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Genotoxic and mutagenic evaluation of water samples from a river under the influence of different anthropogenic activities

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HIGHLIGHTS

- *Allium cepa* and fish were exposed to water samples from a river with anthropogenic influence.
- Water samples with urban, automotive mechanical and family farm waste induced toxic and mutagenic effects.
- Toxic and mutagenic effects on organisms presented relationship with inorganic elements (Al, Si, Ti, Cr, Ni and Cu).
- *Allium cepa* and fish are sensitive instruments for assessment of environmental pollution.

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ABSTRACT

Pollution of aquatic ecosystems is associated with the discharge of mostly industrial and urban effluents, which may cause loss of biodiversity and damage to public health. This study aims to evaluate the toxicity and mutagenicity of water samples collected in the Corrente River, a major waterway in the river basin district of Pedro II, Piauí (Brazil). This river is exposed to intense anthropogenic influence from urban, automotive mechanical and family farm waste, and it is used as the main source of water supply by the population. Water samples were collected during the rainy and dry seasons, at four sites in the Corrente River, and evaluated by physicochemical, microbiological and inorganic elements analyses. The samples were evaluated for mutagenicity using the *Allium cepa* test (toxicity, chromosomal aberration and micronucleus tests) and fish (*Tilapia rendalli* and *Hoplias malabaricus*). The physicochemical, microbiological and inorganic results show a large contribution to the pollution loads at collection points in the town of Pedro II, demonstrating the influence of urban pollution. The Al, Si, Ti, Cr, Ni and Cu contents were determined by PIXE. These same Corrente River water samples demonstrated mutagenic effect for *A. cepa* and fish, as well as toxicity in the *A. cepa* test. The observations of mutagenic effect may suggest that the complex mixture of agents is comprised of both clastogenic and aneugenic agents. This study also showed the need for constant monitoring in places with environmental degradation caused by urban sewage discharges.

1. Introduction

Pollution of aquatic environments is a major problem of

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contemporary society. Population and industrial growth have contributed to this global concern. The main sources of water resources contamination are the discharges of industrial, agricultural and/or domestic effluents, which contain various kinds of chemical compounds. When they are in the aquatic environment, these compounds favor the formation of complex mixtures that can cause problems to health, human welfare and the organisms that inhabit or use it (Hering et al., 2015; Lorente et al., 2015).

Organisms inhabiting areas influenced by effluent discharges can suffer DNA damage, and humans using polluted water are at risk of similar genotoxic effects and of developing cancer (Landrigan and Fuller, 2014). The continued production and release of pollutants into the aquatic environment have made investigating the genotoxic potential of aquatic environments a major task of environmental pollution-control monitoring (Rajaguru et al., 2002). Epidemiological studies fail to evaluate environmental complex mixtures, because they do not characterize all effects, and it is very important to perform biomonitoring using different biomarkers in environments suffering various environmental stresses.

Although it has proven difficult to establish a direct linkage between the ecological effects of pollution and human health, the use of different species as bioindicators has provided the conceptual basis for this connection (Bickham et al., 2000). Among the genotoxicity biomonitoring methods that are widely used in the evaluation of aquatic environments are the micronucleus tests in *Allium cepa* and fish (Fiskesjö, 1993; Bolognesi and Hayashi, 2011; Nunes et al., 2011; Gutiérrez et al., 2015). The advantages of the *A. cepa* test are that it is a fast and inexpensive method, easy to handle, provides reliable results, comparable with other tests performed in mammalian systems (Fiskesjö, 1993; Leme and Marin-Morales, 2009). This test has been internationally validated as a bio-indicator of environmental samples and is used as a preliminary test to evaluate the genotoxic and cytotoxic potential of carcinogens and as an environmental pollution bioindicator (Leme and Marin-Morales, 2009). Assessing environmental risk also requires systems that quantitatively and qualitatively reflect the effects of exposure. Organisms that are in direct contact with contaminated environmental compartments are well suited for inclusion in such systems (Rajaguru et al., 2002). Fish are often used as biomonitoring because they play a number of roles in the trophic web, bioaccumulate toxic substances, and are sensitive to anthropogenic compounds (Al-Sabti and Metcalfe, 1995; Stegeman, 2000). This sensitivity may lead to induction of metabolizing enzymes, inhibition of reproduction and growth, accumulation of hazardous substances, and genetic damage (White and Rasmussen, 1998; Bickham et al., 2000; Andrade et al., 2004).

In northeastern Brazil, the quality of surface and groundwater is compromised due to human activities related to the improper disposal of domestic and industrial solid waste, indiscriminate use of agricultural inputs, deficiency of sanitation systems. The outstanding case is the state of Piauí, which has one of the least developed general sewage systems (4.9%) compared with the Brazilian average (44%) (Araújo et al., 1998; IBGE, 2008). The Corrente River, the river basin district of Pedro II (Brazil) receives wastes from urban, automotive mechanical and family farming. It flows into the Joana Reservoir that was inaugurated in 1996 for purpose of supplying water to the town of Pedro II (IBGE, 2012). In this scenario, the study aimed to evaluate the toxic and mutagenic potential of the different sources of pollution flowing into the Corrente River. For this, water samples were collected at two different periods of the year (rainy and dry seasons). Water samples were collected at four sites in the river and evaluated by the *A. cepa* test (toxicity test and micronuclei) and micronucleus test in redbreast *Tilapia rendalli* and *Hoplias malabaricus*.

2. Materials and methods

2.1. Sampling

Surface water samples were collected at four sites along the Corrente River (Pedro II) (Fig. 1). The municipality of Pedro II (geographic coordinates 04°25′29″South Latitude and 41°27′31″ West Longitude), with its main urban area at an altitude of 603 m, lies 220 km north-northeast of Teresina, the capital of the state of Piauí. It has a population of 37,500, distributed throughout an area of 1,518,186 km^2 (IBGE, 2012).

Table 1 describes each collection site. Samples were collected twice, in April 2011 (rainy season) and September 2011 (dry season). Rainfall during the rainy season was 41.2 ± 24.2 mm and during the dry season was 3.4 ± 1.3 mm. The averages were measured 15 days prior to collection.

The surface water samples were transported to the laboratory under refrigeration and stored at 4 °C for no longer than 4 days according to Standard NBR 9898 (ABNT, 1987). A 6 L volume of water samples was taken from each collection site, and placed in an appropriate flask for biological, physical and chemical analysis (Vargas et al., 2001).

2.2. Allium cepa test

The *A. cepa* test (modified version) was performed as described by Fiskesjö (1993), adapted as described by Nunes et al. (2011). Small bulbs from a population of the common onion (*A. cepa*), of the same variety and uniformly sized were chosen for the experiments. Bulbs were purchased from a single farmer who did not use pesticides. The outer brownish and dry scales and the brownish bottom plate were removed, the ring of the root primordium being left intact. Water samples were placed in ten different test tubes containing one onion bulb each. Then onions were placed directly in flasks with the controls and different samples, and left to germinate at 18–22 °C (Leme and Marin-Morales, 2009).

The negative control was water without chloride and the positive control was copper sulfate (0.0002 g/L). The positive control was selected according to Carvalho et al. (2011). After 2 days, when the roots had reached a length of 1.5–2.0 cm, three roots from each bulb were harvested during the second mitotic cycle to analyze microscopic parameters. The roots were immediately fixed in acetic acid and ethanol (1:3; v/v) for 24 h, transferred to 70% ethyl alcohol, and stored in a refrigerator. The onions were germinated for 5 more days and afterwards the root length of the three largest roots was measured and used as an index of toxicity.

Two fixed root tips from each bulb were rinsed in distilled water, hydrolyzed in 1 N HCl at 60 °C for 8 min and rinsed again to prepare the slides. The carmine acetic staining method was used to stain both the nuclear contents and the cell wall contour.

Microscopic slides were prepared by squashing the root tips in acetic acid 45% (Fiskesjö, 1993). The following microscopic parameters were observed: (a) the mitotic index (MI) (1000 cells per slide); (b) Micronuclei (MN); (c) chromosomal aberrations (CA); (d) Binucleated (BN) cells and; (e) Root length. MNs and BN cells were scored under an oil immersion lens (100×) and 2000 cells from each onion bulb were examined. The result of each test was taken into consideration only if the negative and positive controls yielded negative and positive results, respectively.

2.3. Micronucleus test in fish

The micronucleus (MN) test was performed according to published manuscripts (Al-Sabti and Metcalfe, 1995; Fenech, 2000; Andrade et al., 2004). Peripheral blood samples were drawn from