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RESEARCH ARTICLE

QUATI RIVER (PARANÁ, BRAZIL) CONTAMINATION: MUTAGENICITY AND GENOTOXICITY ASPECTS USING THE *ALLIUM CEPA* TEST

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ABSTRACT

Changes in aquatic ecosystems are resulting from human activities, for example domestical, agricultural and industrial discharges. Nowadays, conventional physical-chemical parameters are not suitable for hazard assessment anymore. The Quati River (Paraná, Brazil) is a very important river for the population of Cascavel city, and in 2011 was contaminated by a leak of industrial sewage. Since then, no environmental study was made on this river. Thus, this study aimed to evaluate the status of the Quati River according to genetic parameters: micronuclei (MN) and chromosomal aberrations (CA) frequencies and the mitotix index (MI); using the *Allium cepa* test in two points of the river (P1 and P2). Distilled water was used as negative control (NC) and MMS as positive control. Were observed statistically increasing in all parameters compared to NC ($p < 0.05$). Shockingly, a fold-increase of 7.97 in P1 and 4.23 in P2 were observed in MN frequencies. We observed that the Quati River is contaminated and that this contamination is possible caused not only by the industrial sewage leak in 2011, but by constantly sewages dumped into the river. We suggest more surveillance on the river and the continuity of environmental studies for monitoring.

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INTRODUCTION

Around 80% of all global diseases are water borne (Milaré 2013). Moreover, most changes in aquatic ecosystems are resulting from human activities as domestical, agricultural and industrial discharges in water sources, a common practice in Brazil (Ohe *et al*. 2003; Monte Egito *et al*. 2007). We know that anthropogenic stressors act on aquatic environments at different scales, but, considering the complex interactions between chemical mixtures and the complexity to manage and protect water sources, conventional physical-chemical parameters are not suitable anymore for hazard assessment. On this purpose, several levels of tests, including biological tests, are used to assess environmental quality and the presence of contaminants on water sources. Thus, reliable and relevant ecotoxicity data provide an opportunity to understand the influence of chemicals on surface waters (Merrington and Van Sprang 2014). In this study, we used the *Allium cepa* test for analysis.

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An important consideration is that this test allows the simultaneous assessment of cytotoxic, genotoxic, and mutagenic effects of a determined environmental sample without performing different assays. It is recommended by the International Program on Chemical Safety to screen micronuclei (MN) and chromosomal aberration (CA) frequencies, as well to determine the mitotic index (MI) of *A. cepa* cells exposed to the river water samples. Previous studies have shown that pollutants with mutagenic and cytotoxic potential may cause DNA instability and cellular division inhibition (Grant 1999; Evseeva *et al*. 2003; Silveira *et al*. 2016a). The assessment of mutagenicity, genotoxicity, and cytotoxicity are critical and decisive steps in chemical mixture evaluations, used to reflect sewage (domestic or industrial) and agrochemical exposure (pesticides, herbicides). Quati river is a water source located inside Cascavel city (350,000 habitants - Paraná, Brazil). This river flows to the Cascavel river, and this is the most important river used daily for water drinking and household use in the region (Orssato 2008). Despite a not published data yet, our group submitted a manuscript where we observed anthropogenic contamination in the Cascavel River. Furthermore, in 2011 there was an episode of accidentally

contamination in the Quati River, with an industrial sewage leak. At our best knowledge, our study is the first to evaluate the status of the river since then. So, considering the importance of the river to its population, its location, and the anthropogenic activities (contamination on 2011 and local history of contamination on near rivers), this study used the *Allium* cepa test and aimed to determine the status of the Quati River according to genetic parameters, to evaluate if there is any trace of contamination.

MATERIALS AND METHODS

Chemical Reagents

Seeds of *Allium cepa* with no pesticides used in fabrication were obtained from Granja Barreira (RS-Brazil). Ethanol 99%, glacial acetic acid, Shiff´s reagent, acetic carmine, and methyl methanesulfonate (MMS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Sample Collection

Quati River is located inside the urban perimeter of Cascavel city (Paraná, Brazil), between south latitude 24º95'22.14" and west longitude 53º43'46.54". Two points were investigated: P1 (24º54'05.38"S - 53º32'00.42"W) and P2 (24º92'61.27"S - 53º47'46.41"W).

Allium cepa test

Protocol were followed as previously published paper (Silveira *et al*. 2016a; Silveira *et al*. 2016b), with slight modifications. Seeds of *A. cepa* were germinated in Petri dishes covered with filter paper, at room temperature (25 ± 5 °C). The sprouts were kept moist with distilled water until they reached 1 cm in length and, after this, the filter paper was replaced, and the seeds were 1 mL treated with the water samples every 8 h. After 72 h of treatment, fixation and staining procedures were performed as proposed by Grant (1982), with slight modifications. Briefly, the fixation was performed using ethanol and glacial acetic acid (3:1) for 24 h. The slides were stained with Shiff´s reagent for 1 h and then with acetic carmine (2%). The treatments were performed in three biological replicates. Each replicate gave rise to the analysis of 5000 meristematic cells divided among 5 slides (1000 cells per slide) for a total of 15000 meristematic cells analyzed per treatment. Mitotic index, micronuclei frequency, and chromosomal aberration frequency were evaluated according to Rank and Nielsen (1994), with modifications. Briefly, MI were characterized by the total number of dividing cells in cell cycle; for CA, abnormalities in the cell cycle in all phases (as c-metaphase, anaphase and telophase bridges, chromosome losses, chromosome breaks, apoptotic cells) were analyzed. For MN, we analyzed only MN in interphase. Distilled water was used as a negative control (NC) and MMS (methyl methanesulfonate – 400 µM) as positive control (PC). Images were captured by an Olympus DP 71 camera connected to an Olympus BX 60 microscope, using the DP manager image software (version 3.1.1.208) (Olympus, Japan). Data distribution and normality were verified by the Kolmogorov-Smirnov test. All comparisons were performed by one-way ANOVA. When significant differences were observed (i.e. $p < 0.05$), Dunnett's test was applied, comparing all groups with the negative control. T-test was used to compare P1 of the first collection period with P1 of the second collection period and the same for P2. SigmaPlot 11.0 (Systat Software, Inc., Chicago, Il, USA) was used to perform the statistical analyses and the graphs were made in GraphPad Prism 6.00 for Windows (GraphPad Software, La Jolla California USA).

RESULTS

Both P1 and P2 showed statistically increasing in all parameters (MI, CA and MN – Figures 1, 2 and 3) compared to NC ($p < 0.05$). According to MI, P1 was 2.01 and P2 was 3.21 fold-increased than NC. For CA, P1 was 1.78 fold-increased and P2 1.65 fold-increased; and for MN P1 was 7.97 and P2 was 4.23 fold-increased than NC.

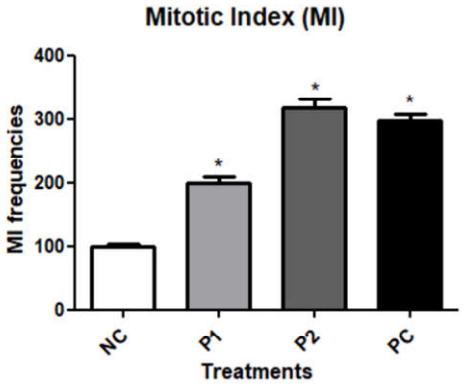

Fig. 1. Frequencies of mitotic index (MI) per 5000 cells. Legend—P1: Point of collection 1; P2: Point of collection 2; NC: negative control; PC: positive control

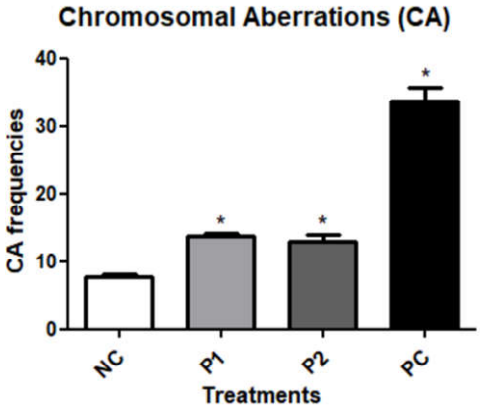

Fig. 2. Frequencies of chromosomal aberrations (CA) per 5000 cells. Legend—P1: Point of collection 1; P2: Point of collection 2; NC: negative control; PC: positive control

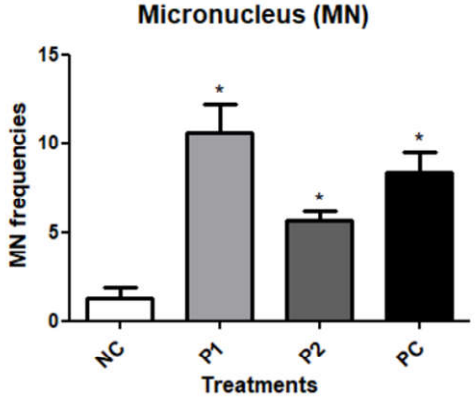

Fig. 3. Frequencies of micronucleus (MN) per 5000 cells. Legend—P1: Point of collection 1; P2: Point of collection 2; NC: negative control; PC: positive control

DISCUSSION

The *A. cepa* test has been used to detect mutagens since the 40s and can be used to assess a multitude of chemical agents, which has contributed to its increasing application around environmental monitoring studies. It is a basic, but recommended bioassay by the World Health Organization (WHO), the United Nations Environmental Program, and the United States Environmental Protection Agency (EPA) (Mauro *et al*., 2014) as an assay for toxicological and genetic evaluation, once this test shows the primary effects of products at low cost (Roberto *et al*., 2016). To our knowledge, our study is the first to perform a cytological screening using the *A. cepa* test in the Quati River, and indeed it has proved to be an excellent tool to evaluate the mutagenic, genotoxic, and cytotoxic potential of the river. Along the years, it has substantially increased the discharge of substances and hazardous chemicals substances into aquatic environments, and this contributed to imbalance the natural ecosystems, what consequently, drew the attention of several researchers and governmental agencies to the living organism´s health (Leme and Marin-Morales, 2009). It is known that mutagenic agents could affect DNA stability and compromise gene functions. If some genes or chromosomes suffer permanent DNA damage, a heritable modification of certain characteristics may be resulted (Roberto *et al*., 2016). Thus, it is very important to characterize the status of rivers across the world, especially those whose water is used for consumption and / or a known contamination has occurred, as the Quati River.

As demonstrated by our results, we observed statistically increasing in MI, CA and MN frequencies in P1 and P2 of the Quati River, a very concerning result. Increased MN and CA frequencies are strong evidence of mutagenicity and genotoxicity (Ribeiro 2003; Leme and Marin-Morales 2009; Sobral *et al*., 2013), while the MI shows the cytotoxicity level in the cells following exposure to river water (Fernandes *et al*., 2007). This data shows us that the river is still contaminated by mutagenic / genotoxic substances or compounds, and that years after the accident whereas industrial sewage was leak to the river the damage was not neutralized. Moreover, it is well knowing that the area around Cascavel city is characterized by intense agricultural production (Gibson and Koifman, 2008), with few or none surveillance on where domestic and industrial sewages are dumped, showing a constant way of contamination in the Quati River and all rivers around the city. In this sense, several studies evaluated the water quality of urban streams and rivers, and all studies showed a correlation with anthropogenic activities, like industrial and urban sewage (Athanásio *et al*., 2014; Lv *et al*., 2015; Li *et al*., 2016; Vaseem *et al*., 2016; Duarte *et al*., 2017). In a nearest river study, Ribeiro *et al*. (2014) observed an increase of MNs frequencies in fishes of the São Francisco River (located in the same city of Cascavel). All this data combined with our results show us the importance of this kind of study and gave idea that the Quati River is contaminated since the accident involving the industrial sewage that was leak to the river, but probably this was not the only case of contamination. Probably there are another site where industrial, agricultural and domestical sewage ends up in the river and could possible contribute to this poor prognosis of the river. We suggest a major surveillance on area, to avoid more damage to the environment, living organisms and to all people that uses this water daily, and the continuity of monitoring studies in the Quati River.

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