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Biomonitoring Hospital Effluents by the *Allium cepa* L. Test

M. D. Bagatini · T. G. Vasconcelos · H. D. Laughinghouse IV · A. F. Martins · S. B. Tedesco

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Abstract Hospital effluents are serious problems in developing countries like Brazil, and when not treated adequately, can cause mutagenic effects on live organisms. Biomonitors, like *Allium cepa* L., which is one of the most used plant species when monitoring effluent genotoxicity, have been used to alert the world population about environmental contamination and genotoxic chemical emissions. The *Allium cepa* test was used to evaluate the genotoxicity of a hospital effluent in Santa Maria, Rio Grande do Sul State, Brazil. During the study, chromosomal disruptions, anaphasic bridges, and micronuclei during telophase were observed, indicating environmental toxicity risk.

Keywords Hospital effluent · Genotoxicity · *Allium cepa* · Toxicology · Cell cycle

In this study, the *Allium cepa* test was used to evaluate the genotoxicity of a hospital effluent in the municipal of Santa Maria, Rio Grande do Sul State (RS), Brazil. In past decades, the scientific community has shown interest in amplifying their knowledge and control on hospital effluents. The impacts caused by toxic agents on the environment and humans, often are not capable of being observed and measured directly. Information obtained through biomonitors permits estimating and comparing these impacts. The studied discharge is relevant since it dispenses potentially toxic substances into the environment, which are treated when passed through the septic tank. Up to now, there are no studies that quantify the effects caused on plants and wildlife that exist around the area of discharge of the effluent. Using this study, the impact of the effluent on directly affected organisms can be analyzed. There are no reports, so far, about diseases proven to occur due to the toxicity of this effluent; however, there is a large amount of substances contaminating the environment. Biomonitors, also known as sentinel organisms, have started to be used to alert populations about dangerous environments. They can be defined as indicator systems that generally include subsystems of a complete organism, used to identify a specific target. Plants are essential members of the ecosystems and are, in general, more sensitive to environmental stress than other systems using environmental biomonitors (Silva et al. 2003). *Allium cepa* is one of the most used plant species in toxicity and genotoxicity tests, particularly when monitoring effluents. Genotoxic chemical emissions from anthropogenic activities into environmental compartments require genotoxicity assays for the assessment of the potential impact of these sources on the ecosystems (Rank 2003). For example, some drugs like cytostatic agents are genotoxic (Bassi and Moretton 2003).

The mitotic index and replication index are used as indicators of adequate cell proliferation (Gadano et al. 2002), which can be measured using *Allium cepa*. The chromosomal aberration method in *Allium* roots is

M. D. Bagatini · S. B. Tedesco (✉)
Laboratory of Plant Cytogenetics, Department of Biology, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Av. Roraima, 1000, Cidade Universitária, Santa Maria, RS CEP 97105-900, Brazil
e-mail: solatedesco@yahoo.com.br

T. G. Vasconcelos · A. F. Martins
Laboratory of Research in Effluent and Residue Treatment (LATER), Department of Chemistry, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil

H. D. Laughinghouse IV
Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

validated by the International Program on Chemical Safety (IPCS), as an efficient test for the analysis and in situ monitoring of the genotoxicity of environmental substances. In this study, the *Allium cepa* test was used to evaluate the genotoxicity of a hospital effluent from a developing country to monitor the risks of environmental contamination.

Materials and Methods

The assays were carried out at the Laboratory of Plant Cytogenetics – Department of Biology and the Laboratory of Research in Effluent and Residue Treatment (LATER) – Department of Chemistry, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil. The samples from the effluent were collected at stations S1 (before the septic tank) and S2 (after the septic tank) from the Outpatient Center (PA) at the Universidade Federal de Santa Maria (UFSM) University Hospital (HUSM) Sewage System, composed of 10 samples, and sampled within 9 a.m. and 6 p.m. on January 3, 2005. After sampling, the samples were immediately filtered, acidified, and cooled to 4°C. The procedure was carried out according to Fiskejö (1993), with modifications. For the *Allium cepa* test, three groups of six bulbs were placed into water to root, where the control group remained in distilled water and the bulbs from the other two groups were treated for 24 h in the distinct samples from the HUSM effluent. Afterwards, the rootlets were collected, fixed in 3:1 (ethylic ethanol:acetic acid), and conserved in ethanol 70% at 4°C. For slide preparation, the rootlets were hydrolyzed in HCl 1 N for 5 min, washed in distilled water, then submitted to the squashing technique (Guerra and Lopes 2002), and colored with acetic orcein 2%. Slides of the onion root-tips were made and for each group of bulbs, 6,000 cells were analyzed, calculating the mitotic indexes (MI), comparing the data statistically with the χ^2 test using the Bioestat 3.0 statistical program (Ayres and Ayres 2003).

Results and Discussion

Cytotoxicity/genotoxicity of the effluent from the PA-HUSM in S1 and S2 were evaluated using the *Allium cepa* test. The results, presented in Table 1, demonstrate that there was a decrease in the mitotic index for each of the samples. This indicates, qualitatively, the presence of compounds with some degree of cytotoxicity present in the effluent. Figure 1 indicates the chromosomal alteration evidencing the presence of genotoxic substances in the effluents.

In the normal process of mitotic cellular division, chromosomes in metaphase should be organized in the metaphasic plate, in the middle of the cell, and through its centrometers (primary constriction) connect to the fuse fibers, which permits that in anaphase, subsequent phase of the cellular division process, the chromatids of each chromosome migrate to the opposite pole of the cell; in telophase, they are already at the poles and called daughter chromosomes; so cytokinesis occurs (cytoplasmatic division, dividing the original cell into two other cells genetically identical).

Live organisms are frequently exposed to environmental agents that possess the capacity to induce chromosomal alterations (mutations), phenomenon that can lead to the development of cancerous processes and cellular death. The detection of these products and their probable affects in live organisms are important in the study of impact caused by animal, plant, and principally, human populations (Costa and Menk 2000; Silva et al. 2003; Paz et al. 2006).

The most common cytogenetic alterations in metaphasic cells are chromosomes in rings and chromosomal disruptions (chromosomes that are released or lose parts, resulting in fragments of chromosomes) or unorganized chromosomes. In anaphase, there is an occurrence of disruptions and/or disorganization of the mitotic fuse, as well as anaphasic bridges, and presence of micronuclei in telophase (Fiskejö 1993; Vicentini et al. 2001; El-Shahaby et al. 2003). In this study, chromosomal disruptions in

Table 1 Number of interphase and dividing cells and the mitotic index of onion root-tip cells

Treatments	Number of cells in division					Cell in division (%) MI	Chromosomal aberrations (%)
	Prophase	Metaphase	Anaphase	Telophase	Interphase		
Control (distilled water)	170	62	43	48	5,477	8.72 a	0.0
S1	144	25	15	12	5,804	3.27 c	4.9
S2	197	85	84	58	5,576	7.07 b	3.6

The *Allium cepa* bulbs were placed for 24 h in water (control) and hospital effluent treatments. The total number of cells analyzed for each treatment and control was 6,000

Means followed with the same letter do not significantly differ at the 5% level by the χ^2 test

Treatment time: Control = 0 h (*t* zero), Treatments: S1, S2 = 24 h

MI mitotic index, *S1* hospital effluent sample before the septic tank, *S2* hospital effluent sample after the septic tank

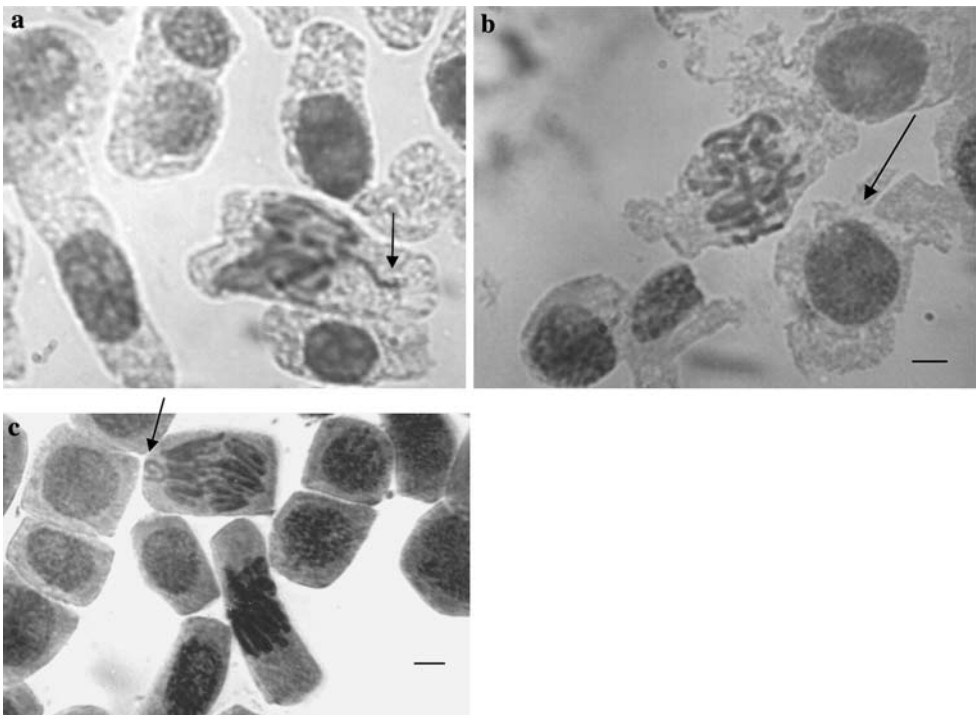

Fig. 1 Cells during the cellular cycle of *Allium cepa*. **a** Cells of *A. cepa* rootlets submitted to S1 (before the septic tank). *Arrow* indicates chromosomal breakage; **b** Cells of *A. cepa* rootlets submitted to S1 (before the septic tank). *Arrow* indicates deteriorated cells; **c** Cells of *A. cepa* rootlets submitted to S2 (after the septic tank). *Arrow* indicates chromosomal breakage. Scale bar = 4μ

metaphase (Fig. 1a), anaphasic bridge formation, and the presence of micronuclei in telophase, as well as morphological alterations (Fig. 1b) were observed. These observed alterations clearly indicate the presence of toxic and mutagenic and/or genotoxic substances in the samples before the septic tank.

The cells with chromosomal alterations indicate the genotoxicity of the studied effluent. The morphological alterations observed in the cells do not demonstrate if the genetic material (organized in the form of chromosomes during cellular division) was affected, only the existence of toxicity in the effluent. In this study, the terms toxicity and genotoxicity serve, respectively, to define that there was some cell alteration in the genetic material through observation of the chromosomes of this plant material during its cell division. After the septic tank, alterations in the chromosomal structure (Fig. 1c) are also observed; however, the MI increases (Table 1), showing that the cellular proliferation also increased.

In relation to the mitotic indexes, the samples from S1 presented a more accented decrease than that in S2. The importance and or relevance of the pre-tank (before the septic tank) is its indiscriminate discharge, which afterwards, passes through a treatment system called a septic tank to eliminate the toxicity of the effluent. This demonstrates a certain capacity of the septic tank to reduce the cytotoxicity of the effluent, but, on the other hand, the residual cytotoxicity reinforces the idea that the treatment system has surpassed its capacity limit.

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