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Evaluation of Mutagenic Effects in a Bioindicator from the Radiation Dose Rates Exposed in Radiological Clinics

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Abstract: Since its discovery in 1896, ionizing radiation has been increasingly used both in medicine and in other areas, a fact that has led to extraordinary advances in health. The use of biomonitors, also known as sentinel organisms, has long been used to warn people of hazardous environments. They can be defined as indicator systems that usually include subsystems of a complete organism used to identify a specific target. In the present study, it is proposed an analysis of the mutational biological effects produced by low doses of ionizing radiation in biological samples placed in radiological examination clinics for environmental monitoring purposes. For this, *Tradescantia pallida* was used to verify dose sensitivity / response to biomonitoring through the micronucleus test. The plants were exposed for 24 hours in previously selected locations in clinics in Sao Paulo, Rio de Janeiro and Curitiba. At each site, three biomonitoring points were chosen. The results obtained at these sites pointed to a small increase in the frequency of micronuclei per biosensor cell analyzed. Through these data, a low dose mutagenic effects scale was constructed. *Tradescantia pallida* has offered a good alternative in the assessment of environmental biomonitoring for prevention and safety of employees and patients, being a great alternative tool for the study of the effects of ionizing radiation and prevention. It can be used as a landscaping adornment and as a warning system.

Keywords: Biomonitoring, Radiation, Regulatory Agencies, Security

1. Introduction

Each year, the amount of radioactive waste generated by research institutions, hospitals and nuclear plants grows in Brazil and worldwide. As a result, there is a growing need for storage of these wastes in the country, which leads to questions by society in general and by the workers themselves, regarding the concern about radiation exposure in the neighborhoods closest to these deposits, in relation to the effects on man and the environment. In Brazil, the monitoring body related to ionizing radiation is the National Nuclear Energy Commission (CNEN), which monitors and lists related standards and regulations. Any nuclear facility requires compliance with Laws Nos. 4,188 / 62 and 10,308 / 01 respectively and standards NE-6.05 - Management of Radioactive Waste in Radiating Facilities, NE-6.06 - Selection and Selection of Sites for Radioactive Waste Disposal and NN-6.09 - Acceptance Criteria for Deposition of Low- and Mid-Level Radiation Levels and NE-3.01 Basic Guidelines for Radiological Protection. Due to the importance of radiometric monitoring and the maintenance and control of the sites that use radiological equipment, this work aimed to study the response of a biosensor to the radiation exposure from these sites, seeking to verify the biological effects of low dose rates on environment in a short period of time.

1.1. Radiological Protection

Principles of Radioprotection

The objectives of radiation protection are to prevent or reduce their somatic effects and reduce the genetic deterioration of people, where the problem of chronic exposure is of fundamental importance. ¬ Justification - Any

activity involving radiation or exposure must be justified in relation to other alternatives and produce a positive net benefit for society. Optimization - All exposures should be kept as low as reasonably achievable. ¬ Dose limitation - Individual doses of workers and individuals from the public should not exceed the annual dose limits established by the CNEN 3.01 nuclear standard (shown in table 1).

The normal exposure of subjects should be restricted in such a way that neither the effective dose nor the equivalent dose in the organs or tissues of interest caused by the possible combination of exposures arising from authorized practices exceed the dose limit specified in the table below unless in special circumstances, authorized by CNEN. These dose limits do not apply to medical exposures.

Table 1. *Annual dose limits established by CNEN.*

Annual Dose Limits [a]			
GREATNESS	**ORGAN**	**INDIVIDUAL OCCUPATIONALLY EXPOSED**	**INDIVIDUAL OF THE PUBLIC**
Effective dose	Full body	20 mSv [b]	1 mSv [c]
Equivalent dose	Crystalline	20 mSv [b] (Amended by resolution CNEN 114/2011)	15 mSv
	Skin [d]	500 mSv	50 mSv
	Hands and feet	500 mSv	——

[a] For purposes of administrative control carried out by CNEN, the annual dose term should be considered as dose in the calendar year, that is, in the period from January to December of each year.
[b] Weighted average in 5 consecutive years, provided that it does not exceed 50 mSv in any year.
[c] In special circumstances, CNEN may authorize an effective dose value of up to 5 mSv in a year, provided that the effective effective dose over a period of 5 consecutive years does not exceed 1 mSv per year.
[d] Average value in 1 cm^2 of area, in the most irradiated region.
Source: Standard CNEN - NN - 3.01 of 03/13/2014. "Basic Guidelines for Radiological Protection" [1]

1.2. Radiation Protection

Care radiations can be controlled by operating with three parameters: time, distance and shielding.

(a) Time - The shorter the time a person is exposed to a radioactive source, the smaller the dose absorbed and the smaller the effects caused by the radiation. Therefore, you should always stay as close to a radioactive source as possible.

(b) Distance - It is recommended to keep as far as possible from a source of radiation. This is because the radiation intensity is inversely proportional to the square of the distance between the target and the source, and thus the dose to which an individual is subject can be rapidly reduced.

(c) Shielding - Term referring to the barriers used to attenuate the beam of radiation emitted by radioactive sources in order to reduce the exposure. To ensure that it is effective, the choice of shielding material should take into account the type of radiation, source activity, and acceptable dose rate outside the shielding material

1.3. Radiation Meters

Radiation detector is a device that, placed in a medium where there is a field of radiation, is able to indicate its presence. From the physical point of view, radiation can interact with matter by transferring its energy (partially or totally) and causing the displacement or removal of electrons from its orbitals, phenomena known as excitation and ionization [2]. Among the types of existing detectors, gas detectors are the most traditional and widespread. Its operation consists of collecting the pairs of ions produced by the passage of radiation in the detector. The collection is made by the electrodes present in the gas chamber, which when ionized by the radiation conducts electricity generating the measurable current pulses. The applicability of each type of gas detector will depend greatly on the details of its construction, the applied voltage, and the nature of the gas contained therein [3, 4]. The most commonly used gas detector to date is Geiger-Muller (GM). It operates with relatively high voltage (500 to 900V) causing the electrons to undergo sufficient acceleration to produce secondary ionizations when they collide with other molecules of the gas, reaching a point at which all the gas is surrounded by successive ionizations irrespective of the amount of incident radiation (amplification) without maintaining a proportion. This characteristic of GM prevents it from being able to distinguish types of radiation because it cannot discriminate energies, but makes it a sensitive detector for application in surface and surface monitoring and is of great value in nuclear medicine. Figure 1 shows the photo of the Geiger-Muller already coupled from the cylindrical probe, which were used in this work [3].

Figure 1. *Ionizing Radiation Monitors Modelo: GP-500.*

2. Materials and Method

2.1. Bioassay: *Tradescantia*

Tradescantia is a plant that is easy to adapt to any environment and can be grown all year round, both outdoors, in subtropical regions and in greenhouses, anywhere in the world. The relatively small size and genetic code made up of six pairs of relatively large chromosomes made it a favorable tool for cytogenetic studies. The influence of chemical and physical agents (especially radiation) on the frequency of mutations has been extensively studied through the analysis of changes observed in *Tradescantia* [4-7]. The evaluation of the genetic alterations of *Tradescantia* can be made by detecting somatic mutations induced by mutagens present in the air, soil and water or detecting chromosomal aberrations induced by clotogenic agents (which promote the breakdown of chromosomes) present in air, soil and water [9]. The stamen filament test, which can be used in the evaluation of somatic mutations, and the pollen tube test and the micronucleus assay, were developed for the detection of chromosomal aberrations during mitosis and meiosis, respectively [9, 10].

Using heterozygous clones for flower color, the occurrence of dominant gene mutation or loss can be easily assessed by staining the color of the stamen filaments. The possibility of evaluating a significant sample (around 300 to 700 filaments or up to 7,500 to 18,000 cells of the filaments in a single flower), by the simple evaluation of the change from blue to pink (identifying the mutant cell index), favors this type of assay for the study of genetic effects of mutagens at low doses of exposure (4). This type of evaluation was known as the *Tradescantia* stamen filament assay and was developed by Sparrow of the Brookhaven National Laboratory in the USA. It was explored as a somatic mutation test in radiobiology, chemical mutagenesis and ambient air monitoring [11].

In addition, extensive studies on the direct and indirect effects of radiation on the mitotic chromosomes of *Tradescantia's* microspores led to the establishment of innumerable principles in the induction of radiation-induced chromosomal breaks and aberrations, as well as the interpretation of the aberrant chromosome and the chromatid configuration. The chromosomes of the root end of *Tradescantia* were used for the study of chemical mutagens and chromosome lesions induced by radiation. And the chromosomes of the mitotic cells of the pollen tube of *Tradescantia* were used in the study of alterations induced by ultraviolet light and ionizing radiation until the end of 1940. From then on, it was possible to grow mature pollen grains in a simple medium of agar- lactose which, when subjected to a chemical mutagen or radiation, could exhibit aberrations in the chromatid. The *Tradescantia* pollen tube test was therefore established for the identification of chromosomal aberrations during mitosis [12].

Although chromosomes of germ cells in meiosis are more sensitive to radiation than chromosomes in mitosis, they were rarely used for the study of radiation-induced aberrations. These aberrations were difficult to analyze and count for consistent quantitative data. It was developed, then, the *Tradescantia* micronucleus bioassay (Trad-MCN) micronucleus, first used by Ma in 1983 [6] for the evaluation of the effects of 1,2-dibromethane on the chromosomes of the cells in meiosis. The basis for the development of this assay was the fact that the major problem in the quantitative evaluation of chromosomal aberrations was in the loss of the chromosomes in metaphase I and their blurred image in the preparations of the cells in meiosis. However, if the agent is applied at the beginning of prophase I and the chromosome proceeds for a period of recovery, the acentric fragments of the chromosomes become micronuclei, in the tetrad phase of meiosis, easily identified under light microscopy [12, 13].

2.2. *Tradescantia* and Ionizing Radiations

Ma in 1998 [14], published a dose-response curve for X-rays obtained by micronucleus assay. It is observed that the response is linear, with an increase in the micronucleus index when the radiation dose is increased. This curve, however, is very unprecise when taking into account low doses of radiation (<5 cGy) that are present in the risk areas. The same type of curve had already been obtained by Sparrow et al. in 1972 [15], even with minimum doses of up to 0.25 cGy, for neutrons and X-rays, using, however, the method of evaluation of the filament of the stamen of *Tradescantia.* A linear relationship was observed between the mutation index and the radiation dose below 5 cGy. At doses between 10 and 100 cGy, a quadratic component was observed in the X-ray response curve, compatible with a probable lesion repair at these higher dose levels. It can be concluded that the effects of radiation at low doses are probably due essentially to single shock events [12].

A series of heterozygous clones for the flower color of *Tradescantia* (blue / pink) are available for mutagenicity tests: BNL 02, KU 27, BNL 4430, KU 9, KU 7, KU 20 and BNL 2031. They are classified according to its rate of occurrence of spontaneous mutations. Depending on this characteristic and sensitivity to the various mutagenic agents, they are better suited to one or another type of experiment, either in the laboratory or in field study [16, 17]. Clones BNL 4430, BNL 02, KU 9 and KU 20 are particularly sensitive to radiation in varying degrees. *Tradescantia* clone 4430 is a diploid (2n = 12) hybrid obtained from the Brookhaven National Laboratory through the cross between *Tradescantia* hirsutiflora and *Tradescantia* subacaulis. The flower of clone 4430 is blue (dominant) and heterozygous for this locus. It presents high radiosensitivity, accompanied by high sensitivity to chemical mutagens as well, which makes this clone especially suitable for studies of the detection of environmental mutagens [13].

Radiobiology data have shown that stamens filaments of clone 4430 are sensitive to minimum doses of 0.25 cGy of X-rays. In addition, *Tradescantia* has been and has been used in environmental monitoring around nuclear power plants for the detection of radionuclides being released by reactors and in the study of the genetic effects of ionizing radiation

resulting from the Chernobyl accident and in tests of ambient air mutagenicity of the regions of Krakow and Poland after that accident. Thus, *Tradescantia* can be used in controlled studies on the effects of ionizing radiation, as well as serving as a sentinel in the detection of leaks or in hazardous environments [18].

There are descriptions of synergistic effects of radiation with alkylating agents, which do not necessarily constitute the sum of the effects of each agent alone [19]. For this reason, the actual risk assessment should be based on studies of these synergies between physical and chemical mutagens. In our country, Saldiva and his group have already published studies on the effects of environmental pollutants on clone 4430 of *Tradescantia*). However, the climatic conditions of Brazil, a tropical country with heat, humidity and rainfall, proved to be adverse for the ideal clone cultivation, with inhibition of the growth and flowering of the plants. In addition, when grown outdoors, it suffered constant attacks of insects and parasites, limiting their use in the "real world" [20-22].

Tradescantia pallida var. purpurea, a plant of the same family as *Tradescantia* (Commelinaceae), which is easily found in flowerbeds and gardens of the city of São Paulo, began to attract the interest of the group. It is a tetraploid species, extremely resistant to parasites and insects, that sprouts and grows easily, blooming year-round. To check its validity as a biological monitor for the genotoxicity of ionizing radiation, relative to clone 4430, they performed another study using the micronucleus assay [24]. In this, irradiation of the plants demonstrated that the popular variant is equally sensitive to low doses of radiation as clone 4430 [25].

Data and findings from experiments with *Tradescantia* are generally consistent, accurate, and reliable. In particular, the response to low levels of radiation and chemical mutagens is rarely obtained with other biological assays [21, 23].

Sparrow, cited by Xiao [26] compared the radiosensitivity of several organisms, including mammalian cells, with that of *Tradescantia* and found that the D0 values between 100 and 180 R obtained with the study of the other organisms overlap with the values of D0 obtained for *Tradescantia* in more than one study (149 R, 153 R, 170 R). Thus, it seems reasonable to consider that *Tradescantia* exhibits comparable radiosensitivity to that of mammalian cells [27-29].

The innumerable similarities between the genetic makeup of higher plants and mammals may lead one to believe in similar effects of a mutagen on plant and mammalian DNA. There are, however, great organizational and physiological differences between these organisms, especially morphogenesis and metabolism, which can lead to different reactions to a chromosome lesion. In humans, for example, only a minimal fraction of the DNA damage may or may not lead to mutations, whereas in *Tradescantia* the vast majority of lesions result in mutation. A certain increase in the frequency of initial damage will increase the rate of incidence of mutations in both the human and *Tradescantia* levels [30-32]. Thus, a relative increase in the frequency of somatic mutations in *Tradescantia* may indicate a proportional increase in the risk of mutations in humans.

2.3. Experimental Procedure

In this research, three sites were selected for Biosensor exposure in clinics in Rio de Janeiro, São Paulo and Curitiba, because they have space in the premises for radiological examinations, taking into account their characteristics:

(a) Clinic A - Rio de Janeiro - Radiological exams and attendance of up to 300 people per day.
(b) Clinic B - São Paulo - Radiological exams, tomographies and resonances, attendance of up to 500 people per day.
(c) Clinic C - Curitiba - Radiological exams, attendance of up to 500 people per day.

At each site, a radiometric survey was carried out in its surroundings to select the exposure points using the MRA GP500 monitor model 7237 / 03.44. Three points were selected near the x-ray examination room. a point A within two meters of the door outside the examination room, Point B at the door outside the exam room, and a point C within the room 0.50 cm from the examination table (Figures 2 and 3).

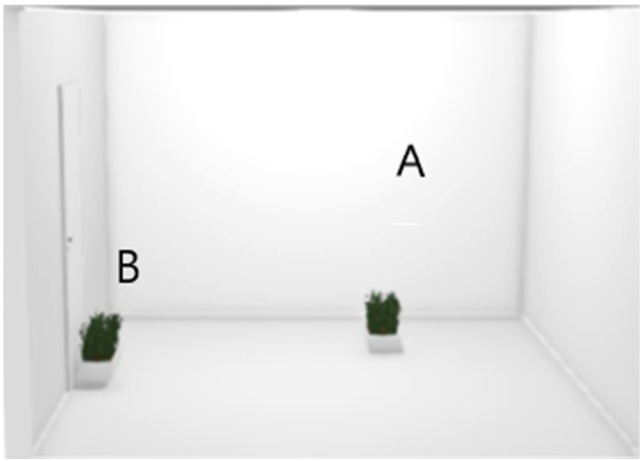

Figure 2. *Illustration of the exposure of biosensors in the studied locations. Point A is 2.0m from the on-site door within the exam room and Point B– on the on-site door within the exam room.*

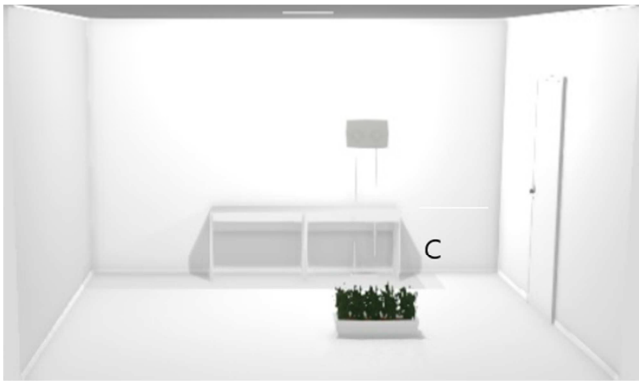

Figure 3. *Illustration of the exposure of biosensors in the studied locations. Point C - location within the examination room.*

After selecting the points, the vessels containing the *Tradescantia* pallida plants were positioned so that at least 10 samples were exposed at each point for 24 hours. After exposure, the samples were placed in water for a period of 6-8 hours, long enough for the meiosis process to continue and the pollen grain stem cells to reach the tetrad stage. When the tetrad phase is reached, it is possible to see the micronuclei. In the final step, the tetrads are fixed, in acetic acid and alcohol solution, in proportion (1:3), following the protocol published by T. H. Ma [5-7].

For the preparation of the blades, once the inflorescences have been selected, they should be well kneaded and subjected to a drop of carmine (contrast agent), to better visualize the stages of tetrads. Lightly tighten the coverslip, so that the tetrads can be viewed by the microscope in the same plane; heat them under the flame of a lamp at a temperature of 80 ° C; the residue is removed and the coverslip is sealed with enamel. The expectation of counting is 300 tetrads per coverslips and, by means of a table, the tetrad count per micronucleus / lamina is determined. In each repository of radioactive waste, from each selected group, 10 samples were analyzed, totaling 3000 cells per group, which were titrated as belonging to the control group, group A and group B, and group C, respectively, according to the rates of dose at each site. [8, 9]

3. Statistical Analysis

For the analysis of the data the program for statistical treatment, SPSS 22.0 for Windows [24] was used. The variance parameter was determined, for the purpose of comparing the counts relative to the three groups in each region, for a significance level of 0.05, Student's t-test was also used in comparisons between samplings (two in two groups), obeying the protocol [9, 10].

4. Results and Comments

For each deposit of radioactive waste, a total of 9000 cells were analyzed. All counts were compared to the control group (Co), which coincides with the control group at the culture site, in which the measured dose rate was 0.26 µGy / h. Table 1 shows the resulting dose rates for each selected group and site.

Table 1. *Dose rate (µGy / h) resulting from each selected group and location.*

Groups / Local	Co* (µGy/h)	A (µGy/h)	B (µGy/h)	C (µGy/h)
Clin-A	0,26	0,61	31,8	27,1
Clin-B	0,26	0,39	25,8	36,5
Clin-C	0,26	0,47	28,7	36,7

* Co is the control group at the growing site.

The Figure 4 represents the number of micronuclei per 100 cells analyzed, resulting from the exposure of the biosensor, which relates the dose rates to the observed mutational effects in each selected group and site. The curve in question showed a small growth, even for the low doses.

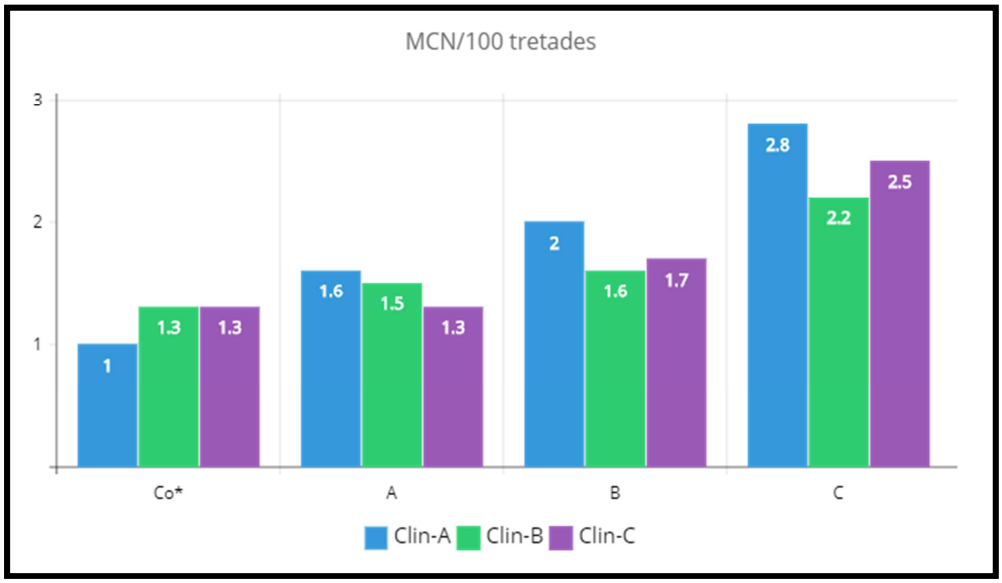

Figure 4. *Results according to the groups versus MCN / 100 tetrad.*

First, in order to verify the influence of the biosensor during transport to the place of exposure, the MCN / 100 tetrad frequencies at the points of the cultivation control, Co (negative), were compared with the frequencies in the other local control groups, radioactive waste. In this comparison, no significant difference was found ($p > 0.05$), which leads to the conclusion

that the biosensor did not suffer stress damage during transport.

When the group Co was related to groups A and B, different responses were observed between them. For group A, in clinics A and B, respectively, no significant difference was observed. For point C, at point C within the room, an increase in mutation frequency was noted, with a significant difference ($p < 0.05$). For clinic B, a greater difference was found in points B and C and in clinic A only for point C; only point A at a distance of 2.0m from the entrance door of the examination room did not show a significant increase when compared to the culture site.

The curve of figure 5 demonstrates the linear relationship found between the dose rates and the frequency given in number of micronuclei per 100 trétrades.

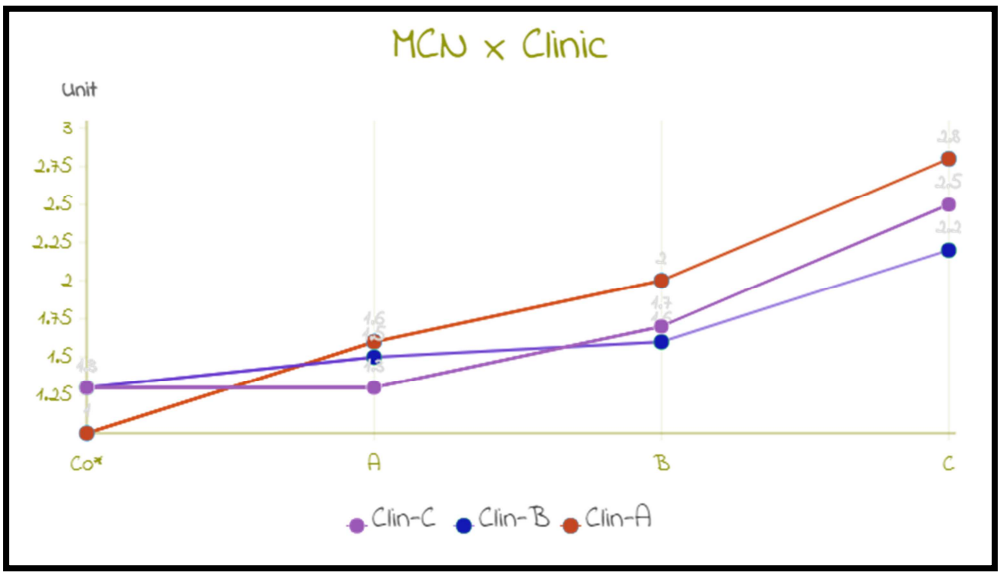

Figure 5. *Mutagenic scale as a function of the local dose rate.*

5. Final Considerations

Studies using species of *Tradescantia*, compare their sensitivity to the effects of exposure to radiation, with those due to the action of genotoxic agents [16-19]. The mutagenic scale shown in figure 2 is consistent with that obtained by Suyama [20], when studying a methodology of biomonitoring tests with *Tradescantia* when submitted to X-ray exposure. Villalobos-Pietrini [25] used this methodology with the biosensor when comparing them to that of the *Tradescantia* clone 4430, having recorded the increase of the mutational frequency of 7 MCN / 100 to 17MCN / 100, by subjecting them to a dose of 0, 8 Gy from a Co_{60} source. It has recently been shown that the sensitivity of *Tradescantia* to the effects of radiation serves to correlate the dose rates of gamma radiation that is subjected to the mutational frequency observed in low doses [13], by micronuclei methodology. From the responses provided by the biosensor, *Tradescantia pallida*, the advantage of using this methodology is to observe a high amount of mutational changes in a short period of time, being able to anticipate the effects caused in the environment and, consequently,, to the man, according to the degree of occupation. Therefore, it is recommended to use this methodology in the periodic monitoring, since this biosensor can be introduced into the environment, due to its ease of planting, propagation and excellent acclimatization, and may even be used in the prevention of radiological accidents.

The results of low-dose, well-characterized studies can help to develop new ways to use low dose radiation in clinical environments, as well as to fully determine specific dose and dose limits for certain levels, which comes to Agree to the studies carried out by Truong [26].

With reference to the salutation of the collaborator and the public, it was clarified that although some points present a higher rate of mutagenic effects in the biosensor, it is still within the expected and the norms not being detrimental to both. However, it is suggested that an organ certified and recognized by CNEN should do the employee who uses the dosimeter with diary and the readings.

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