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GAMMA RADIATION INFLUENCE ON *CLADONIA SUBSTELLATA* VAINIO (LICHEN) AND ITS EFFECTS ON LIMESTONE ROCKS.

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ABSTRACT

The lichens play an important role in decomposition of the rocky substrate, through the chemical weathering of their substances. This work aimed to determine the influence of gamma Ray on usnic acid production of *Cladonia substellata* and the influence of chelates formation in limestone rocks. Samples with 2.5g of *C. substellata* were packed on paper envelopes for irradiation submission to gamma on a Co-60 source. They received 10 different doses: 5, 7, 10, 15, 20, 30, 40, 50, 60 and 80 Gy. Lichen irradiation was conducted on the gamma irradiator (Co-60), and packed over powdered limestone. Lichen samples were analyzed by High Performance Liquid Chromatography (HPLC) and rocky samples by X-Ray Diffractometry. The X-ray diffractogram obtained from the analysis of limestone not subjected to the action of lichen - control sample was compared with limestone subjected to *C. substellata* irradiated at doses 10, 30 and 80Gy. Increased production of the usnic acid and changes on the rocky samples were noted. We realized that *C. substellata* increments the usnic acid biosynthesis as the gamma radiation dose is increased, but there is a limit to it. The chelating effect of the usnic acid on limestone was proportional to the produced amount of the substance, which could be extrapolated to natural conditions, where excessive radiation may influence pedogenesis and ecological succession.

1. INTRODUCTION

The soil is formed from the combined influence of climate and biotic activities, modified by topography, which acts on the originary materials for a certain period of time [1].

Regarding the biotic activities, the lichen stands up, because not only they indicate the air quality, but also are part of a numerous reactions on the living system, acting on the soil formation, interacting with bugs and other small animals, providing food and shelter.

Regarding the soil forming function, the lichen enables succession of the rocky substrate colonization, since they can live directly on the rocks, creating a biogeophysical and biogeochemical intemperized cover. The biogeochemical process consists on the release of lichen substances that will act on the chemical degradation (or decomposition) of the rock [2].

The production of those substances on differentiated amounts is a way of adapting to the natural adversities or anthropic interference [3]. They also respond to an exogenous radiation supply. Considering all the previously noted, this work aimed to determine the influence of gamma ray on usnic acid production of *Cladonia substellata* and its compound increase on chelates formation in limestone rocks [4].

2. DATA SOURCES & METHODOLOGY

2.1. Lichen

For this experiment, we collected tuffs of *Cladonia substellata* Vainio at the Guaribas Biological Reserve, Mamanguape – PB. Part of it was identified by morphological and chemical characteristics of the thallus and deposited in the UFP Herbarium, Department of Botany (UFPE), under the registration number 46687.

2.2. Limestone

Limestone rock samples were collected on the island of Itapessoca, located in Goiana-PE.

2.3. Lichen Irradiation

Samples with 2.5g of *C. substellata* were packed on paper envelopes for irradiation submission to gamma on a Co-60 source. They received 10 different doses: 5, 7, 10, 15, 20, 30, 40, 50, 60 and 80 Gy. Lichen irradiation was conducted on the gamma irradiator (Co-60 – irradiator, Radionies Laboratory, dose tax was 9,06 Gy. H-1, on 11/04/2004) at the Nuclear Energy Department – UFPE.

2.4. Setting up the Experiments

Samples (46g) of powdered rock were packed on Petri dishes, with protective dome, in three repetitions (by triplicate), receiving each 2,5g of *C. substellata* irradiated with gamma radiation.

2.5 Gathering the Samples

90 days after the experiments were set up, lichen and rocky samples were collected from each Petri dish and packed each one separately for further analysis.

2.6 Obtainment of the Extracts

The *C. substellata* samples from control group (not irradiated) and from the irradiated group were extracted by drainage system with organic solvents.

2.7. Sample Analysis

2.7.1. High Performance Liquid Chromatography (HPLC)

The organic extracts (1mg/ml) and the usnic acid Merck-pattern or purified (0,1mg/ml) were injected into Hitachi Liquid Chromatographer coupled to an UV detector at 254 nm.

2.7.2. X-Ray Diffractometry

X-Ray diffractometry analysis were performed with limestone samples before and after being subjected to lichen irradiated at doses of 10, 30 and 80 Gy, to verify possible transformations on the mineralogical composition of the rock.

3. RESULTS

Thru Performance Liquid Chromatography (HPLC) analysis, it was possible to quantify the metabolized USN by *C. substellata* samples subjected to different gamma radiation doses. A variation on the peak area corresponding to the USN was observed, and also small variations on its retention time (RT) in the row. The contents of the metabolized usnic acid by the various *C. Substellata* samples vary depending on the dose. This can be best assessed by calculating the peak areas of the chromatograms (Figure 1).

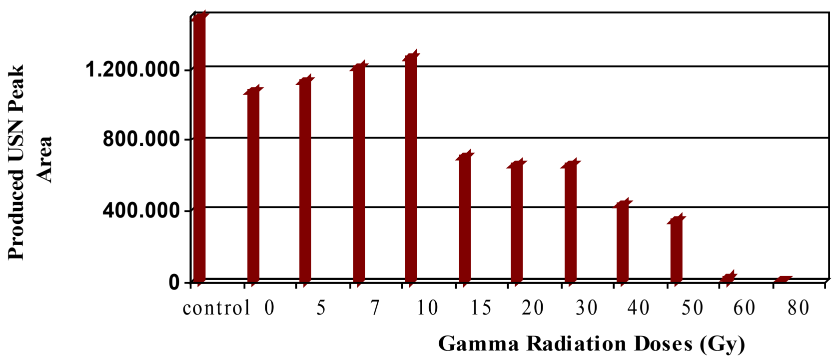

Figure 1- Produced usnic acid (USN) peak area in organic extracts of *C. substellata* (mg USN/ mg extract), subjected to different doses of ^{60}Co gamma radiation.

In natural conditions the lichen produces greater amount of USN. However, after 90 days of incubation, as observed with the lichen not subjected to radiation (0 Gy), this substance is produced at lower levels, due to laboratory conditions. Still it is possible to increase the production of USN increasing the gamma radiation dose, which is growing by the dose of 10 Gy. This behavior indicates a higher metabolic activity for the USN production, as a defense mechanism or protection of the thallus, or even adapt to new imposed conditions. Moreover, the lichen has a tolerance limit to radiation, shown by the production of the USN. This falls from the 10 Gy dose, reaching maximum levels at 60 Gy and 80 Gy.

The lichen, when subjected to gamma or UV radiation, tends to produce greater quantities of its phenols, because since they act as photoreceptors and/or photo-inductors, they protect the interior of the lichen thallus, preserving its biological functions [5]. *Cladonia verticillaris* thallus promotes greater synthesis of fumarprotocetraric acid - its main compound - when directly exposed to the sun in the natural environment [6]. The same lichen, when protected by the shade of the canopy of *Anacardium occidentalis*, prioritizes the accumulation of chlorophyll and other photosynthetic pigments. The existence of late effects on *Cladonia sylvatica* and *C. verticillta* irradiated with acute or chronic doses of gamma radiation [7; 8; 9]. Furthermore, the authors emphasize the internal or external structural damage instead of the physiological behavior, except for the brown pigment production, which can deal with pheofitine, resulting from the chlorophyll degradation.

The X-ray diffractogram obtained from the analysis of limestone not subjected to the action of lichen - control sample (Figure 2 - A) was compared with limestone subjected to *C. substellata* irradiated at doses 10, 30 and 80Gy (Figure 2 - B, C, D), showed changes in the height of some peaks as well as the disappearance or emergence of other new peaks, different from the control sample. Thus, we observed changes in the mineralogical composition of the limestone subjected to contact with irradiated *C. substellata*.

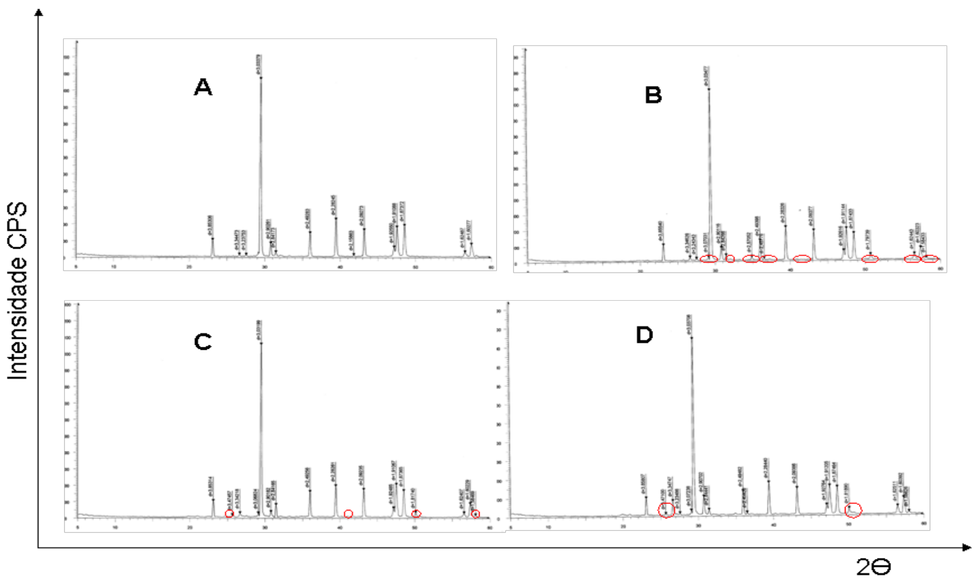

Figure 2: X-Ray Difratograms of limestone samples kept in contact with *Cladonia substellata* thallus subjected to gamma radiation. A:

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natural rock – control sample; B: 10 Gy; C: 30 Gy; D: 80 Gy.
Control sample additional detected peaks.

The samples of *C. substellata* irradiated with 80 Gy provided little modification on the limestone, since its difratogram was similar to the control material. On the other hand, the irradiated lichens (10 and 30 Gy) were different. The limestone subjected to irradiated *C. substellata* at 10 Gy presented eight peaks, unlike the ones from control sample, while the 30 Gy irradiated material showed four peaks. This data corroborate with the information obtained by HPLC, showing that the increase on the usnic acid production is proportional to the increase of the gamma radiation dose.

Mineralogical changes that were observed by x-ray diffraction may be compared to the observed the contact of *Parmelia conspersa* with its granite substrate, detecting the presence of kaolinite crystals, halloysite and amorphous silica [10]. This author proved also that the most frequent mineral formed on the Rhipocarpon geographicum interface with the granite is the halloysite, while micas and feldspars show alterations to the goethite. Also, showed that the *Umbilicaria pustulata* lichen does not generate minerals in nature as in the laboratory, producing montmorillonite instead.

4. CONCLUSIONS

Gamma radiation inducts an increase on the usnic acid production on *C. substellata* lichen, probably as a defense mechanism against the increased gamma radiation action, but there is a limit to it, which determines the production decrease of the acid on higher doses.

Given the differentiated biosynthesis of the usnic acid, because of the gamma radiation dose on the *C. substellata* samples, the degrading effect on limestone was proportional to the amount of this acid on the lichen thallus.

It's likely that on a natural environment, lichen exposed to high natural radiation doses or its sources may interfere on the pedogenesis process and/or ecological succession on the ecosystems.

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