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The influence of heavy metals on toxicogenetic damage in a Brazilian tropical river

Leomá A. Matos [a], Ana C.S. Cunha [a], Aline A. Sousa [a], Jéssica P.R. Maranhão [a], Nathanyel R.S. Santos [a], Matheus de M.C. Gonçalves [a], Sandra M.M. de M. Dantas [a], João M. de C. e Sousa [b, c, *], Ana P. Peron [b], Felipe Cavalcanti Carneiro da Silva [b, c], Marcus V.O.B. de Alencar [c], Md. Torequl Islam [c], Raí Pablo Sousa de Aguiar [c], Ana A. de C. Melo-Cavalcante [c], Claúdia C. Bonecker [d], Horácio F.J. Junior [d]

[a] Departamento de Ciências Biológicas, Universidade Federal do Piauí, Campus Ministro Petrônio Portela, Av. Universitária, CEP - 64.049-550, Brazil
[b] Departamento de Ciências Biológicas, Universidade Federal do Piauí, Campus Senador Helvídio Nunes de Barros, Av. Cícero Duarte, Picos, Piauí, CEP - 64600-000, Brazil
[c] Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Piauí, Campus Ministro Petrônio Portela, Bairro Ininga, Teresina, Piauí, CEP - 64049-550, Brazil
[d] Departamento de Ciências Biológicas, Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, Av. Colombo 5790, Maringá, Paraná, CEP - 87020-900, Brazil

HIGHLIGHTS

- Poti river carries polluted water.
- Poti river water exhibited genotoxic effects in *Oreochromis niloticus.*
- Heavy metal-induced significant toxic effects.

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ABSTRACT

Anthropogenic activities in tropical rivers favor the eutrophication process, which causes increased concentration of heavy metals. The presence and bioaccumulation of metals are directly related to the presence of genotoxic damage in aquatic organisms. Thus, we evaluated the presence of heavy metals (Fe, Zn, Cr, Cu and Al) and performed toxicogenetic tests in surface (S) and bottom (B) of water samples of the Poti river (Piaui/Brazil). Cytotoxicity and genotoxicity tests were performed in *Allium cepa*, and micronucleus (MN) and comet assay were performed in *Oreochromis niloticus.* The chemical analysis showed concentrations above the limit for Cu, Cr, Fe and Al according to Brazilian laws, characterizing anthropogenic disturbance in this aquatic environment. Toxicogenetic analysis presented significant cytotoxic, mutagenic and genotoxic effects in different exposure times and water layers (S and B), especially alterations in mitotic spindle defects, MN formations, nuclear bud and DNA strand breaks. Correlations between Fe and cytotoxicity, and Al and mutagenicity were statistically significant and point out to the participation of heavy metals in genotoxic damage. Therefore, Poti river water samples presented toxicogenetic effects on all bioindicators analyzed, which are most likely related to heavy metals pollution.

1. Introduction

All living organisms are interacting with the aquatic environment, and environment degradation by human activities may cause DNA damage in these aquatic organisms (Akinboro et al., 2011; Nunes et al., 2011). Changes in the rate of cell division and/or

* Corresponding author. Departamento de Ciências Biológicas, Universidade Federal do Piauí, Campus Senador Helvídio Nunes de Barros, Av. Cícero Duarte, Picos, Piauí, CEP - 64600-000, Brazil.
E-mail address: jmarcelo@ufpi.edu.br (J.M.C. Sousa).

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DNA structure are harmful to the cells, which can interfere with vital processes such as DNA replication and gene transcription. In addition, these alterations may also cause gene mutations and chromosomal aberrations that contribute to cancer development and cell death (Ossana et al., 2013). The detection of pollutants in aquatic environments, and their likely effects on organisms are important for studying the impact on animals, plants and especially human population (Leme and Marin-Morales, 2009).

Heavy metals are potentially genotoxic and carcinogenic, and are known as oxidative stress inducers, stimulating the production of reactive oxygen species (ROS), which cause DNA damage and cell death (Lushchak, 2011). Heavy metals bioaccumulate in the environment and may increase the risk of various degenerative diseases, including cancer (Beyersmann and Hartiwig, 2008). The presence of high concentrations of heavy metals is related to the drastic reduction of water quality and mainly related to human activities (Kumar et al., 2015).

The complexity of the pollutants in environmental samples demands a multitude of genotoxicity tests, with increasing simplicity, sensitivity, and affordability (Tabrez et al., 2011). In this sense, to evaluate toxicogenetics effects of complex mixtures from river water samples, ecotoxicological tests (cytotoxicity, genotoxicity and mutagenicity) are carried out in microorganisms, animal cells and plants, alone or combined (Žegura et al., 2009; Mazzeo et al., 2013).

In aquatic environments, fishes are often used as biological indicators of water quality, and biomonitors for the presence of metals and pollutants (Torres de Lemos et al., 2007). Fishes also provide information of pollutants' bioavailability that contribute to the process of biomagnification (metals) and the risks for human health, since is part of human diet. Data from bioassays using fishes have shown good correlation with genotoxicity in human cells exposed to mutagens (Marcon et al., 2010).

Plants are also excellent biological systems, because they are good bioindicators of toxicity, with high sensitivity to detect cytotoxic and mutagenic agents through different genetic mechanisms, including point mutations and chromosomal aberrations. The impact of the mutagens in these tests is indicated by the inhibition in the growth of root and shoot (Siddiqui et al., 2011a, b). The species *Allium Cepa* (2n = 16) is one of the best systems for evaluating cytotoxicity and mutagenicity of environmental substances (Leme and Marin-Morales, 2009), and is widely used in monitoring the effect of pollutants, including heavy metals, cyanotoxins and hydrophilic and lipophilic chemicals (Bianchi et al., 2011).

The Poti river, a tropical and shallow river from the semi-arid region of Brazil, has lentic characteristics, and is stratified mainly during the dry season. The river runs through the urban area of Teresina (Piauí, Brazil), and has suffered direct consequences from city development, mostly because of an incomplete sewage system. Low levels of sewerage coverage impair the river by increasing the pollutant load, especially during the dry season, reducing the water quality index (WQI) as a result of its artificial eutrophication (Silva et al., 2010).

Fast urbanization and industrialization have resulted in the tremendous release of xenobiotic compounds into the environment (Tabrez and Ahmad, 2011). Thus, it is suggested to carry out studies to detect the presence of metallic pollutants and other chemical and biological contaminants in the aquatic environment and their possible toxicogenetics effects. In this sense, this study aimed to evaluate genotoxic, mutagenic and cytotoxic effects in water samples of the Poti river within the urban area of Teresina, and correlate the possible genetic damage to metallic pollutants, including aluminum (Al), copper (Cu), chromium (Cr), iron (Fe) and zinc (Zn).

2. Materials and methods

2.1. Study area and collection points

The Poti river is located in northeast of Brazil, and its source flows from the state of Ceará to Piauí with its mouth in the city of Teresina (Piauí, Brazil), where it find its affluent, the Parnaiba River. Poti River is classified according to the Brazilian laws (CONAMA, 2005), as class 2 river (intended for human consumption, agriculture, recreation and fishing activity). The three sampling sites selected for the river are equidistant from each other, within 26 km of urban area (Fig. 1). Point 1 (P1) is located at 5°6′53.16″S and 42°43′52.81″W; Point 2 (P2) is located at 5° 3′51.50″S and 42°48′24.82″W and point 3 (P3) is located at 5°2′2.76″S and 42°49′48.57″ W, approximately 1000 m from its mouth. The P1 is upstream the urban area of Teresina, being influenced only by rural communities and sand and rock extractors; P2 is located within the city, which represents the intermediate zone of the city, and is influenced by almost two thirds of the urban area; and P3 is located downstream the city, and has suffered influence of dredging and horticulture activities. Each point was evaluated for both surface (S) and bottom (B) water sampling.

Water samples used as controls (positive and negative) for testing *Oreochromis niloticus* and *Allium cepa* were from fish farming tanks and dechlorinated water, respectively. Collection of water samples were in September 2014, during dry season (hottest period of the year) (Oliveira and Silva, 2014).

2.2. Chemical analysis water

Water samples were collected manually, in triplicates, at 25 cm from the surface (S) and near the bottom (B) of each point. For sampling, it was used polyethylene bottles (500 ml) for surface collections and Van Dorn bottle with horizontal flow (5 L) for bottom collections. Immediately after collection, samples were stored and chilled to 4 °C, transported and analyzed, within 24 h, in order to evaluate the levels of iron (Fe) (mg/l), zinc (Zn) (mg/L), copper (Cu) (mg/L) and chromium (Cr) (mg/L) through flame atomic absorption spectrophotometry (according to APHA et al., 2005). Water samples were acidified and, subsequently, subjected to acid digestion and concentration for flame atomic absorption spectrophotometer (Varian-AA50B model) analysis. Aluminum (Al) (mg/L) quantification was determined by the Standard Methods for the Examination of Water and Wastewater (Rice et al., 2012). Water samples from local fish farming tanks were used as control (CO). The sum of all metals' concentrations by point and layers were considered as accumulated metals (Marcon et al., 2010).

2.3. Bioassay O. niloticus

Specimens of *Oreochromis niloticus* from local fish farming tanks with approximately same weight (300 g), size (15–20 cm) and age (2 months), were used for the comet assays (genotoxicity), micronucleus (mutagenicity) and nuclear/cellular abnormalities (cytotoxicity) tests. Fishes were acclimated (29 ± 2° C, pH 7.8 ± 0.3) in 350 L tank (Duarte et al., 2012) and subsequently transferred to aquarium filled with Poti river waters.

A total of 24 tanks of 15 L were used, where each aquarium received one fish, and each point/layer was measured in triplicates (NC, P1S, P1B, P2S, P2B, P3S, P3B and PC). One third of the aquarium water was renewed daily, during the 6 days of exposure time (144 h). Water from fish farming tanks was used for negative (NC) and positive (PC) controls. Cyclophosphamide (4 mg/L), injected intraperitoneally and below the pectoral fin, was the cytotoxic,