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Use of a bioindicator system in the study of the mutagenetical effects in the neighborhoods of deposits of radioactive waste

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Abstract

The purpose of the research described in this paper was to analyze the biological mutational effects caused by low doses of ionizing radiation on biological samples placed nearby and around deposits of radioactive waste, as a way of monitoring the environment close to them. In order to do this, the plant *Tradescantia pallida* was chosen, and through micronuclei tests the sensitivity of the dose/response to bio-monitoring could be observed. The plants were exposed for a period of 24 h in previously chosen sites around Brazil, within the proximity of nuclear waste deposits. In each location, three points were chosen for bio-monitoring. The results obtained at these locations showed a small increment in the frequency of micronuclei per cell of the biosensor. From these data, a scale of mutagenesis effects due to low-dose radiation was built up. *T. pallida* is a good alternative for environmental bio-monitoring in tropical climates, as it is an excellent alternative tool in the studies of the effects of ionizing radiation on the environment.

Keywords: *Tradescantia*; Low dose; Radioactive deposit

1. Introduction

Each year, the amount of radioactive waste from research institutions, hospitals and nuclear power plants in Brazil and around the world is growing, and so the need to store this waste grows too. Waste storage induces questions for society concerning the amount of radiation exposure to man and the environment in the neighborhoods of waste deposit sites.

In Brazil, the responsibility for inspecting the deposits of nuclear waste is legally attributed to the National Commission for Nuclear Energy (Comissão Nacional de Energia Nuclear—CNEN). Usually, the stored nuclear waste is of low or medium activity; this material is previously compacted and maintained in steel drums that are stored in initial, intermediary or permanent deposits. The permanent deposits are protected by thick concrete walls and may house the materials for short or midterm intervals of time. There is, in Brazil, only one permanent deposit for waste of small to medium activity where part of the material resulting from the cesium-137 accident in Goiânia (1987) is stored. The construction of other prominent deposits is under consideration. However, selection for the location of these deposits depends on a technical analysis that includes details of different levels of data and information. There is also a need to comply with the laws no. 4.118/62 and 10.308/01, respectively, and the regulations NE-6.05—Management of radioactive waste in radioactive installations (Gerência de Rejeitos Radioativos em Instalações Radiativas) (CNEN, 2002a), NE-6.06—Selection and choice of locations for deposits of radioactive waste (Seleção e Escolha de Locais para Depósitos de Rejeitos Radioativos) (CNEN, 2002b), NN-6.09—Criteria of acceptance for the deposits of low and medium levels of radioactive waste (Critérios de Aceitação para

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Deposição de Rejeitos Radioativos de Baixos e Médios Níveis de Radiação) (CNEN, 2002c) and NE-3.01—Basic directives for radiological protection (Diretrizes Básicas de Proteção Radiológica) (CNEN, 2005).

Due to the importance of radiometric monitoring and the maintenance of radioactive waste deposits, this paper is aimed at what response a biosensor will show, when exposed to radiation originated from such deposits, in order to find out the biological effects of low doses of radiation on that environment, in a short period of time.

2. Materials and methods

2.1. Biotesting

Tradescantia pallida (Rose) Hunt. variety *purpurea* Boom is a small ornamental plant from the family Comelinacea the characteristics of which make it useful for experiments involving genetic damage to cells, especially those originating from exposure in a genotoxic environment.

To develop and experiment biosensors, there is a need to ensure that they are compatible with environmental conditions where they will be used. Hence, the choice of *T. pallida* resulted from its good adaptation to the adverse climatic conditions in the various regions around Brazil. This plant can be found in many streets and gardens of the cities all over the country. It is a tetraploid species that has remarkable resistance to both parasites and insects. It blooms all year round and needs little care and attention to grow.

T. pallida allows us to obtain response curves of biological damage versus dose, based on the micronuclei methodological system developed by Ma for *Tradescantia* clone 4430 and *Vicia faba* (Ma, 1982; Ma et al. 1994). This methodology has been widely used by various groups of researchers to evaluate the damaging effects of genotoxic agents and to obtain a prognosis for human health.

2.2. Experimental procedure

In this paper, we have chosen four regions around Brazil, because they contain nuclear waste deposits and because of their peculiar characteristics.

(1) The radioactive waste deposit at the Institute of Nuclear Engineering (IEN), located in the city of Rio de Janeiro: this deposit is considered of intermediate level. Some of the waste is stored for future use, other waste is removed to a permanent deposit.
(2) The radioactive waste deposit at the Nuclear Power Plant in Angra dos Reis (UNA), located at the coastline of the state of Rio de Janeiro: it is considered to be an initial deposit; it contains richer active waste of low and medium activity. This deposit is under the custody of the Electronuclear Corporation, and is supervised by CNEN.
(3) The radioactive waste deposit at the Institute for Nuclear Energy Research (IPEN), located in the city of São Paulo: it is considered of intermediate level, however, it has a huge store of waste.
(4) The radioactive waste deposit at Abadia de Goiás (ABADIA): this is the only permanent waste deposit in Brazil for small and medium activity.

Radiometric readings were carried out at the surroundings of each of these deposits using a MRA GP500 monitor, model 7237/03.44. At each waste deposit, three locations were selected in accordance with the levels of dose rate: (1) CW (Control Waste deposit site) location where the dose rate was close to the dose rate measured at the garden where *T. pallida* was cultivated referred to as CG (Control Garden), (2) NE (Nearby the Entrance door of the waste deposit) and (3) DE (along the waste deposit, but 1 m Distant of its Entrance door).

Once the locations had been selected, vases containing *T. pallida* were placed, in such a way that 10 samples were exposed in each location, over an interval of 24 h. After being exposed, the samples were placed into water, for at least 6–8 h. This is time enough for the meiosis process to take place and for the mother cells of the pollen grains to reach their tetrad phase. When the tetrad phase is reached, it is possible to see the micronucleus (MCN). In the final stage, the tetrads are fixed, in a solution of acetic acid and alcohol (1:3, v/v), in agreement with the protocol published by Ma (1982).

To prepare the slides for microscope observation, chosen inflorescences are mashed and treated with a drop of carmine (contrasting agent) to observe the different stages of the tetrads. The slide is squeezed slightly to visualize the tetrads under the microscope, on the same plane. Then, the preparation is heated over a Bunsen burner at 80 °C; the residuals are removed and the slides sealed with enamel. Three hundred tetrads per slide were counted, and by way of a table the number of micronuclei/slide was determined (MCN/100). For each of the three locations, at each of the four sites, 10 samples were analyzed, totaling 3000 cells that were labeled as pertaining to the control negative group (CG), groups CW, NE and DE, respectively.

2.3. Statistical analysis

To analyze the data the SPSS 9.0 for Windows program for statistic treatment was used (SPSS, 1999). The parameter variance was determined, in order to compare the counts in relation to the three groups from each region, to a level of significance of 0.05; Student's *t*-test was also used when comparing the samples, in compliance with the protocol from Ma (1983).

3. Results and commentaries

A total of 9000 cells were analyzed for each waste deposit. Every count was compared with the control group