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Cytotoxic, genotoxic and mutagenic evaluation of surface waters from a coal exploration region

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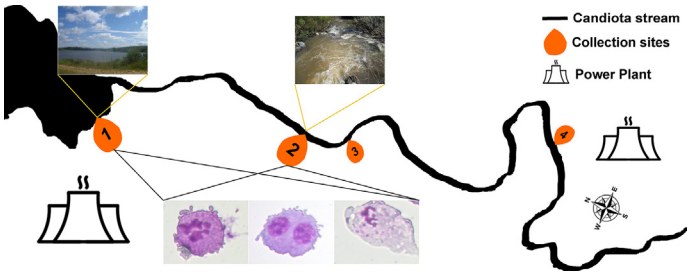
HIGHLIGHTS

- Toxicity of surface waters impacted by coal exploration was investigated *in vivo* and *in vitro*.
- Water samples inhibited cell proliferation.
- Water samples increased frequencies of V79 cell death, apoptosis, and necrosis.
- No genotoxic and mutagenic activities were observed.

GRAPHICAL ABSTRACT



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ABSTRACT

Coal mining generates a considerable amount of waste, which is disposed of in piles or dams near mining sites. As a result, leachates may reach rivers and streams, promoting the wide dispersion of contaminants in solution and as particulate matter. The present study evaluated the cytotoxic, genotoxic, and mutagenic action of surface waters collected around a thermoelectric power plant and the largest mining area in Brazil (Candiota). Four sites in Candiota stream were selected, and samples were collected in winter and summer. Water samples were analyzed using the comet and CBMN assays in V79 and HepG2 cells. Furthermore, genotoxicity of water samples was evaluated *in vivo* using the SMART in *Drosophila melanogaster*. In addition, polycyclic aromatic hydrocarbons and inorganic elements were quantified. The results indicate that water samples exhibited no genotoxic and mutagenic activities, whether *in vitro* or *in vivo*. On the other hand, surface water samples collected in sites near the power plant in both summer and winter inhibited cell proliferation and induced increased frequencies of V79 cell death, apoptosis, and necrosis. The cytotoxicity observed may be associated with the presence of higher concentration of inorganic elements, especially aluminum, silicon, sulfur, titanium and zinc at sites 1 and 2 in the stream, as well as with the complex mixture present in the coal, in both seasons. Therefore, the results obtained

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point to the toxicity potential of water samples with the influence of coal mining and combustion processes and the possible adverse effects on the health of exposed organisms.

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1. Introduction

Coal comes second after oil as energy source in the world, meeting 25% of the global demand. In Brazil coal supplies 6% of the country's total energy consumption, and is used mainly as fuel in thermoelectric power plants. The country holds 0.1% of the world's coal reserves, 89.25% of which is located in Rio Grande do Sul (RS) state. Importantly, located in RS, Candiota is the city where Brazil's largest coal field is found, with about 12 billion tons that represent 38% of the country's coal (ANEEL, 2008).

Despite its importance as energy resource, the extraction, processing, and use of coal pose potential pollution hazards. Large quantities of coal dust particles are emitted during coal extraction and processing, increasing environmental pollution. Coal is a complex, heterogeneous mixture of organic and inorganic components, though heavy metals may also be present. The main organic components of coal are hydrocarbons, which may generate polycyclic aromatic hydrocarbons (PAHs), the major source of organic pollution caused by the fuel (Liu et al., 2008).

Acid mine drainage (AMD) is mainly generated by pyrite tailings by percolation of rainwater in coal mine residues (Akcil and Koldas, 2006). When AMD reaches surface and/or groundwater bodies, it may degrade water quality (Banks and Banks, 2001), altering water pH levels and releasing heavy metals, whose high toxicity potential has been widely described (Bell and Bullock, 1996; Luptakova et al., 2012).

The impact of coal extraction, processing, and use on water sources, soil, air quality, and biota has been described in the region of Candiota (Braga et al., 2004; Divan Junior et al., 2009). Interaction of coal and combustion by products with biological systems induces genotoxic or mutagenic effects in several organisms, such as mollusks (Leffa et al., 2010; De Souza et al., 2015), rodents like *Ctenomys torcuatus* (Da Silva et al., 2000), *Rattus rattus* and *Mus musculus* (León et al., 2007) as well as bats (Jose Zocche et al., 2010), human cells (Menezes et al., 2013) and occupationally exposed humans (Celik et al., 2007; León-Mejía et al., 2011; Rohr et al., 2013; Leon-Mejia et al., 2014).

Considering the lack of studies about the biological effects of water samples from coal mining and burning regions and taking into account that the water used to supply Candiota city is pumped from Candiota Stream, the present study evaluated the cytotoxic and genotoxic effects both *in vitro* and *in vivo* of surface water collected in Candiota Stream at different distances from the power plant.

2. Materials and methods

2.1. Collection sites and sampling

Four collection sites were selected on the vicinity of the PresidenteMédici thermoelectric power plant stream, in the city of Candiota (Rio Grande do Sul, Brazil) aiming at to evaluate the influence of prevailing wind on pollutant dispersion. Latitude and longitude of collection sites were as follows: (S1) Site 1, 31°32′23.42″S and 53°40′30.62″ (before power plant); (S2) Site 2, 31°33′23.10″S and 53°40′16.52″ (in front of power plant); (S3) Site 3, 31°33′37.24″Sand 53°39′59.18″O (200 m after power plant); (S4) Site 4,31°34′10,04°S and 53°39′43.01″O (5 Km after power plant). The fourth site was located in the watercourse.

Surface water samples (1000 mL) were collected in two seasons, summer and winter in appropriately tagged flasks (Eaton et al., 2005) and transported to the TOXIGEN laboratory at ULBRA following standard procedures.

2.2. Physicochemical parameters

The physicochemical variables evaluated were pH, alkalinity, water and air temperature, water hardness, chlorides, conductivity, total fluoride, total orthophosphate, nitrate, nitrite, Kjeldahl total nitrogen, sulfate, and chemical oxygen demand (COD).

2.3. Quantification of inorganic chemical elements

Water samples were filtered using the vacuum-filtration method and filters were submitted to particle-induced X-ray emission (PIXE) analyses. The experiments were carried out at the Ion Implantation Laboratory (Institute of Physics, Federal University of Rio Grande do Sul). The PIXE technique provides multi-elemental analysis in a straightforward manner by identifying characteristic X-rays emitted from a sample irradiated with a proton beam (Johansson et al., 1995). A 3-MV Tandetron accelerator provided 2 MeV proton beams with an average current of 3 nA and 0.5 nA for the irradiation of water and sediment samples respectively. The samples were irradiated during 400 s. During the experiments, the pressure inside the reaction chamber was kept at approximately 10^{-6} mbar. The X-rays produced by the samples were detected using a Si (Li) detector with an energy resolution of approximately 150 eV at 5.9 keV. The PIXE system was calibrated using a range of reference materials. The standardization procedure adopted in this work followed the methodology described by Johansson et al. (1995) and includes all relevant experimental parameters for the analysis of the PIXE spectra. For the quantitative analysis, the spectra were fitted with the GUPIXWIN software package (Campbell et al., 2000). The data are expressed as ng/cm^2.

2.4. Quantification of PAHs

The PAHs analyzed in this study were: acenaphthalene, acenaphthene, anthracene, benzo (a) anthracene, benzo (a) pyrene, benzo (b) fluoranthene, benzo (g, h, i) perylene, benzo (k) fluoranthene, crisenodibenzo (a, h) anthracene, phenanthrene, fluoranthene, fluorene, indene (1,2,3 cd) pyrene, naphthalene, and pyrene, according to EPA recommendations. PAHs were analyzed through the 3510C (1996) and 8270D (2007) methods by gas chromatography/mass spectrometry (GC/MS) technique.

2.5. Cell culture

V79 and HepG2 cell lines were purchased from Banco de Células do Rio de Janeiro (Rio de Janeiro, Brazil). Cells were cultured in 75-cm^2 culture flasks (TPP) with DMEM medium (Gibco) supplemented with 10% of fetal bovine serum (FBS) (Cultilab) and antibiotics (1% of penicillin/streptomycin solution and 0.1% of gentamycin solution, both from Gibco). Cells were maintained at

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