

**THE STUDY OF THE MUTAGENETICAL EFFECTS OF LOW DOSE LEVEL
EXPOSURE TO IONIZING RADIATION USING A BIOINDICATOR SYSTEM CLOSE
TO DEPOSITS OF RADIOACTIVE WASTE**

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Abstract

The purpose of this paper is to analyze the biological mutational effects caused by low doses of ionizing radiation in biological samples placed close to and around deposits of radioactive waste, as a way of monitoring the environment close to these deposits. In order to do this the *Tradescantia pallida*, was chosen, and through micronuclei tests one could verify the sensitivity of the dose/ Response to bio-monitoring. The plants were exposed for a period of 24 hours in previously chosen sites around Brazil, within the proximity of deposits of nuclear waste. In each location, three points were chosen for bio-monitoring. The results obtained at these locations shows a small increment in the frequency of micronuclei per cell analyzed by the biosensor. From this data, a scale of mutagenesis affects from low dosage radiation was built up. The *Tradescantia pallida* is a good alternative in environmental bio-monitoring for 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, deposit radioactive

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 with this the need to store this waste around the country also grows. This need induces the society in general and workers in

particular about exposure to radiation of the neighborhoods close to these deposits, what affects does this radiation have on man and the environment.

In Brazil, the organ responsible for inspecting the deposits of nuclear waste is the National Commission for Nuclear Energy (Comissão Nacional de Energia Nuclear) (CNEN). The stored nuclear waste can be of low or medium activity, the material is previously compacted and kept in steel drums. They can be kept 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, material that gave rise to the accident with cesium 137, in 1987 in Goiânia. 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) [1], NE-6.06 – Selection and Choice of Locations for Deposits of Radioactive Waste (Seleção e Escolha de Locais para Depósitos de Rejeitos Radioativos) [2], NN-6.09 – Criteria of Acceptance for the Deposits of Low and Medium Levels of Radioactive Waste (Critérios de Aceitação para Deposição de Rejeitos Radioativos de Baixos e Médios Níveis de Radiação) [3] and NE-3.01- Basic Directives for Radiological protection (Diretrizes Básicas de Proteção Radiológica) [4].

Due to the importance of a radio metric monitoring and the maintenance of radioactive waste deposits, this paper is aimed at what response a biosensor will have, when exposed to radiation coming from these deposits, to find out the biological effects of low doses of radiation on that environment, in a short space of time.

2. Materials and methods.

2.1. Biotesting

The *Tradescantia* is a small ornamental plant 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 that it meets the environmental conditions found in Brazil, the choice of this plant, which comes from the family *Comelinácea*, the *Tradescantia pallida* (Rose) Hunt. Variety *Purpúrea* Boom was due to its adaptation to the adverse climatic conditions in the various regions around the country, this plant can be found in many streets and gardens of the cities all over the country. This is a tetraploid species that has notable resistance to both parasites and insects. It blooms all year round and needs little care and attention to grow.

The *Tradescantia pallida* allows us to obtain response curves of biological damage versus dosage, based on the micronuclei methodological system developed by T.H. Ma for a *Tradescantia* clone 4430 and the *Vicia Faba* [5,6]. This methodology has been widely used by various groups of researchers when wanting to evaluate the damaging effects of genotoxic agents and obtaining a prognosis for human health.

2.2. Experimental procedure

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

- 1- The Radioactive Waste Deposit at the Institute of Nuclear Energy (IEN), located in the city of Rio de Janeiro, this deposit is considered intermediate level. Some of the waste is stored for future use; others are removed to a permanent deposit.
- 2- The Radioactive Waste Deposit at the Nuclear Power Station in Angra dos Reis (UNA) - located on the coastline of the state of Rio de Janeiro this is considered to be an initial deposit, it contains richer active waste of low and medium activity. This deposit is under the custody of Eletronuclear, and supervised by CNEN.
- 3- The Radioactive Waste Deposit at the Institute for Nuclear Energy Research (IPEN) - located in the city of São Paulo and is 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.

Radio metric readings were taken in each of these deposits, their surroundings, to choose the points of exposure, using a MRA GP500 monitor, model 7237/03.44. Once the locations had been selected, vases containing the *Tradescantia pallida*, in such a way that ten samples are exposed in each location for an interval of 24 hours. After being exposed the samples are then placed into water, for at least six to eight hours. This is enough time for the meiosis process to continue and the mother cells of the pollen grains reach their tetrad phase. When the tetrad phase is reached it is possible to see the micronucleus. In the final stage of, the tetrads remain fixed, in a solution of acetic acid and alcohol, to the proportion of (1:3), in line with the protocol published by T.H. Ma [5].

To prepare the slides, once the inflorescences are chosen, they are mashed and submitted to a drop of carmine (contrasting agent), so as to say the different stages of the tetrads. Squeeze the slide slightly, so that the tetrads can be visualized by the microscope on the same plane; heat over a Bunsen burner to a temperature of 80°C; remove the residuals and seal slides with enamel. The expected count of 300 tetrads per slide and by way of a table one can determine the number of tetrads the micronuclei/ slide. In Each deposit of radioactive waste, of each selected group, ten

samples were analyzed, totaling 3000 cells the group, that were labeled as pertaining to the control group, group A and group B respectively, in accordance with the levels of dosage in each location.

2.3. Statistical analysis

To analyze the data a program for statistic treatment was used, SPSS 9.0 for Windows [7]. 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, the test t-Student was also used when comparing the samples (two in every two groups), in compliance with the protocol from T.H. Ma [8].

3. Results and commentaries

Relatively for each deposit of radioactive waste, a total of 9000 cells were analyzed. Every count was compared with a control group (Co) which coincided with the control group from the location of cultivation, where the dosage rate measured was 0.26 $\mu\text{Gy/h}$. In table 1 is a sample showing the dosage rates resulting from each group and the selected locations.

Table 1 – Dosage rate ($\mu\text{Gy/h}$) the results from each group and the chosen locations.

<i>Groups</i> <i>Location</i>	Co* ($\mu\text{Gy/h}$)	C1 ($\mu\text{Gy/h}$)	A ($\mu\text{Gy/h}$)	B ($\mu\text{Gy/h}$)
<i>IEN</i>	0.26	0.44	21.9	35.1
<i>UNA</i>	0.26	0.35	25.4	46.5
<i>IPEN</i>	0.26	0.44	30.0	137
<i>ABADIA</i>	0.26	0.26	2.2	3.1

*Co is the control group from the cultivated location.

Figure 1 represents the number of micronuclei per hundred cells analyzed, the results of the exposure of the biosensor, that relates the dosage rates to the mutational effects observed in each group and chosen location. The curve in question showed a slight growth even that load dosage rates.

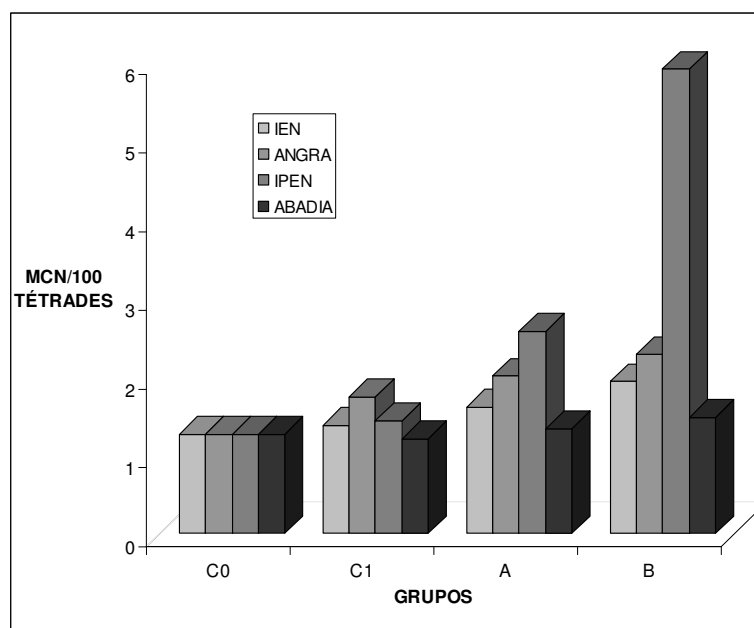


Figure 1 – Results in function of groups versus MCN/100 tetrads.

First of all, to verify the influence suffered by the biosensors during transportation to the location of exposure, the frequencies MCN/100 tetrads were compared on the control points of the cultivation, Co (negative), with the frequencies in the other local control groups, from the radioactive waste deposits. From this comparison no significant difference was found ($p>0.05$), which leads one to conclude that the biosensor did not suffer any damages from stress during transportation.

When relating group Co with the groups A and B, it was noticed that there were different responses among them. For group A, at the IEN and Abadia deposits, respectively, there was no significant difference. For group A, at the ANGRA and IPEN (intermediate level) deposits, an increase in mutational frequency was noticed, detecting a significant difference ($p<0.05$). For group B, a great difference was found at the deposits of ANGRA, IEN and IPEN; only the deposit at Abadia showed no significant increase when compared with the Location cultivation.

The curve and Figure 2 shows the linear relationship found between the dosage rates and the frequency given in numbers of micronuclei per 100 tetrads.

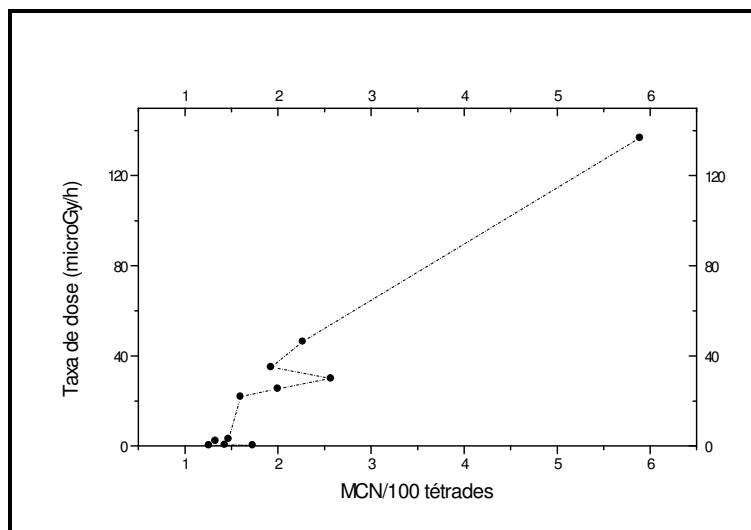


Figure 2 – Mutagenesis scale in relation to dosage rate.

Recent studies, using the species *Tradescantia*, compare its sensitivity to the effects of exposure to radiation with those from genotoxic agents [9,10,11,12]. The mutagenesis scale shown in figure 2 is coherent with that obtained by Suyama et al [13], when studying a methodology of biomonitoring tests with the *Tradescantia*, when exposed to x-rays. Villalobos-Pietrini [14] used this method with the biosensor when comparing the *Tradescantia* clone 4430, having registered an increase in the mutational frequency from 7 MCN/100 to 17MCN/100, when they were submitted to a dosage of 0.8 Gy from a source of Co^{60} . Recent studies have shown that the sensitivity of the *Tradescantia* to the effects of radiation serve as a way of connecting gamma radiation dosage rates to which it was submitted to the mutational frequency from low dosage rates [15], using the micronuclei methodology.

The answers Acquired from the biosensor, *Tradescantia pallida*, results in an advantage, when using the present methodology, as one can observe a high quantity of mutational alterations in a short space of time, as such one is capable of anticipating the effects caused on the environment and as such on the human being, as a result of the level of occupation. It is recommended, therefore, the use of this method for periodic monitoring, as the biosensor can be introduced into the environment, due to its ease of planting, propagation and excellent acclimatization, and may also be used in the prevention of radiological accidents.

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