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Mutagenic potential of fine wastes from dimension stone industry

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ABSTRACT

The industrial treatment of dimension stones, such as marbles and granites, includes a stage of plate polishing, in which resins and abrasives are used, producing a fine grained waste with high moisture content. These wastes pass through decantation tanks in order to separate the solid and liquid phases. Until now, there is no knowledge about the mutagenic effects that this effluent can cause to organisms exposed to it. Thus, this study evaluated the mutagenic potential of dimension stone polishing wastes in onion root cells and fish erythrocytes. The onion seeds were germinated in Petri dishes with filter paper moistened in the liquid phase of the effluent. After germination, the onion roots were prepared for analysis of chromosomal aberrations in meristematic cells. The fishes were exposed during 72 h to the solid phase of the effluent diluted in pure groundwater. Blood samples were used for counting of micronucleus and nuclear abnormalities. The onion seeds had similar germination and mitotic index in all treatments. However, it was observed in the seeds exposed to the polishing waste, numbers significantly higher of micronucleus, nuclear buds and other chromosomal aberrations when compared with the negative control. The fishes exposed to the waste showed numbers significantly higher of micronucleus when compared with the negative control. The fishes from all treatments showed significant increase in nuclear abnormalities when compared to the negative control. We concluded that the analysed wastes have mutagenic potential at the studied conditions; this effect can be related to the high content of phenolic compounds identified in the samples.

1. Introduction

Brazil is between the main dimension stone producer countries, exporting blocks and plates of marble and granite for use as surface coverings. From January to October 2014, Brazil exported approximately $1 billion worth of rock plates (Abirochas, 2014) with 75% produced in the state of Espírito Santo (ES). According to Villaschi Filho and Sabadini (2000), the oldest production centre in Brazil is located at Cachoeiro de Itapemirim, a municipality in the southern portion of ES, which is the source of the majority of Brazilian production; however, it has been receiving attention due to the large quantity of waste generated.

After sawing of blocks, the produced plates are polished to confer gloss to the final material. This process involves the use of abrasives that are based on magnesium, diamond or phenolic resins. The generated waste is taken to settling tanks where the liquid and solid phases of the wastewater are separated. The liquid phase returns to the processing plant, and the solid phase continues to be deposited until the maximum capacity of the tank is reached. When this capacity is reached, the liquid phase is released in water bodies and the solid phase is deposited directly onto soil without proper sealing.

Several substances disposed of by industrial processes in water bodies can cause genomic mutation. These substances show binding affinity to the genetic material and can potentially cause DNA damage. They are known as genotoxic or mutagenic agents (Boer And Hoeijmakers, 2000).

Among the organisms used to monitor aquatic pollution, the most commonly used are fish, molluscs and plants. Onion, *Allium cepa*, has stood out among higher plants as a model in tests to detect the mutagenic action of chemical substances or contaminated water (Matsumoto et al., 2006). The efficiency of onion as a test organism is due to the knowledge regarding the duration of its cell cycle, its rapid root growth, high tolerance to various cultivation conditions and large chromosomes in reduced number, characteristics that are essential for genotoxicity studies (Evseeva et al., 2003; Egito et al., 2007).

The use of fish as test organisms in toxicological monitoring has also been shown to be efficient because it precisely evaluates agents that potentially cause genetic damage in the aquatic

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environment. Fish have nucleated erythrocytes, which allows easy visualisation of micronuclei. According to Vijayan et al. (1996), tilapia, *Oreochromis niloticus*, is a species that is recognised to have high sensitivity for the detection of pollutants, rapidly responding to environmental alterations.

The economic relevance of the dimension stone industry and the volume of wastes generated emphasise the necessity of knowing the potential impacts that these materials can cause to the environment. Thus, the present study aimed to analyse the mutagenic potential of the waste generated during processing of dimension stones in onion root meristems and fish erythrocytes.

2. Materials and methods

The experiments were performed with wastes collected at a dimension stone processing company located in the municipality of Cachoeiro do Itapemirim, in the southern portion of the state of Espírito Santo (ES), Brazil. The water that supplies the processing plant comes from a lake located near the property. After polishing marble and granite plates, the water that circulates through the polisher is directed to the settling tanks to decrease the quantity of suspended sediment, after which the water is reused in the process. The settling tanks are emptied when their storage capacity is exhausted, and the settled solid waste is removed for disposal. Filling of the settling tanks is then reinitiated.

2.1. Study of the liquid phase of the waste

Two sampling points were selected for the liquid phase of the polishing waste, one at the inlet of the settling tank (TI) and another at the outlet (TF). One sampling point was in the supply lake of the company (LA).

Water temperature (t), pH, electrical conductivity (EC), turbidity (T) and dissolved oxygen (DO) were measured *in situ* using portable equipment. Sample collection was performed according to NBR 9898 (ABNT, 1987), and the samples were sent to the Hydrogeology Laboratory of the Centre for Agrarian Sciences of the Federal University of Espírito Santo (*Laboratório de Hidrogeologia do Centro de Ciências Agrárias da Universidade Federal do Espírito Santo*) for analyses of alkalinity (ALK), chloride (CHLO) and total phenols (PHEN). Alkalinity was determined by titration with a base according to NBR 13736 (ABNT, 1996), and the analyses of chloride and total phenols were performed by colorimetry using a scanning UV–vis spectrophotometer.

2.2. Experiment with Allium cepa

Seeds of *Allium cepa* from the same lot were germinated on Petri dishes lined with filter paper moistened in a solution according to the following treatments: (1) Control–negative control group containing distilled water; (2) Lake-water collected from a lake that supply the company; (3) Waste-liquid phase of the polishing waste collected at the inlet of the settling tank; and (4) Resin-dilution of the polishing resins with distilled water (2.5 mL L^{-1}). In each plate were placed 60 seeds. The plates were kept under a 12 h light/dark cycle and controlled temperature (24 °C) in incubator. After reaching approximately 2.0 cm, the total number of germinated seeds was counted and the germination index (GI) was calculated, which represents the percentage of germinated seeds in each treatment, represented by the formula:

$$GI=TN \times 100/PN$$

where TN is the total number of germinated seeds and PN the total number of seeds in each plate.

The roots were fixed in alcohol:acetic acid (3:1) for 24 h. Next, they underwent hydrolysis in 1 N HCl for 5 min at room temperature. The meristematic region (the first three millimetres of the root) from each of five roots was cut off, stained with a drop of acetic orcein and covered with a coverslip. The material was crushed, providing better spreading of the cells, and was analysed with a light microscope at 400× magnification. To assess the cytotoxic potential a total of 2000 cells were counted per slide, differentiating cells at interphase and at different stages of mitosis to calculate the mitotic index (MI), which corresponds to the ratio between the number of dividing cells (DC) and the total number of cells observed (CO).

During counts the micronucleated cells, nuclear budding and chromosomal aberrations were quantified, in order to determine mutagenicity.

The data obtained with the onion root meristems were subjected to statistical analysis using Bioestat 5.0 software and the non-parametric Mann–Whitney test for comparisons between pairs of treatments.

2.3. Study of the solid settled waste

The solid phase of the waste was collected according to the procedures of NBR 9898 (ABNT, 1987), the concentrations of lead, chromium, cadmium, silver, aluminium, iron, sodium, phenol, fluoride and chloride were measured by leaching test according to NBR 10005 (ABNT, 2004a), and a solubilisation test was performed according to NBR 10006 (ABNT, 2004b). The results were used for classification of the wastes according to NBR 10004 (ABNT, 2004c).

2.4. Experiment with Oreochromis niloticus

The experiment with *O. niloticus* was conducted with animals approximately 10.0 cm in length obtained from the fish farming department of the Federal Institute of Espírito Santo-Alegre Campus (*Instituto Federal do Espírito Santo-Campus de Alegre*). Fishes were exposed for 72 hours in 50 L tanks, in four treatments with 10 individuals each: (1) Control-containing pure groundwater; (2) Lake-containing 50 L of pure groundwater mixed with 2.0 Kg of sediment collected in the lake that supplies the marble factory; (3) Waste-containing 50 L of pure groundwater mixed with 2.0 Kg of polishing waste collected from the inlet of the settling tank; and (4) Resin-containing 50 L of pure groundwater and 10 mL of the resin used in the polishing process. The tanks were kept under constant aeration.

After 72 h of exposure, a 0.1 mL blood sample from each fish was used for the preparation of two slides with a blood smear per individual. The slides were stained with panoptic dye and were analysed under a light microscope with 1000× magnification. A total of 6000 erythrocytes were counted per fish (3000 per slide). Micronuclei that were smaller than the nucleus, clearly separated, not refringent and that showed the same staining as the nucleus were quantified. Cells bearing other nuclear alterations were also analysed according to Carrasco et al. (1990).

The data obtained with the fish erythrocytes were subjected to statistical analysis using Bioestat 5.0 software and the non-parametric Mann–Whitney test for comparisons between pairs of treatments.

3. Results and discussion

3.1. Effects of the liquid phase of the waste on germination of A. cepa

Results from the analyses of the liquid phase of the waste from the processing plant and the supply water are shown in Table 1. In