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Genotoxicity assessment in aquatic environment impacted by the presence of heavy metals

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ABSTRACT

The aim of this study was to access the genotoxic potential of Extremoz Lake waters in Northeastern Brazilian coast, using the *Allium cepa* system, piscine micronucleus test and comet assay. In addition, heavy metal levels were quantified by atomic absorption flame spectrometry. The results of the *A. cepa* system showed significant changes in the frequency of chromosome aberrations and in the mitotic index compared to negative control. No significant changes were observed in micronuclei frequency in the erythrocytes of *Oreochromis niloticus*. The comet assay showed a statistically significant alteration in the level of DNA breaks of *O. niloticus*. Chemical analysis detected an increase in heavy metal levels in different sampling periods. These results point out a state of deterioration of water quality at Extremoz Lake, caused by heavy metal contamination and genotoxic activity. It is recommended to establish a monitoring program for the presence of genotoxic agents in this water lake.

1. Introduction

Natural environments located near urban and industrial areas are often contaminated by pollutant discharges (Claxton et al., 1998; White and Rasmussen, 1998). These discharges may contain chemical agents that are not eliminated during effluent treatment, resulting in release of contaminants into the environment (Rank and Nielsen, 1998).

Physical–chemical analyses are often conducted in order to detect the presence of chemical agents potentially hazardous to the aquatic environment and to human health. However, the chemical identity of many substances released into nature, as well as their resulting metabolites, is still not well characterized, making it necessary to assess the potential impact caused by exposure to these chemical agents on aquatic organisms and water consumers (Van der Oost et al., 2003). The characterization of biological consequences of toxic exposure based only on chemical procedures is almost impossible due to their limitations in predicting synergic and antagonic effects of contaminants, in complex environmental mixtures (Helma et al., 1998). Biological assays are capable of characterizing the effects of environmental contaminants caused by chronic and/or acute exposure, without prior knowledge of the chemical components present in the water (Ohe et al., 2004).

Several studies have used genotoxicity tests as a tool for investigating the quality of groundwater and surface waters (Siddiqui and Ahmad, 2003; Egito et al., 2007; Hoshina et al., 2008). These tests are able to detect agents that potentially cause damage to DNA and adversely affect human health (Moller, 2005; Norppa, 2004) and the environment (Dixon et al., 2002). Exposure of individuals to genotoxic environmental chemicals has been associated to the occurrence of cardiovascular diseases, premature aging and the emergence of neoplasias (De Vizcaya-Ruiz et al., 2008; Farmer and Singh, 2008; Garinis et al., 2008). DNA damage in aquatic animals may be associated to reduced growth, abnormal development and decreased survival of embryos and adults (Lee and Steinert, 2003). Several studies have linked the induced chromosomal aberrations, DNA strand breaks and micronucleated blood cells with embryo developmental delay and changes in the structure and function of the reproductive endocrine system in fish species (Hagger et al., 2005; Keiter et al., 2006; Liney et al., 2006). These alterations may lead to a reduction

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in population size and even to species extinction (Bickham et al., 2000; Theodorakis, 2001).

Owing to the low concentrations at which some pollutants are found in the environment, a number of genotoxicity analyses require the extraction/concentration of environmental chemicals. However, during these procedures, some chemical agents may be modified or lost. The use of sensitive organisms, in short-term assays, such as plants and aquatic animals, including those performed as *in situ* tests, stands out as suitable alternatives (Majer et al., 2005).

Metals are natural components present in ecosystems. They are important elements, indispensable for biochemical and physiological processes in living beings. However, many of these elements, when found at high levels, may have adverse effects on human health. Several metals with toxic and/or genotoxic properties released into nature from domestic or industrial sources are risk factors for the development of neurodegenerative disorders, arthritis and cancer (Goyer et al., 2004; Il'yasova and Schwartz, 2005; Lu et al., 2005; Sivulka, 2005).

This article aimed to test the genotoxic activity of water samples from Extremoz Lake, Brazilian Northeast, which is an important water supply system for a population of thousands people. Also, levels of cadmium, lead, zinc, chromium, copper, nickel and manganese were measured.

2. Material and methods

2.1. Water sampling

Extremoz Lake is located in metropolitan region of Natal city, Rio Grande do Norte state, Brazil. It supplies around 160,000 residents and it is also used for recreation and fishing purposes. In recent years, urban growth and the establishment of an industrial park have threatened Extremoz Lake water quality and consequently pose a risk to population health and to the ecological balance of this important lake ecosystem.

Water samplings were carried out in April 2005, October 2006 and January 2008. Five different sites were sampled in the first sampling date (Fig. 1). Site 1 (5°43′19.81″S/35″17.14′26″W) and 2 (5°42′30.25″S/35°19′55.32″W) are located near tributaries rivers which supply the lake, site 3 (5°43′15.46″S/35°17′03.03″W) is located near the water collection by the water company for further treatment and supply of potable water , site 4 (5°43′05.10″S/35°16′59.60″W) probably receives effluents from beverages industry and site 5 (5°42′27.24″S/35°16.55′28″W) is located in convergence region of other four sampling sites. In samplings performed between October 2006 and January 2008 water collection was carried out only in site 5. The same local was chosen for fixation of the floater to conduct *Allium cepa* test.

2.2. Biological material for genotoxicity tests

Homogeneously sized *A. cepa* bulbs ($2n=16$) were acquired from local market at Natal city. *Oreochromis niloticus* (Perciformes, Cichlidae), popularly known as tilapia, were collected in the study area using fish nets. For the test groups 10 individuals were collected in September 2006 and 16 animals in July 2007. The negative control consisted of 11 individuals kept in aquaria with periodically renewed distilled water, and fed *ad libitum* for 6 months at 25 °C.

To carry out the genotoxicity tests, the blood of the fish was collected immediately after sampling by branchial puncture, using heparinized syringes, packed in heparinized microtubes and kept in ice during transport to the laboratory. The analyses conducted with blood samples were carried out at the same day of sampling.

The experiments with fish were conducted before the publication of Brazilian law no. 11794 in October 2008, known as Lei Arouca and the creation of Institutional Committee for Animal Experimentation. However, our procedure did not violate these regulations.

2.3. Water chemical analysis

The levels of cadmium, lead, zinc, chromium, copper, nickel and manganese were determined by atomic absorption flame spectrometry according to APHA (1998). Immediately after collection, the water samples were acidified. Samples were then submitted to acid digestion and subsequent concentration. The quantification of metals was performed on an atomic absorption spectrometer (Varian-AA50B). Each sample was quantified in duplicate.

2.4. A. cepa system

To characterize the cytotoxic and genotoxic potential of Extremoz Lake waters, the *A. cepa* test was conducted, according to Fiskesjo (1985) and Ma et al. (1995), *in situ* and under laboratory conditions, with some modifications. Initially, the bulbs were germinated in distilled water for 48 h at room temperature in laboratory. After germination, the bulbs were exposed in the laboratory to the water samples collected in the study area or transported to the site. For the *in situ* assay, bulbs were installed in a floater with their roots into the water where they remained exposed for 24 h.

These roots were collected immediately after the exposure period and fixed in ethanol–acetic acid (3:1) during 24 h, to analyze meristematic cells. After the 24 h recovery time the roots from the other group were also collected and fixed as previously described (analysis of region F1). After fixation, the roots were washed in distilled water and hydrolyzed in 1N HCl at 60 °C for 10 min and stained with Schiff reactive for 2 h. The roots were further washed in distilled water and placed on glass slides with a drop of 2% acetic carmine solution. After selection of the meristematic region or F1, the coverslip was placed on these regions and the cells were squashed over the slide surface by slight pressure. Ultrapure water and methyl methanesulphonate (MMS) 10 mg/L were used as negative and positive controls, respectively.

Genotoxic potential was assessed using analysis of chromosome aberrations and micronuclei in the root cells of *A. cepa* by observing 100 and 1000 cells in each bulb, respectively. Cytotoxic potential was determined by means of the mitotic index in slides prepared with the meristematic region, given that it exhibits intense mitotic activity. Mitotic index (MI) was defined as the number of dividing cells per 1000 cells. Both analyses were carried out under 400 × magnification in optical light microscope. Replicates of five bulbs were analyzed for each treatment.

2.5. Comet assay

To assess the levels of DNA damage induced by the chemical agents present in the Lake Extremoz waters, peripheral blood erythrocytes of *O. niloticus* were evaluated using the comet assay according to Tice et al. (2000).

Microscopic slides were previously coated with 1.5% normal agarose. A cell suspension containing the erythrocytes was prepared by total blood dilution in low melting point agarose 0.75% in phosphate-buffered saline (PBS; pH 7.4)

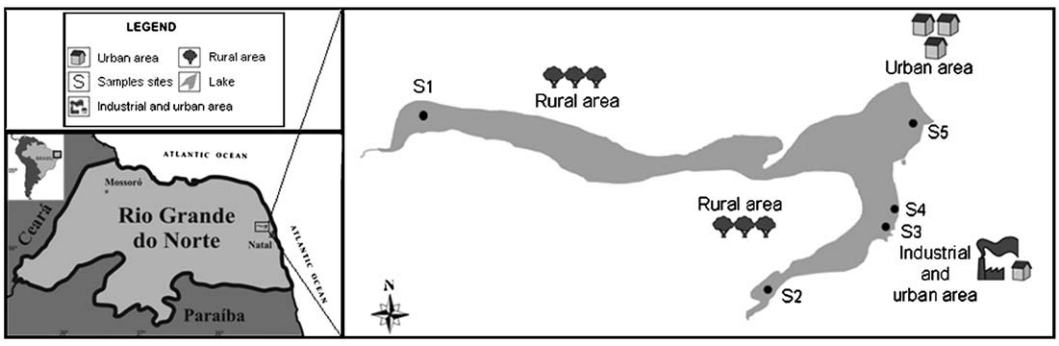

Fig. 1. Map of the sampling sites (S1–S5) on Extremoz Lake, RN, Northeastern Brazil.