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In vitro genotoxic effect of secondary minerals crystallized in rocks from coal mine drainage

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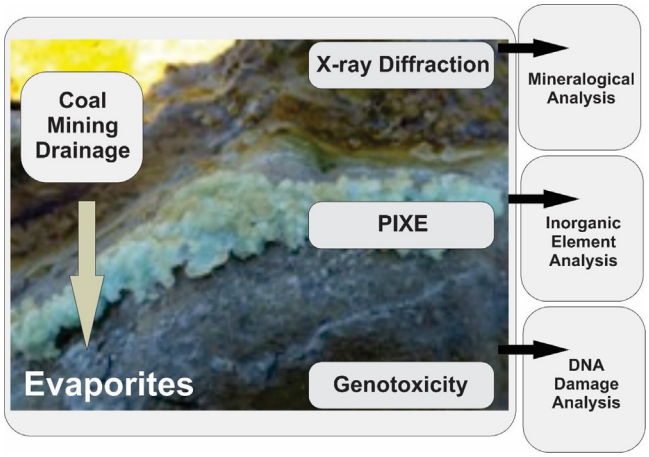
HIGHLIGHTS

- Melanterite, halotrichite, hematite, gypsum and halite were found in evaporite.
- DNA damage increased in V79 cells exposed to evaporites samples.
- High concentrations of chromium, iron, nickel and zinc were observed in evaporites.
- Evaporites of coal area have been assessed and characterized for the first time.

GRAPHICAL ABSTRACT



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ABSTRACT

Coal processing generates a large volume of waste that can damage human health and the environment. Often these wastes produce acid drainage in which several minerals are crystallized (evaporites). This study aimed to identify secondary minerals, as well as the genotoxic potential of these materials. The samples were collected at two sites along the Rocinha River in Santa Catarina state (Brazil): (1) directly from the source of the acid drainage (evaporite 1), and (2) on the river bank (evaporite 2). The samples were characterized by X-ray diffraction and by particle-induced X-ray emission techniques. *In vitro* genotoxicity testing using Comet assay and Micronucleus test in V79 cells was used to evaluate evaporite samples. Our study also used System Biology tools to provide insight regarding the influence

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Micronucleus test
System biology

of this exposure on DNA damage in cells. The results showed that the samples induced DNA damage for both evaporites that can be explained by high concentrations of chromium, iron, nickel, copper and zinc in these materials. Thus, this study is very important due to the dearth of knowledge regarding the toxicity of evaporites in the environment. The genetic toxicity of this material can be induced by increased oxidative stress and DNA repair inhibition.

1. Introduction

The surface area of Brazil is more than 8.5 million km^2 with a great diversity of land and geological formations and a wide range of minerals. Mineral extraction in the south of the Brazil began in 1855 [1]. Coal is the most frequent resource utilized to generate power [2], with 41% of the world energy. However, social and environmental damage during extraction and consumption are the main restrictions of coal use. Currently, the biggest coal reserve, with approximately 28.8 billion tons, is located in the state of Rio Grande do Sul. It is followed by the reserves of Santa Catarina and Paraná, with approximately 3.4 billion and 100 million tons, respectively. In Santa Catarina alone more than 6 million tons are extracted yearly [1].

The most dangerous environmental and social impacts caused by coal mining in Brazil mainly affect the water bodies, soil and landscape of the surrounding areas. Another important, hazardous environmental impact is acid drainage formation. The wastewater is discharged increasing the sulphate and iron concentration and decreasing the pH concentration at the drainage site [3]. Furthermore, in coal processing areas crystallized minerals (evaporites) are formed due to water evaporation. This evaporite formation process in the coal mining environment forms aggressive minerals that cause great environmental damage due to the large quantity of polluting elements present in waste water drainage [4].

The different contaminants found in coal as well as its by-products (*e.g.* nitrogen dioxide, sulphur dioxide, ash, soot, and clinker) and waste are considered serious environmental contaminants due to their mobilization and bioaccumulation in the food chain, water contamination and toxic effect on biota [5]. These toxic residues released into the environment induce DNA damage in different organisms and tissues, indicating exposure hazards and the need for biomonitoring [6,7,8].

Due to the composition of coal and its derivatives (hydrocarbons and toxic metals) various studies have evaluated the genotoxicity of these materials (such as coal dust and coal fly ash). *In vitro* assays showed that different concentrations of coal dust, coal fly ash and bottom ash particles induced cytotoxic, genotoxic and mutagenic effects in exposed cells [9,10]. Testing the toxicity of surface water samples collected in the coal mining areas, it was observed that these samples induced toxicity in cells probably due to the presence of higher concentrations of inorganic elements, especially aluminum, silicon, sulfur, titanium and zinc [11].

Thus, the study aimed to evaluate genotoxic effects of evaporite samples derived from coal mining drainage using the Comet assay and Micronucleus test in V79 cells. In addition, evaporite samples were also submitted to inorganic element and geochemical analysis. System Biology tools were used to provide insight into the influence of evaporite exposure on DNA damage in exposed cells. This is the first *in vivo* and *in silico* toxicology research on evaporite samples (abundant material formed in all coal mining) from coal mine drainage.

2. Materials and methods

2.1. Sampling procedures and preparation

The evaporite samples collected from the Lauro Muller coal area (Fig. 1) include precipitated neoformed minerals and the underlying material (Fig. 2). The neoformed compounds studied were found at the base of the waste pile where the drainage system is inefficient (Fig. 1b). Neoformed materials were collected along Rocinha River in January 2014. An evaporite 1 sample was collected directly from acid drainage (Fig. 1b), while evaporite 2 was collected on the banks of the Rocinha River, Santa Catarina (Fig. 1b, c). The samples were collected by hand using latex gloves or using a Ponar dredge depending on the depth and the flow, and pH (potential of hydrogen) was measured using a pH meter. The coal mining drainage sediment samples were protected against light using aluminum foil and were transported to the laboratory at 4 °C in a cool box. In the laboratory the sediment samples were air dried. The dry samples were sieved to ensure a maximum particle size of 65 μm and kept in the refrigerator at 4 °C until analysis [12,13].

In this study, about 100 g were collected in each evaporite sample. To perform the cellular viability and genotoxic tests (Micronucleus and Comet assay), the evaporite samples were dissolved in sterilized water (at concentrations of 0.003, 0.006, 0.013, 0.025 and 0.050 mg/mL) under a laminar flux hood and divided into bottles to be used in analyses. For the analysis of inorganic composition (PIXE technique), the water samples were filtered in a vacuum with filter papers (125 mm) and for geochemical analysis the evaporite samples were homogenized and compacted on the sample holder to ensure the surface flatness required for this technique.

2.2. Mineralogical and inorganic elements analysis

For mineralogical analysis, the evaporite samples were studied in a Philips powder diffractometer fitted with a Philips "PW1710" control unit, Vertical Philips "PW1820/00" goniometer and FR590 EnrafNonius generator. The equipment was prepared with a graphite diffracted beam monochromator and copper radiation source (λ(Kα1)=1.5406Å), operating at 40 kV and 30mA. The Powder Diffraction X-Ray pattern (PDXR) was found by measuring the scintillation response to Cu Kα radiation versus the 2 q value over a 2q range of 2–65°, with a step size of 0.02° and counting time of 3s per step. The semi-quantification of the individual crystalline phases (minerals) in each sample was determined using the Match! program Copyright© 2003-2011 CRYSTAL IMPACT, Bonn, Germany.

The inorganic elements were analyzed by the PIXE (Particle-Induced X-ray Emission) technique. The evaporite samples were dissolved in water and subsequently filtered by a 125 mm porosity filter. These filters were dried at ambient temperature and were placed in the target holder inside the reaction chamber. During the experiments, the pressure inside the reaction chamber was ~10–5 mbar. The experiments were carried out at the Ion Implantation Laboratory of the Institute of Physics, Federal University of