Ecotoxicology and Environmental Safety 74 (2011) 826–833

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

# Genotoxicity and mutagenicity of water samples from the Monjolinho River (Brazil) after receiving untreated effluents

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ARTICLE INFO

*Article history:*
Received 16 August 2010
Received in revised form
8 November 2010
Accepted 22 November 2010
Available online 21 December 2010

*Keywords:*
*Allium cepa*
Chromosome aberration
Micronucleus
Cytotoxicity
Aquatic toxicology

ABSTRACT

Cytotoxicity, genotoxicity and mutagenicity assays, using the *Allium cepa* test-system, were carried out in order to evaluate the effects of domestic and industrial effluents in the Monjolinho River in different seasons of the year. In the summer and intermediate seasons, chromosome aberration, micronuclei, cell death and inhibition of the mitotic index were observed in water samples collected at different sites. In the winter, either chromosome or cellular alterations were not observed. Through chemical analysis, we infer that the excessive metals such as Pb, Ni and Cu were mainly responsible for the effects observed in *A. cepa* cells. Limnologic analysis like electrical conductivity, dissolved oxygen and the presence of nitrogen and phosphated compounds showed that the river's contamination is due to organic matter discharge along its course. Moreover we note that this river had a higher self-depurative capacity at the end of its course, before its confluence with the Jacaré-Guaçu River.

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## 1. Introduction

Monjolinho River is responsible for the São Carlos (São Paulo, Brazil) city's water supply and receives contributions from various tributaries impacted by domestic and industrial sewage containing oil, dies, metals, starch, pesticides, among other substances (Campagna et al., 2008; Côrtes et al., 2000; Espíndola et al., 2000; Fracácio, 2006; Pelaéz-Rodriguês, 2001). São Carlos has an industry-based economy. It has over 700 business establishments (SEADE, 2008), being the greatest part represented by the metallurgical industry, transformation of non-metallic minerals (Côrtes et al., 2000).

Toxic substances in the ecosystems are often persistent in the environment and affect not only the fauna and flora associated with them, but human beings as well, both by food and water supplies (OECD, 1994). Aquatic environments, which often serve as temporary or final receptors of a great variety and high amount of contaminants (Gherard-Goldstein et al., 1990; Marinelli et al., 2000), end up serving as a means of transport for several toxic substances, which consequently contaminate all the watershed (Marinelli et al., 2000).

Domestic wastewater is composed of highly varied substances with considerable amounts of suspended matter and heavy metals (White and Rasmussen, 1998). Industrial discharges may even contain other substances such as dies (Caritá and Marin-Morales, 2008), petroleum-derived hydrocarbons (Hoshina and Marin-Morales, 2009; Hoshina et al., 2008; Leme and Marin-Morales, 2008), chromium (Matsumoto et al., 2006), among others, which are capable of causing serious DNA damage to the cells of exposed organisms.

Current concerns about more efficient and complex evaluating methods of environmental impacts are due to the fact that contaminants, when introduced into the aquatic system, present toxicity by its own presence or by compounds formed during their degradation process; and it may interfere in the organisms' physiology, influence both genetic and survival aspects and, consequently, alter population structure (Fracácio et al., 2000).

In order to evaluate genotoxic effects of environmental chemical substances, different test-systems have been used. Among the biological systems, high plants are, according to Grant (1994), considered excellent genotoxicity indicators both for its high sensitivity to detect mutagens in different environments and for its capacity to evaluate such effects by means of several genetic endpoints, from point mutations to chromosome aberrations. Among high plants, the species *Allium cepa* is recognized as one of the best test-systems used to evaluate genotoxic potential of environmental substances due to its high sensitivity, good correlation with other test-systems, easy handling, low cost, as well as for having large chromosomes with a reduced number ($2n=16$) (Fiskesjö, 1985; Leme and Marin-Morales, 2009).

Several authors have shown the efficiency of this test-system in the detecting toxic, cytotoxic, genotoxic and mutagenic effects of urban and industrial effluents and pesticides, which cause great

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doi:10.1016/j.ecoenv.2010.11.006

environmental impacts when they reach water resources. Leme and Marin-Morales (2008) detected the presence of chromosome aberrations and micronuclei in meristematic cells of *A. cepa* root tips exposed to water samples from the Guaecá River (Brazil), which was impacted by an oil spill containing mainly total petroleumand polycyclic aromatic hydrocarbons. By using the same test-system, Hoshina and Marin-Morales (2009) also observed genotoxic and mutagenic effects upon the Atibaia River's water (Brazil) after the effluents of a petroleum refinery had been discharged into it. These results confirm the good sensitivity of *A. cepa* to detect genetic effects induced by petroleum derivatives on environmental samples.

Genotoxic and mutagenic evaluation of effluents containing metals can also be efficiently done through *A. cepa* cell assays, as shown in the study carried out by Matsumoto et al. (2006). The authors collected water samples at the Bagres Stream (Brazil), which receive effluents from chromium-contaminated tanneries, and observed significant induction of chromosome aberrations and micronuclei. Studies carried out by Vujošević et al. (2008) at the Sava River (Croatia), impacted by urban, industrial and agricultural effluents, showed that the *A. cepa* test-system can be successfully used to detect toxic, cytotoxic and mutagenic effects upon river waters containing a mix of contaminants from different sources. The authors observed inhibition of root growth, mitotic index variations and high frequencies of chromosome aberrations and micronuclei in the cells of this test-organism.

The city of São Carlos (Brazil), considered an important technological center in the state of São Paulo, has the Monjolinho River as its main source of water supply. This river receives several effluents from the city of São Carlos and, therefore, the quality of its water has been increasingly impaired. In order to monitor its water quality, in this study, assays indicators of cell death, chromosome aberrations and micronuclei in meristematic cells of *A. cepa* have been developed.

## 2. Materials and methods

### 2.1. Collection sites of water samples

In 2005, water samples were collected at six different sites of the Monjolinho River in different seasons of the year (spring, summer, autumn and winter). The collection sites were chosen according to the water and sediment quality described by Barreto (1999), Fracácio et al. (2000) and Marinelli et al. (2000), as follows: site 1 (P1), approximately 3 km away from the main source of the Monjolinho River; site 2 (P2), at the entrance of São Carlos municipality's urban area; site 3 (P3), inserted into an urbanized area; site 4 (P4), middle-upper part of the Monjolinho River, right after it drains the urban area, before the first site into which *in natura* domestic sewage is discharged; site 5 (P5), middle part of the river, located in a rural area, downstream the first *in natura* sewage discharging site in the municipality; site 6 (P6), located in a rural area, a few meters upstream the river mouth, where it flows into the Jacaré-Guaçu River (Fig. 1).

### 2.2. Test-organism

All assays were carried out with only one variety (Baia Periforme) of *A. cepa* seeds to avoid different responses in the several stages of the tests.

### 2.3. Cytogenotoxicity assays

*A. cepa* seeds were placed into ultra pure water (Milli-Q) to germinate up until the roots got 1 cm long. Later on, the roots were placed on individual Petri dishes containing water from collection sites, where they were left for 20 h. Control tests were carried out by using methyl methanesulphonate—MMS (positive control—PC) in the 10 mg/l concentration and ultra pure water (negative control—NC).

Once this time was over, part of the roots in all assays was collected and fixed. The rest of the material was transferred to the Petri dishes containing ultra pure water for a 48-hour recovery period. After that, new collections and fixations were done for all the assays. The materials were fixed in alcohol-acetic acid (3:1), for 6 to 18 h at room temperature. Next, the fixator was replaced for freshly prepared fixator and materials were stored in a refrigerator.

For slide preparation, the previously fixed root tips were washed in distilled water and hydrolyzed in HCl 1 N for 8 min. The roots were then incubated in Schiff's reagent for approximately 2 h. Next, the meristematic regions were cut, covered with a coverslip and carefully squashed into a drop of 2% acetic carmine solution. Five slides were prepared per each collection site, using five root tips derived from five different individuals, for both treatment periods (20 and 48 h).

About 1000 cells from each slide were analyzed, totaling around 5000 analyzed cells per each collection site.

Cytotoxic effects were analyzed by quantifying both mitosis cells and cells in death process. Cell death was considered when cells showed vacuolated and swollen cytoplasm or heteropycnotic, condensed and/or fragmented nuclei (Majno and Joris, 1995). Genotoxic effects were quantified by Chromosomal Aberrations (CA) analysis such as C-metaphases, chromosome adherences, multipolar anaphases, chromosome bridges, polyploidy, bi-nucleated cells, chromosome losses and breaks and micronuclei (MN) in the different mitosis phases. CA, MN and CD (cell death) frequencies and mitotic index (MI) were calculated according to the formula: frequency $=(A/B)\times 100$; where $A$ is equivalent to the total number of cells with a parameter to be analyzed (CA, MN, CD and mitotic cells), and B corresponds to the entire number of analyzed cells. All the obtained results were statistically compared by means of the Mann–Whitney test, considering significant all values with a lower than 0.05 significance level.

### 2.4. Physical–chemical analysis of the water

Determining some physical–chemical parameters of the water can help understand the obtained results, once the water quality alterations may interfere in metabolism or change chemical and structural properties of molecules in the exposed biological systems. In this study, pH, electrical conductivity, dissolved oxygen and water temperature of the analyzed sites were determined by means of *in situ* monitoring using the Horiba U-10 water quality checker with multiple sensors. Metals (atomic absorption spectrophotometry, in blaze and graphite furnace, according to APHA, 1995) and inorganic nutrients (inorganic phosphates, total phosphorus, nitrites, nitrates and ammonia) present in the water were also evaluated (according to APHA (1995), Golterman et al. (1978), Koroleff (1976), and Mackereth et al. (1978)). For metal analyses, the water samples were fixed with P.A. nitric acid (1.5 ml/l) and kept in the refrigerator for later analysis. For nutrient analysis, water samples were frozen up until analysis time, which was done using the colorimetric method (UV–vis spectrophotometers).

Rainfall was obtained by measuring daily heights of rain throughout the year 2005.

## 3. Results

### 3.1. Cytotoxic, genotoxic and mutagenic effects

Cell analysis of *A. cepa* root tips exposed to different water samples collected from the Monjolinho River showed that during the summer (warm and wet season), the water samples interfered in cell division of the test-organism, inducing CA, MN, MI alterations and CD in at least one of the studied sites. In the winter (cold and dry season), the CA, MN, MI and CD results did not differ from the ones observed for an NC. In the autumn (transition between summer and winter), significant CA presence was observed for water samples collected at P5. The water samples collected at all sites in the spring (transition between winter and summer) presented potential to inhibit cell division. Water samples from P2 and P6 also induced CD (Table 1).

After *A. cepa* root tips were submitted to water samples collected at the Monjolinho River, they were transferred to an ultra pure water for 48 h. Cell analysis for this treatment did not indicate an MN induction for water samples collected in four seasons; however, they induced the CA formation for water samples from P2 (summer) and P4 (spring). CD analysis indicated that water samples from P3 and P4, collected in the summer, still induced cytotoxic effects upon *A. cepa*. For an MI, the inhibition of cell division was observed for water samples from P3, P4, P5 and P6 collected in the summer, and for water samples from P5 collected in the autumn (Table 1).

All the CA found in *A. cepa* cells, after being exposed to water samples from the Monjolinho River for both the 20-h tests and recovery period, are represented in Fig. 2.

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