animals near the houses.

Cytogenotoxic study is an important tool to detect changes in living organisms due to water contaminants. Silva and Nascimento (2013) analyzed the cytogenotoxic alterations in *A. cepa* cells exposed to the sample water of Tietê River (São Paulo, Brazil) in order to evaluate the water quality and the risks to biological communities and related these effects to complex interactions of domestic and industrial pollutants released in the river. In our study, the genotoxic and the mutagenic effects were observed in *A. cepa* cells exposed to water at sample P1 and P2, it can be attributed to the increases in heavy metal concentrations at these sites. Moreover, between these two points, chromosomal instability due to micronucleus, nuclear bud, and other chromosomal alterations was observed. P2 presented the worse water quality as compared to others points due to higher levels of dissolved Cu, dissolved Fe, and Mn, thus it manifested higher DNA damage levels. Maceda et al. (2015) related the genotoxic effect of Cu with the micronucleic formation in fish cells. Arambasic et al. (1995) exposed different organisms, such as *Allium cepa* L., *Lepidium sativum* L., and *Daphnia magna* to Cu, Pb, Zn, phenol, and sodium, and Cu was the most toxic substance. Freshwater fishes, such as *Rasbora sumatrana* and *Poecilia reticulata* were exposed to different heavy metals, and it was propounded that Cu was the most toxic element for both species (Shuhaimi-Othman et al., 2015). Benites et al. (2014) evidenced the changes in *Danio rerio* cells exposed to the Uruguay River's water with high Cu concentration. No significant alterations were found in GI; however, other studies with *Allium cepa* presented a root growth reduction due to Cu exposure. Palacio et al., (2005) evaluated the value of GI in *Allium cepa* exposed to several heavy metals and identified that Cu and Pb significantly reduced the root growth. Fiskesjo (1985) asserted that Cu significantly impeded the growth of *Allium cepa* seedlings.

In our study, all sample points manifested high Fe concentrations. According to Jadoon and Malik (2017), Fe can increase free radicals' production rates in living organisms, thus causes further DNA damage. Chandra et al. (2005) found that high concentrations of Fe, Cr, and Ni in metal waste leachate caused cytogenetic alterations in *A. cepa* cells. Lasier et al. (2000) investigated the effects of Mn on *Ceriodaphnia dubia* and *Hyalella azteca* and concluded that Mn caused chronic toxicity from 3.9 mg/L and acute toxicity from 6.2 mg/L. Doroftei et al. (2010) characterized the *A. cepa* radicles exposed to Mn in the presence of disorganized nuclei and cytogenetic damages. In P3, although the concentrations of Cu, Al, Fe, and Mn were above acceptable standards according to CONAMA nº 357 (Brasil, 2005), no mutagenic effect was detected. It can be assumed that this complex mixture reduced the rates of cell division and chromosomal damage. Li et al. (2015) identified the progressive MI reduction in *Helianthus annuus* (sunflower) exposed to Al increase. Marin and Santos (2008) also expressed that Al caused toxicity in plants and reduced the density and the growth of roots. Francisco et al. (2018) noticed a decrease in MI in meristematic cells of *A. cepa* exposed to Al. Fenech et al. (1999) affirming that DNA damage was dependent on the rate of cell division. P3 manifested low germination and mitotic indexes as compared to samples P1 and P2, thus no significant cytotoxic effect was observed at this P3.

5. Conclusion

The monitoring of the Demetrio stream by utilizing various bioindicators is essential for the evaluation of its water quality, considering that this one of the main affluent of the Gravataí River that supplies with drink water the metropolitan region of Porto Alegre (RS, Brazil). The results found in this study demonstrated the low environmental quality of the Demetrio stream according to CONAMA Resolution nº 357 (Brasil, 2005), it can be attributed to natural soil toxicity of the region as well as to anthropic actions. Demétrio Stream were classified as Class 4 that is, the water is suitable only for navigation and to landscape harmony, according to their color patterns and high Cu concentration. Toxic elements, such as Mn and Cu, were dumped in the stream, thus resulting in a severe degradation in the Demetrio stream's environmental quality. With the use of cytogenotoxic tests performed for the assessment of water quality, the main genotoxic alterations were observed in the water from P2, upstream near the area with the highest urban density, which also exhibited the highest concentration of Cu in addition to high concentrations of Fe, and Mn; these high metal concentrations might be related to the alterations obtained in the water from P2.

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Annex A. Principal Component Analysis (PCA).

Table A1. Eigenvectors of principal component analysis (PCA) using microbiological and physicochemical parameters in the sampling points of the Demetrio stream.

Variable	PC1	PC2
Al	0.3112	-0.09333
Cl	0.2717	0.2958
Cu	0.2219	0.4143
Fe	0.3152	0.01288
Fl	0.3152	-0.01035
Mn	0.2952	0.2048
NO_3^-	0.1232	-0.5369
SO_4^{2-}	0.2897	0.2301
Turbidity	0.3106	-0.09995
Color	0.3147	0.03424
pH	0.3101	-0.1056
Temperature	0.2821	-0.2606
Coliforms	0.1544	-0.5085

PC1: Principal Component 1; PC2: Principal Component 2.