Available online at www.sciencedirect.com

ScienceDirect

Applied Radiation and Isotopes 66 (2008) 535–538

Applied Radiation and Isotopes

www.elsevier.com/locate/apradiso

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

Teresa Cristina dos Santos Leal[a,*], Verginia R. Crispim[b], Marcos Frota[c], Alphonse Kelecom[a,c], Ademir Xavier da Silva[b]

[a]Post-graduation in Environmental Science (PGCA), UFF, Niterói, Brazil
[b]Nuclear Engineering Program/COPPE/UFRJ, Rio de Janeiro, Brazil
[c]Laboratory of Radiobiology and Radiometry (LARARA), Department of General Biology, UFF, Niterói, Brazil

Received 12 May 2007; received in revised form 2 October 2007; accepted 12 November 2007

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

*Corresponding author.
E-mail address: teresa@tcleal.eng.br (T. Cristina dos Santos Leal).

0969-8043/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.apradiso.2007.11.003

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

Table 1
Dose rates (μGy/h) at each location for each waste deposit site

Locations (abbreviation)	Waste deposit sites Dose rates (μGy/h)			
	IEN (Institute of Nuclear Engineering)	UNA (Nuclear Power Plant at Angra dos Reis)	IPEN (Institute for Nuclear Energy Research)	ABADIA (Abadia of Goias)
Control garden (CG)	0.26	0.26	0.26	0.26
Control waste site (CW)	0.44	0.35	0.44	0.26
Nearby the entrance (NE)	21.9	25.4	30.0	2.20
Distant of the entrance (DE)	35.1	46.5	137	3.10

CG: negative control (garden where *T. pallida* was cultivated); CW: positive control (location of the waste deposit site where the dose rate was close to the dose rate measured at the garden); NE: location nearby the entrance door of the waste deposit; DE: location 1 m distant of the entrance door of the waste deposit.

Table 2
Number of micronuclei per hundred cells (MCN/100) for each location in the neighborhoods of deposits of radioactive waste as a function of dose rate

Locations (abbreviation)	Waste deposit sites MCN/100 tetrads			
	IEN (Institute of Nuclear Engineering)	UNA (Nuclear Power Plant in Angra dos Reis)	IPEN (Institute for Nuclear Energy Research)	ABADIA (Abadia of Goias)
Control garden (CG)	1.26	1.26	1.26	1.26
Control waste site (CW)	1.37	1.73	1.43	1.20
Nearby the entrance (NE)	1.60	2.00	2.57	1.33
Distant of the entrance (DE)	1.93	2.27	5.90	1.47

(CG) from the location of cultivation where the dose rate measured was 0.26 μGy/h. Table 1 shows the dose rates at each location for groups in each waste deposit.

Table 2 presents the number of micronuclei per hundred cells (MCN/100) analyzed for each group. The results tend to indicate higher micronuclei frequency per tetrads at the locations of higher dose rates.

First of all, to verify the influence suffered by the biosensor during transportation to the location of exposure (waste deposit sites), the frequencies of MCN/100 tetrads observed for the negative control CG (cultivation garden) were compared with the frequencies at the positive control groups CW (radioactive waste deposit sites). 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.

On comparing the NE and DE groups to the control group (CG), different responses could be observed. Thus, no significant difference was observed for the NE groups at IEN and ABADIA deposits. On the contrary, for groups NE at UNA and IPEN deposits (intermediate level) showed a significant increase ($p<0.05$) in mutational frequency. For group DE, a great difference was found at the deposits of UNA, IEN and IPEN; only the deposit at ABADIA showed no significant increase when compared with the CG control group (cultivation location).

Fig. 1 shows the relationship found between the dose rates and the number of MNC/100 tetrads. This relationship is approximately linear.

Recent studies, using the species *T. pallida*, compare its sensitivity to radiation exposure with that to genotoxic agents (Celebuska-Wasilewska, 1992; Ichikawa, 1991, 1992; Gomes et al., 2002). The mutagenesis scale shown in Fig. 1 is coherent with that obtained by Suyama et al. (2002), when studying a biomonitoring methodology using *Tradescantia* exposed to X-rays. Vilalobos-Pietrini et al. (1999) 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 17 MCN/100, when the plants were submitted to a dose of 0.8 Gy delivered by a ^{60}Co source. Recent studies have shown that the sensitivity of *Tradescantia* to the effects of radiation is a way to correlate gamma-radiation dose rates to which the plant is submitted to the mutational frequency resulting from the exposition to low-dose rates, using the micronuclei methodology (Santos Leal et al., 2005).

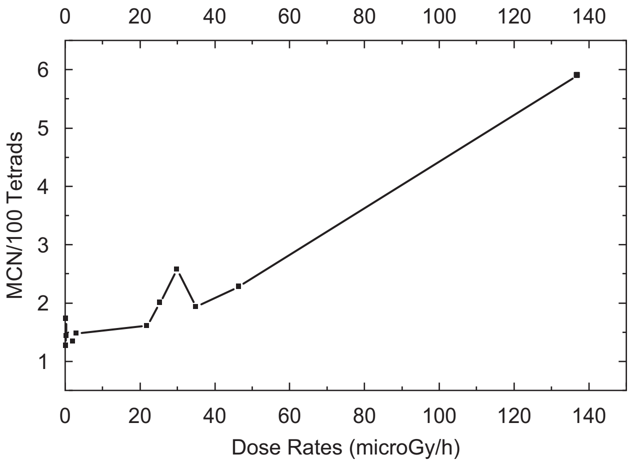

Fig. 1. Mutagenesis scale relating dose rate to number of MCN/100 tetrads.

The biosensor *T. pallida* exhibits a noticeable quantity of mutational alteration in a short time following radiation exposure. Hence, the effects caused on the environment might be anticipated, and by extension on the human being, as a result of its occupation exposition level. The use of this method is recommended, therefore, for periodic monitoring, as the biosensor can be introduced into the environment, due to its easy planting, propagation and excellent acclimatization, and may be used, in addition, in the prevention of radiological accidents.

References

Celebuska-Wasilewska, A., 1992. *Trandescantia* stamen hair mutation bioassay on the mutagenicity of radioisotope-contaminated air following the Chernobyl nuclear accident and one year later. Mutation Research 270, 23–29.

CNEN—Comissão Nacional de Energia Nuclear, 2002a. Gerência de Rejeitos Radioativos em Instalações Radiativas—Norma CNEN NE-6.05, Brazil.

CNEN—Comissão Nacional de Energia Nuclear, 2002b. Seleção e Escolha de Locais para Depósitos de Rejeitos Radioativos—Norma CNEN NE-6.06, Brazil.

CNEN—Comissão Nacional de Energia Nuclear, 2002c. Critérios de Aceitação para Deposição de Rejeitos Radioativos de Baixo e Médio Níveis de Radiação—Norma CNEN NN-6.09, Brazil.

CNEN—Comissão Nacional de Energia Nuclear, 2005. Diretrizes Básicas de Proteção Radiológica—Norma CNEN NN-3.01, Brazil.

Gomes, H.A., Nouailhetas, Y., Almeida, C.E.B., Mezrahi, A., 2002. Biological response of *Tradescantia* stamen-hair to high levels of natural radiation in the Poços de Caldas Plateau. Brazilian Arquives of Biology and Technology 45, 301–307.

Ichikawa, S., 1991. Validity of simplified scoring methods of somatic mutations in *Tradescantia* stamen hairs. Environmental and Experimental Botany 31, 247–252.

Ichiwaka, S., 1992. *Tradescantia* stamen-hair as an excellent botanical tester of mutagenicity; its response to ionizing radiations and chemical mutagens, and some synergistitic effects found. Mutation Research 270, 3–22.

Ma, T.H., 1982. *Tradescantia* micronucleus bioassay and pollen tube aberration test for in situ monitoring and mutagen screening. Environmental Health Perspectives 37, 45–164.

Ma, T.H., 1983. *Tradescantia* micronuclei (Trad-MCN) test for environmental clastogens. In: Kolber, A.R., Wong, T.K., Grant, L.D., Dewoskin, R.S., Hughes, T.J. (Eds.), On vitro Toxicity Testing of Environmental Agents. Current and Future Possibilities. Part A: Survey of Test Systems. Plenum Press, New York, pp. 191–214.

Ma, T.H., Cabrera, G.L., Chen, R., Gill, B.S., Sandhu, S.S., Vandenberg, A.L., Salamone, M.F., 1994. Aamone *Tradescantia* micronucleus bioassay. Mutation Research 310, 221–230.

Santos Leal, T.C., Crispim, V.R., Gomes, H.A., 2005. The study of the effects of low dose level exposure to ionizing radiation using a bioindicator system. Applied Radiation and Isotopes 62, 313–316.

SPSS, 1999. SPSS 9.0 for Windows. SPSS Inc., Chicago, IL. 〈http://www.spss.com.br/spss〉.

Suyama, F., Guimarães, E.T., Lobo, D.J.A., Rodrigues, G.S., Domigos, M., Alves, E.S., Carvalho, H.A., Saldiva, P.H.N., 2002. Pollen mother cells of *Tradescantia* clone 4430 and *Tradescantia pallida* var. Purpurea are equally sensitive to the clastogenic effects of X-rays. Brazilian Journal of Medical and Biological Research 35, 127–129.

Vilalobos-Pietrini, R., Flores-Marques, A.R., Meneses, M.A., Tavera, L., Balcazar, M., Gómez-Arroyo, S., 1999. Genetic effects observed in tetrads of *Tradescantia* induced by radon. Fundamental and molecular mechanisms of mutagenesis. Mutation Research 426, 215–219.