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BIOMONITORING OF SURFACE WATER TOXICITY RELATED TO URBAN AND INDUSTRIAL WASTEWATER RELEASE

BIOMONITORAMENTO DA TOXICIDADE DE ÁGUAS SUPERFICIAIS RELACIONADA AO LANÇAMENTO DE EFLUENTES URBANO E INDUSTRIAL

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ABSTRACT

Water toxicity was biomonitoried by *Tradescantia pallida*, relating the results to physicochemical and qualitative analysis. Two clusters were formed by hierarchical analysis of micronuclei percentage (MN), dissolved oxygen (DO), electrical conductivity (EC) and turbidity. The first cluster corresponded to a stream that receives urban untreated sewage, where the highest MN (6.1%) corroborated the physicochemical results (1.90 mg.L^{-1} DO, 575.0 µS.cm^{-1} and 115.6 NTU). The other cluster includes sampling sites with pollutants from agriculture, livestock and industry. Some of them presented high MN even with acceptable values of physicochemical parameters, according to the Brazilian legislation. So for a better quality information on water pollution monitoring it is essential to recognize the variables that affect the biotic components, in a holistic approach of aquatic ecosystem. In this sense the bioassay with *T. pallida* becomes a useful tool that can be applied in many tropical and subtropical regions, because of its widely distribution.

Keywords: environmental health; water quality; bioindicator; Trad-MN; bioassay.

RESUMO

A toxicidade da água foi biomonitorada com Tradescantia pallida, relacionando os resultados com análises físico-químicas e qualitativa. Dois grupos foram formados por meio de análise hierárquica da porcentagem de micronúcleos (MN), oxigênio dissolvido (OD), condutividade elétrica e turbidez. O primeiro grupo corresponde a um córrego que recebe efluente urbano não tratado, onde a maior porcentagem de MN (6,1%) corroborou os resultados físico-químicos (1,90 mg.L^{-1} de OD, 575,0 µS.cm^{-1} e 115,6 UNT). O outro grupo inclui córregos que recebem poluentes da agricultura, pecuária e indústria. Alguns deles apresentaram alta porcentagem de MN mesmo com valores aceitáveis de parâmetros físico-químicos, de acordo com a legislação brasileira. Assim, para monitoramento da poluição da água é essencial reconhecer as variáveis que afetam os componentes bióticos, numa abordagem holística do ecossistema aquático. Neste sentido, o bioensaio com T. pallida torna-se uma ferramenta útil que pode ser aplicada em muitas regiões tropicais e subtropicais, devido à sua ampla distribuição geográfica.

Palavras-chave: saúde ambiental; qualidade da água; bioindicador; Trad-MN; bioensaio.

INTRODUCTION

Water quality issues are a major challenge that humanity is facing in this century, especially in developing countries, where it is urgent to improve sanitation and environmental conservation, in a current water shortages scenario (SCHWARZENBACH *et al.*, 2010; LOYOLA & BINI, 2015). Without adequate treatment of wastewater, the health of the water body receptor is impacted, changing the interactions between biotic and abiotic components of the aquatic ecosystem and also affecting other water users. The lack of proper sanitation is still a great problem of freshwater quality in Brazil. Over 70% of cities have no municipal basic sanitation policy and 62% of the urban population is covered with collecting sewage, with only 30% of sewage being treated (IBGE, 2010).

About 6,000 deaths per year could be prevented if all Brazilian regions had equal adequate sanitation (FUNASA, 2010). Constant urbanization and pressure leads to a significant increase in environmental preventable diseases, when there is no efficiency in water use practices, appropriate land use or pollution control. Consequently, there is an extra cost to the public coffers, especially with hospitalizations for health problems related to poor water quality (WHO, 2015).

The development of efficient methods for diagnosing environmental quality is an important advance in the search for solutions to social and environmental impacts caused by inadequate water management (BUSS; BAPTISTA; NESSIMIAN, 2003). It is observed the need for a more detailed diagnosis that considers interaction of management and planning instruments of water resources in sanitation (ANA, 2013). In this sense, biomonitoring has been proven effective in the integrated analysis of water quality, considering the biological system as a whole, in a complementary manner to the physicochemical parameters traditionally used.

Due to the increasing restriction of the use of laboratory animals, tests with bacteria, plants and cell cultures have been gaining prominence. Among these, plant biomonitoring stands out for providing information on the bioavailability of contaminants and can be used to evaluate the effects of contaminants at low concentrations, such as heavy metals (MIELLI; SALDIVA; UMBUZEIRO, 2009). These bioindicators can also be used for determining contamination patterns in large areas and over time, with more representative results for the exposed population.

The genus *Tradescantia* belongs to the Commelinaceae family and comprises about 500 species, which are found mainly in subtropical and tropical areas (MIŠÍK *et al.*, 2011). *Tradescantia pallida* (Rose) D.R.Hunt is an ornamental herbaceous species, which has been used in outdoor air quality studies (SANTOS *et al.*, 2015), genotoxicity of sewage (THEWES; ENDRES JR.; DROSTEI, 2011) and exposure to ozone, naphthalene and X-rays (SUYAMA *et al.*, 2002; ALVES *et al.*, 2008; LIMA; SOUZA; DOMINGOS, 2009).

As well as others species of the same gender, *T. pallida* has also proven to be an efficient pollution bioindicator, for its easy propagation and high sensitivity to genotoxic agents. By its favorable genetic characteristics, it is possible to analyze the environmental toxicity by Trad-MN bioassay that assesses mutation in pollen grain mother cells (tetrads) (MA, 1981; RODRIGUES *et al.*, 1997).

As a complement of biomonitoring studies, the Rapid Assessment Protocols (RAP) of rivers provide environmental diagnosis in a wide view, and have been used by several researchers in order to facilitate the understanding of the holistic approach of aquatic ecosystems. These protocols are a useful environmental management tool to compare various areas of the same hydrographic basin, characterizing them in a qualitative way (CALLISTO *et al.*, 2002; MACHADO *et al.*, 2015).

In this context, this study aimed to evaluate the toxicity of a watershed polluted by urban and industrial wastewater, using *T. pallida* as a bioindicator and relating the results to physicochemical and qualitative analyses.

MATERIAL AND METHODS

Study area

The study area is located at Uberaba city, Triângulo Mineiro, in Minas Gerais State, with more than 318,000 inhabitants, and about 96% of them living in urban areas (IBGE, 2015). The research was conducted at the Conquistinha River watershed, which receives about 22% of untreated urban and industrial wastewaters. The main urban expansion area of Uberaba is located in this watershed. Conquistinha River is a direct tributary of the Grande River, and can be a source of water supply for human consumption in the future.

Data collection

We collected water samples in eight sites in Conquistinha river watershed, more precisely in the streams: Desbarrancado (A), Três Córregos (B), Sucuri (C and D) and Conquistinha river (E-H) (Figure 1). Data collection campaigns were made in April 2015, at the end of the rainy season.

The following water physicochemical parameters were analyzed for each sampling site: temperature,

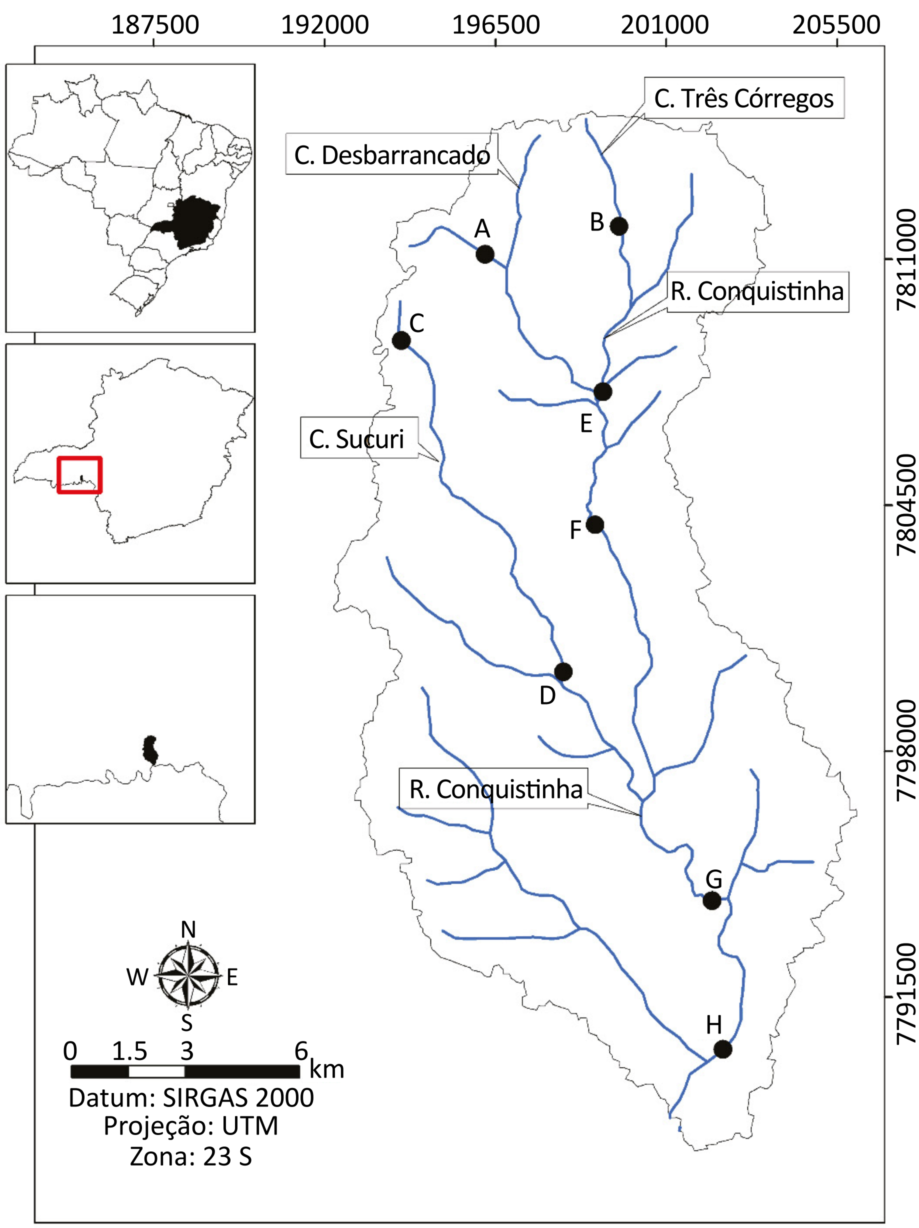

Figure 1 – Sampling sites at the Conquistinha River watershed, Uberaba, Minas Gerais, Brazil.

dissolved oxygen (DO), pH, electrical conductivity and turbidity. They were analyzed in field through Vernier sensors coupled to a LabQuest datalogger.

Flower stems of *T. pallida*, with approximately 10cm length, were placed in tap water for 24 hours for adaptation. Then, they were exposed for over six hours to the negative control (tap water), positive control (0.2% formaldehyde) and water samples from the eight sites at the Conquistinha River watershed. After exposure, the inflorescences were placed again in tap water for a period of 24 hours to recover, then fixed in a solution of acetic acid and ethanol (1:3) and stored in 70% ethanol.

Slides for micronuclei analysis were prepared as proposed by Ma (1981). The micronucleus count was conducted through an optical microscope at 400x magnification in a random group of 300 tetrads. At least five slides per sample site were analyzed. The frequency of micronuclei was expressed in percentage.

The environmental assessment of the surroundings of each sample site was done by applying a RAP, modified from Callisto *et al.* (2002) (Chart 1). A score between 0 and 4 was attributed to each parameter, which corresponds to the environmental integrity. The analyzed stretches were classified according to the environmental quality by adding the scores of each parameter in four condition categories: good (31 to 40 pts), regular (21 to 30 pts) and poor (0 a 20 pts). The stretches were also evaluated for human activities on site by their presence or absence — observed during data collection — in a qualitative analysis.

Statistical analysis

Micronuclei (MN) data were submitted to an analysis of variance (ANOVA), in order to determine differences between the sampling sites. When the level of significance was reached ($p<0.05$), we applied the Tukey test for multiple comparisons. The results of the physicochemical parameters and biomonitoring were analyzed by the Pearson correlation coefficient.

A cluster analysis (CA) was also used in order to detect the similarity groups between the sampling sites, based on MN and physicochemical data. We calculated

Chart 1 – Rapid assessment protocol of habitat diversity in watershed stretches.

Parameters/ score	Good (4)	Regular (2)	Poor (0)
1. Occupation of the banks of the water body (main activity)	Natural vegetation	Pasture/ agriculture	Residential/Commercial/ Industrial
2. Erosion on the banks of the water body and siltation in its bed	Absent	Moderate	Severe
3. Anthropogenic changes	Absent	Changes from domestic sources (sewage, garbage)	Changes from industrial sources (factories, steel mills, channeling the river course…)
4. Full coverage in water bed	Partial	Total	Absent
5. Water odor	Absent	Sewage	Industrial/ oil
6. Water oily	Absent	Moderate	Abundant
7. Water transparency	Transparent	Blurred	Opaque or colored
8. Sediment odor	Absent	Sewage	Industrial/ oil
9. Sediment oily	Absent	Moderate	Abundant
10. Sediment types	Stone/ gravel	Mud/ sand	Cement/ canalized

hierarchical agglomerative CA on the normalized data set by means of the Ward's method, using squared Euclidean distances as a measure of similarity. Results of CA were represented with a dendrogram.

RESULTS AND DISCUSSION

Results of the genotox test with *T. pallida* physicochemical and qualitative analyses are shown in Table 1, as well as the physicochemical and qualitative analyses. All sampling sites showed greater toxicity, by MN percentages, than the negative control (1.1%±0.4). In our results, MN ranged from 1.5 to 6.1%, similar to those found in samples collected in rivers of southern and southeastern Brazil (UMBUZEIRO *et al.*, 2007; COSTA *et al.*, 2014).

Trad-MN bioassays are more sensitive to environmental contaminants than other plant bioassays, as root tip cells and somatic stamen hair analysis, used in paralel experiments (MIŠÍK & MICIETA, 2002). Most studies were conducted with a hybrid between *T. hirsutiflora* and *T. subacaulis*, the clone 4430; but recently, several Brazilian research groups have been using *T. pallida* for environmental monitoring, obtaining satisfactory results (MIŠÍK *et al.*, 2011). This species is broadly distributed, more adapted to tropical climates and more resistant to deseases than the clone.

Differences in the percentage of MN indicate some spatial variation for water pollution at the watershed studied. Water collected in sampling site A presented the highest MN (6.1%±1.0). This value was more than the double of any other sampling site and 25% higher than the positive control. The second highest MN (5.1%±1.0) was found in the last sampling site (H). Water samples from C, D and E had the lowest MN (2.1% on average).

The A sampling site had the highest electric conductivity (575.0 µS.cm^{-1}) and turbidity (NTU 115.6), and the lowest DO concentration (1.90 mg.L^{-1}). With the exception of that site, DO concentrations ranged from 6.2 to 8.1 mg.L^{-1} with an average of 7.5 mg.L^{-1} (Chart 1). Only B and E sites presented low values of electrical conductivity, with less than 50 µS.cm^{-1}. On average, pH was 7.3 and temperature was 22.4°C.

Two clusters were formed by the hierarchical analysis of MN, DO, electrical conductivity and turbity (Figure 2). The first one corresponds only to sampling site A. By calculating the Pearson coefficient, MN had a strong correlation with the DO concentration ($p<-0.79$) and moderate correlation with electrical conductivity and turbidity ($p=0.57$ and $p=0.63$, respectively).

The moderate correlation of Trad-MN bioassay results with electrical conductivity and turbidity indicates that this biomonitoring cannot exclude traditional analyses,

Table 1 – Environmental diagnosis of the Conquistinha River watershed, Uberaba, Minas Gerais, Brazil.

Sampling site	MN (%)	EC (µS.cm^{-1})	Turbidity (NTU)	DO (mg.L^{-1})	pH	T (°C)	RAP (score)
A	6.1±1.0*a*	575	115.60	1.90	7.20	21.60	Poor (18)
B	4.1±0.9*b*	36	22.40	6.22	7.56	25.28	Regular (28)
C	2.3±0.3*c*	226	17.50	7.95	7.10	21.50	Good (32)
D	1.5±0.4*c*	172	50.20	8.05	7.10	22.60	Good (32)
E	2.5±0.7*c*	30	20.20	7.80	7.80	27.70	Good (32)
F	3.0±0.7*bc*	250	32.90	6.86	7.30	20.20	Regular (24)
G	2.8±0.6*bc*	206	56.20	8.10	7.10	21.10	Good (38)
H	5.1±1.0*ab*	219	47.20	7.47	7.00	19.60	Poor (22)

MN: micronuclei frequencies ± standard deviation; sifferent lowercase indicate significant differences by Tukey test ($p<0.05$); EC: electrical conductivity; DO: dissolved oxygen; T: temperature; RAP: Rapid Assessment Protocol.
Source: modified from Callisto *et al.*, 2002.

but complement them. It is noteworthy that the Trad-MN bioassay indicates the toxicity of water, but should be used with caution, because of its low discriminatory power, requiring further analysis to infer the source of pollution. Nevertheless, positive MN results are important in genotoxic risk assessments, indicating the need for further testing with some more relevant species bioindicator (MIELLI; SALDIVA; UMBUZEIRO, 2009).

The percentage of MN found in sampling site A was similar to Cristais River (6.2%), downstream of a textile industry, at southeastern Brazil (UMBUZEIRO *et al.*, 2007). This site is located at Desbarrancado Stream, one of the first tributaries of Conquistinha River, with the nascent and most of its length within the urban perimeter, polluted especially by urban untreated sewage. The A site could be viewed separately in one of the clusters of Figure 2, since the poor water quality inferred by biomonitoring results corroborated the physicochemical parameters analyzed.

The A site does not fit into any of the four freshwater ratings established by CONAMA Resolution n° 357/2005 (CONAMA, 2005), according to DO concentration and turbidity results. Values above the threshold of electrical conductivity (100 $\mu S.cm^{-1}$) were observed in most of the sampling sites, especially in site A (CETESB, 2014). High electrical conductivity of the water may be explained by diffuse pollution with the flow of sediments and rural effluents, which is intensified in the absence of riparian vegetation, as evidenced in the watershed studied.

The other cluster of Figure 2 includes sampling sites that receive pollutants from agriculture, livestock and industrial effluents. Water samples collected in some of these sites, as B and H, caused high toxicity to *T. pallida*, even with acceptable physicochemical conditions according to the current Brazilian legislation. The B site presented micronuclei about 40% higher than the E site, also considered no impacted, probably due to its location close to the urban area and a few meters from an agricultural substrate factory. The highest percentage of micronuclei observed in H site may be related to contamination by industrial effluent, since this site is located in one of the industrial districts of the city, 1.5 km from of the Conquistinha River mouth, in Grande River, an important source of water supply.

After about 3 km from the confluence with the Desbarrancado stream (A site), the Conquistinha River is considered impacted, with an increase of up to 700% of the electrical conductivity (CETESB, 2014). However, regarding to turbidity and DO concentration, all sites fit between freshwater classes I and II, which can be intended for human consumption supply after conventional treatment (CONAMA, 2005).

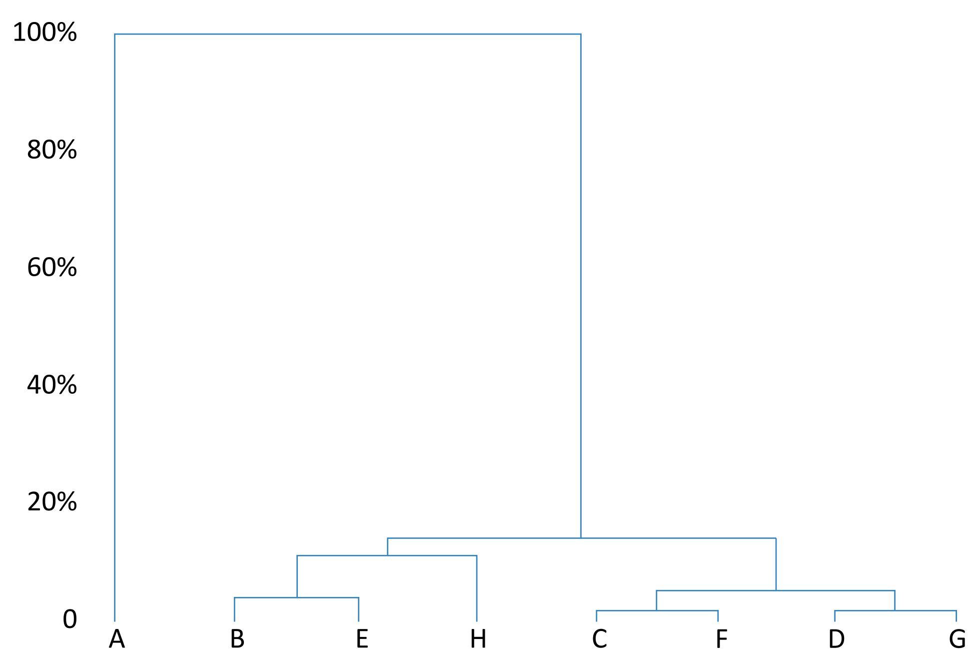

Figure 2 – Dendrogram showing clustering of sampling sites according to water quality characteristics.

According to the environmental assessment given by the application of the RAP in three tributaries of Conquistinha River, the A site was severely impacted (18 pts), the B site was moderately changed (28 pts) and C and D sites were in better conservation condition (32 pts). The four sampling stretches at Conquistinha River (E-H) ranged from regular to good environmental conditions (22 to 32 pts).

In general, almost 70% of the evaluated stretches had margins with high degree of deforestation, soil erosion process and siltation of the stream bed, with the use of land surrounding mainly for sugarcane cultivation (44%) and pasture (33%). Domestic sewage release was observed in sampling site A, and industrial sewage, probably in the last sampling site (H). Bad sewage smell was also evident in A, E and H sites.

Besides the lack of sanitation evident in the watershed studied, the high degree of deforestation in the analyzed stretches compromises the future availability of water resources. Currently, there are extremely few water resources in good conditions of conservation outside protected areas, even with the obligation to preserve the riparian vegetation in Permanent Preservation Areas tracks (PPAs).

Deforestation has been demonstrated to cause several changes in environment biodiversity, and influence litter accumulation in the remnant patches (MACHADO *et al.*, 2015). Among various impacts of deforestation, there is the reduction of water infiltration into the soil and supply aquifers, increasing runoff and soil loss. Thus, the conservation of watersheds riparian vegetation offers more and better water quality.

CONCLUSIONS

Before the occurrence of extreme events of prolonged droughts in a current context of climate changes, agriculture and urban expansion, coupled with the absence of conservation measures, greatly increase the vulnerability of aquatic ecosystems. River basins can have their hydrological behavior altered, which leads to the urgent need to improve the water infrastructure in Brazil, facing the actual water shortage.

Genotox test with *T. pallida* was sensitive to the complex mixture of water pollutants in the watershed studied, since the percentage of MN found in the sampling sites were, on average, 2.2 times higher than the negative control. It was also possible to detect spatial variation in water pollution, even with acceptable values of physicochemical parameters, according to the Brazilian legislation.

So for better quality information on water pollution monitoring, it is essential to recognize the variables that affect the biotic components, in a holistic approach of the aquatic ecosystem. In this sense the bioassay with *T. pallida* becomes a useful tool, which may be applied in many tropical and subtropical regions, due to its widely distribution.

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