



Thesis Abstract

Assessment of the mutagenic, genotoxic and cytotoxic potential of the waters of the Preto River in the area influenced by São José do Rio Preto, SP

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Due to increasing population and industrial expansion observed in recent decades, there has been a substantially greater burden on the environment due to domestic, industrial and agricultural sewage, which can have a severe impact on ecosystems and pose potential damage to human health as well. A wide range of harmful pollutants can be found in domestic effluent, such as chemicals from various categories, in addition to contamination by various biological agents. On the other hand, industrial effluents contain organic and/or inorganic pollutants, depending on the industrial activity. Based on these findings, this study aimed to investigate, by means of biological tests with two test organisms, the possible presence of contaminants with cytotoxic, genotoxic and mutagenic potential, which are dumped along the Preto River, an important river that flows through the region of São José do Rio Preto, SP. The biological material used in this study consisted of seeds of *Allium cepa* (onion) and one species of fish (Tilapia: *Oreochromis niloticus*). Water samples were taken seasonally in August 2006 and 2007 (dry season) and March 2007 and 2008 (rainy season), at six different sites: Site 1 (S1), 8 km before the damming; Site 2 (S2), 1 km before the damming; Site 3 (S3), place of sewage discharge; Site 4 (S4), opposite bank of sewage discharge; Site 5 (S5), end of the damming; Site 6 (S6), 1 km after damming. Chemical analyses were performed for all collected samples. For the study, 100 seeds of *A. cepa* were allowed to germinate on Petri dishes with water samples from the six different sites along the Preto River and ultra-pure water (negative control), and in the presence of an aneugenic substance (Trifluralin - positive control). For most of the collection points and periods studied, root meristem cells of *A. cepa* exposed to the water samples collected along the Preto River showed no significant differences in mitotic index when compared to the negative control. In addition to the cytotoxic potential, the genotoxic potential of chemicals in the samples of water was evaluated. The most frequent chromosomal aberrations were: C-metaphases, anaphase bridges, chromosome breaks and losses, and binucleate cells. Our results showed significant genotoxicity for the periods

of August 2006 (for S3, S5, and S6), March 2007 (for S1, S2, S3, and S6), August 2007 (for S1, S3, S4, and S6), and March 2008 (for S1, S3, and S6). Significant values of mutagenicity were also recorded in August 2006 (for S6) and August 2007 (for S3 and S6), obtained by the micronucleus test. Alteration in nucleolar morphology was another parameter used to evaluate the cytotoxicity of pollutants in the water samples. In August 2006, the river water showed an increase in nucleolar area in *A. cepa* cells in relation to the negative control. In March 2007, only the cells exposed to the water of S3 showed increased nucleolar area. In August 2007, the river water caused a decrease in the area of a single nucleolus for S3, while increased area in a single nucleolus was found in S6, when compared to the negative control. A decrease in the number of cells containing a single nucleolus was observed for tests with samples of all locations and periods, accompanied by an increased number of nucleoli per cell. Blood samples of fish after a 72-h exposure to the various water samples were taken for the preparation of blood smears on slides. All analyses showed non-significant results concerning micronucleated cells in the control group, with the exception of cells exposed to the water sample of S3 (collected in August 2007). Nuclear changes were also recorded. These changes were notched, lobed, broken-egg, and blebbed nuclei, karyolysis, and binucleate cells. Analysis of the comet assay scores showed significant results for S3 and S6, for all periods of the study, confirming the genotoxic action in *A. cepa*. These results can be explained by the fact that these collection sites are at the end point of the runoff network. Our data suggest that waters of the Preto River may be hazardous due to contamination, mainly by urban and industrial effluents, and also serve as a warning for the necessity of better monitoring of water resources for their safe use.

Key words: *Allium cepa*; *Oreochromis niloticus*; Chromosomal aberrations; Nuclear abnormalities; Comet assay; Water pollution