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In vitro chromosomal abnormalities detection for cost effective tests of cytogenetic environmental contamination

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Monitoring meristematic cells of *Allium cepa* L is a very efficient cytogenetic material for *in situ* detection of cytogenetic activity by environmental chemicals, that constitute a cost-effective, suitable toxicity test that provide scientific data evaluation of hazardous substances contamination into environment and its direct health effects [1, 2]. The *Allium cepa* test was introduced by Levan (1938) and has showed a wideworld application for regulating the use of alimentar industrial activities, toxicity screening of urban, agricultural systems and industrial wastewaters and soil contamination [1, 2]. In the assessment of cito-genotoxic and mutagenic environmental contamination this work proposed an in vitro chromosomal abnormalities detection test in meristematic cells of *Allium cepa*, considering the chromosomal abnormalities index (CA) and the mutagenic potential measured by the presence of cells bearing chromosome breaks and micronuclei, and by calculating the mitotic index (MI). Water samples were collected in a micro basin located at the central portion of Araras city (inside S. Paulo) characterized by an intense agropastoral and cane sugar cultivation. A continuous *in vitro* exposure of *Allium cepa* seeds was carried out in Petri dish. Control tests were carried out with distilled water (negative control) and trifluraline herbicide at a concentration 0,019 ppm (positive control). After five days of seeds exposure to samples, the roots were collected with about 2 cm in length and fixed in Carnoy 3:1, washed in distilled water for 5 min. and submitted to the Feulgen reaction. After staining the samples were rinsed with distilled water until the excess reagent was completely removed. Slides were prepared with a drop of acetic carmine (2%) and later covered with cover slips. Cover slips were carefully removed in liquid nitrogen, and slides were mounted in Entellan and examined under a light microscope at 400x magnification. The results showed cytotoxic effects with significant increase in mitotic index values and genotoxic abnormalities such as nuclear buds, C-metaphases, stickness metaphases, polyploidy anaphases and chromosome loss. Mutagenicity was evidenced by the presence of micronucleated cells and cells with chromosomal breaks. In conclusion, this study revealed the test sensitivity to detect cito and geno-toxicity with description of important cytotoxic, genotoxic and mutagenic effects that could be useful for environmental contamination evaluation.

1. Bianchi, J. *et al., Ecotoxicology and Environmental Safety*, **74**, 826-833, 2011.
2. Caritá, R. and Marin-Morales, M.A., *Chemosphere*, **72**, 722-725, 2008.

Table 1 – Frequency, mean and standard deviation of chromosomal abnormalities índex (CA) and mutagenic index (MI) observed in meristematic cells from *Allium cepa*.

Samples	CA		MI	
	Frequency	**Mean±std**	**Frequency**	**Mean±Std**
Negative Control	0.47	4.8±1.3	0.17	1.8±0.4
Positive control	5.13	52.4±10.9*	2.05	21±1.5*
P1	3.08	31.2±6.1*	0.15	1.5±0.5
P2	2.33	23.6±4.4*	0.45	4.6±1.8*
P3	3.69	37±1.5*	0.63	6.4±1.1*
P1	0.68	7.2±3.5	0.17	1.8±0.4
P2	1.24	12.8±3.6*	0.19	2±0.7
P3	1.04	10.6±4.5*	0.13	1.4±0.5

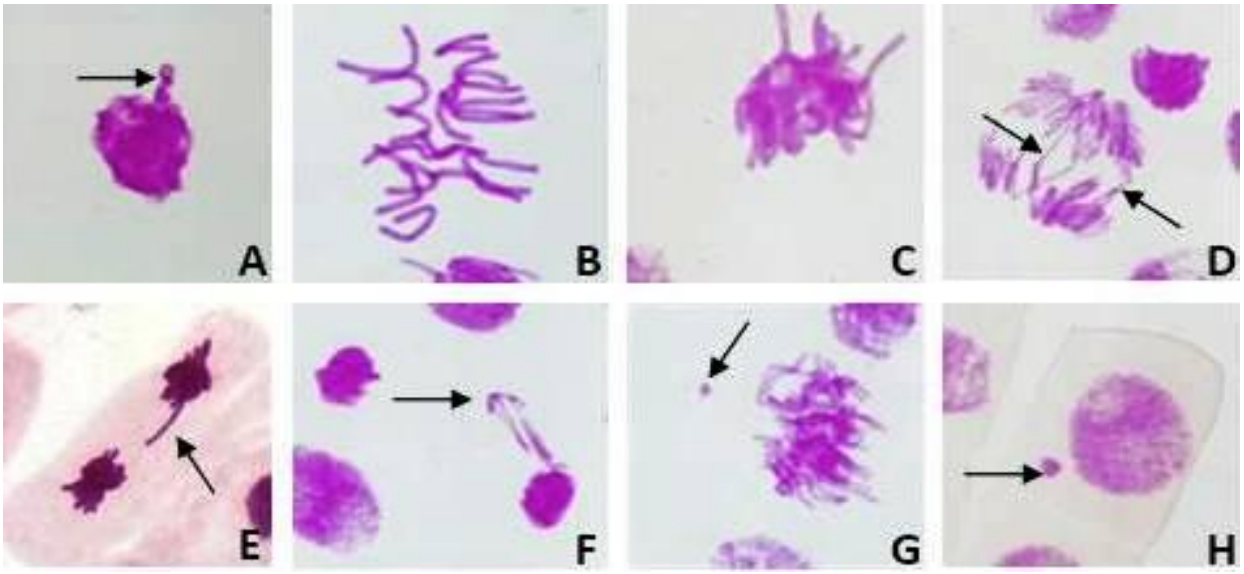

Figure 1 – Mitotic and chromosomal abnormalities obtained by meristem cells of *Allium cepa* exposed to water samples . A. nuclear bud (arrow); B. C-metaphase; C. Metaphase with Tack, D. Polyploidy, multipolar anaphase with chromosome bridge (arrows); E. Late telophase with chromosome (arrow); F. Telophase with chromosome loss; G. Polyploid metaphase with chromosomal breakage (arrow); I. Interphase cells with micronuclei (arrow).

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