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EFFECT OF IONIZING RADIATION ON THE COLOR OF BOTANICAL COLLECTIONS – EXSICCATA

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ABSTRACT

Conservation and preservation strategies are essential to manage botanical collections specially for dried herbarium specimens also known as exsiccates, usually referring to a set of identified specimens belonging to taxa and distributed among all herbaria around the world. Particularly, these collections are very sensitive to the attack of fungi and insects. In recent years, disinfection by ionizing radiation has become an effective strategy to preserve cultural heritage objects and archived materials with excellent results. In this work, the effects on color properties of gamma radiation on exsiccates samples were studied. Thus, six exsiccates, botanical pressed and dehydrated samples were selected from the Dom Bento José Pickel Herbarium (SPSF), situated at São Paulo (Brazil). Three of these samples comes from Asteraceae family and were collected in 1946, 1984 and 1986, while three other samples belong to Solanaceae family and were collected in 1953, 1984 and 2007. Families of selected botanical collections are very susceptible to biodegradation. The irradiation was performed at the Multipurpose Gamma Irradiation Facility at IPEN applying absorbed doses of 1 kGy, 6 kGy and 10 kGy, which are values of absorbed dose for disinfestation and disinfection. Results were analyzed using colorimetry with CIELAB color space scale and scanning electron microscopy. The results showed that there were no significant changes on colorimetric morphological properties of the samples.

1. INTRODUCTION

Botanical collections contain exsiccates, which is a dehydrated vegetal specimen usually dried in an oven, compressed, attached to paperboard and labelled with specimen and gathering (name of specimen, place and date of sample collection, name of collector). These vegetal materials are subjected to fungal attack and insect pests threatening their entirety. Preservation and conservation methods are essential to maintain the wholeness of these botanical collections.

Herbarium is a collection of plant or fungi specimens technically or scientifically preserved to long term studies. These specimens are mainly used to learn about the characteristics of flora and mycota of a region, country or continent. These characteristics include morphology, taxonomy, biogeography and history, for example. Eventually, Herbarium designate also a place that houses an herbarium and it accounts for the whole entirety of exsiccates and botanical collections [1].

Exsiccates are composed of tree leaves, flowers, branches and fruits (Fig. 1). It is made of organic complex material which includes essentially cellulose, lignin, starch, traces of grease, water and crystals. Each vegetal specimen has different properties and responses to stimuli. Some of them chemically deteriorate after the process of herborizing. That deterioration interferes on exsiccates from further chemical analyzes. The lost morphological features of these collections (leaves color, fruits, flowers, smells, tree height, type of vegetation) should be written on field cards and added at the label related to exsiccates. In mostly Herbarium, Asteraceae and Solanaceae samples are some of most sensitive specimens concerning insect pests and fungal attack [2], [4].

Figure 1: Typical exsiccate samples.

The plants of the family Asteraceae have cosmopolitan distribution, which almost 1700 genus and 30000 species. In this regard, 290 genus and 2100 species are found in Brazil [5]. Several Asteraceae are grown as ornamental plants, for instance the margarida (*Leucanthemum vulgare*), crisântemo (*Dentranthema grandiflorum*), dália (*Dahlia pinnata*), gazânia (*Gazania rigens*) and zínia (*Zinnia peruviana*). Other Asteraceae are the girassol (*Helianthus annuus*), alface (*Lactura sativa*), chicória, almeirão, escarola (*Cochorium intybus*) and alcachofra (*Cynara scolymus*). Asteraceae also includes some medicinal plants, carqueja (*Baccharis* spp.), camomila (*Matricaria recutita*), guaco (*Mikania* spp.), estévia (*Stevia rebaudiana*) and mil-folhas (*Achillea millefolium*). Eventually, Asteraceae are common in Brazilian open vegetable formations, especially in the Brazilian cerrado, whereas they are hardly found inside dense forest [6].

Solanaceae are also a family of plants that have cosmopolitan distribution, notably in the Neotropical region. In general, 150 genus and 3000 species of Solanaceae are found in the world, among which 34 genus and 488 species are found in Brazil. The biggest genus is called *Solanum* [7]. Several Solanaceae are edible plants recognized by their economical role, for instance, tomatoes (*Solanum lycopersicum*), potatoes (*Solanum tuberosum*), peppers (*Capsicum* spp.), eggplants (*Solanum melongena*) and Brazilian jiló (*Solanum gilo*). Other are ornamental plants, manacá-de-cheiro (*Brunfelsia uniflora*) and petúnia (*Petunia hybrida*). Numerous Solanaceae accumulate alkaloids and are extremely toxic, for instance, the tabaco

(*Nicotiana tabacum*). Some Solanaceae are native from Brazil, as the maria-pretinha *(Solanum americanum*), a very common crop-invasive plant, the fruta-do-lobo (*Solanum lycocarpum*), one of the most characteristic species of the Brazilian cerrado with large rounded fruits, the jurubeba (*Solanum paniculatum*) used as a medicinal plant, the dama-da-noite (*Cestrum* spp.) and the joá-de-capote (*Physalis angulata*) [6].

Commonly, botanical preservation and conservation methods comprise some techniques for preserving the herbarium's collection. These techniques include some specific assemblages of field and greenhouse materials, as well as concentrated conservation in the purge of insects and mites to avoid the deterioration of the collection [8]. As far as conservation methods of collections is concerned, the use of gamma radiation as a treatment method appears to be better than other traditional methods. Firstly, some advantages are the easy application and instantaneous effectiveness, the absence of chemical residues and the non activation of material nuclei. Moreover, products subjected to gamma radiation do not require quarantine, eradication of insects and fungi because radiation acts at any stage of their life cycle. Disinfection of cultural heritage artifacts and gamma radiation cellulose-based materials has been successfully applied in recent years. Their results demonstrate that there is no significant change (side effects) in the irradiated material and this material will remain stable over the lasting useful life [9], [10]. Studies shown that an absorbed dose of 0.5 kGy (minimum dose) is appropriate to eradicate xylophagous insects, however if the goal is to eliminate fungi, then absorbed doses between 6 kGy and 10 kGy need to be applied. Nevertheless, side effects of radiation may be remarkable and particularly affecting each material. Evaluation and comprehension of side effects of radiation is essential. These side effects are related to polymers including cellulose, lignin and proteins [11].

In this study, exsiccata samples were irradiated using gamma rays with absorbed doses between 1 kGy to 10 kGy. The selected ranged dose promotes disinfestation and disinfection. Samples were evaluated by colorimetry and scanning electron microscopy to verify colorimetric and morphological properties changes.

2. MATERIALS AND EXPERIMENTAL

2.1. Exsiccates Samples Selection and Preparation

Initially, six representative botanical pressed and dehydrated samples – exsiccata - were selected from the Dom Bento José Pickel Herbarium (SPSF), located in São Paulo, Brazil. The families of the selected botanical collections were Asteraceae and Solanaceae. Table 1 and Fig. 2 show the main identification, classification and general characteristics, respectively of the selected materials.

For this study, only two exsiccate samples were selected: SPSF-4021 and SPSF-08821, because the other samples are still being analyzed. However, there is a short-term perspective to continue studies with the other samples.

Table 1: Exsiccata samples identification and classification

Sample No.	Botanical families	Species	Collection date
SPSF-4021	Asteraceae	*Baccharis crispa* Spreng.	1946
SPSF-10516	Asteraceae	*Critoniopsis quinqueflora* (Less.) H. Robinson	1984
SPSF-10553	Asteraceae	*Baccharis regnellii* Sch. Bip ex Baker	1986
SPSF-4074	Solanaceae	*Solanum swartzianum* Roem. & Schult.	1953
SPSF-08821	Solanaceae	*Solanum pseudoquina* A. Sr.-Hil.	1984
SPSF-39975	Solanaceae	*Solanum mauritianum* Scop.	2007

Figure 2: Exsiccata samples general characteristics. Asteraceae (a) *Baccharis crispa*, (b) *Critoniopsis quinqueflora*, (c) *Baccharis regnellii. Solanaceae (d) Solanum swartzianu, (e) Solanum pseudoquina, (f) Solanum mauritianum*

2.2. Irradiation by Gamma Rays from Cobalt-60 Sources

Exsicatas samples were irradiated with gamma rays from cobalt-60 at the Multipurpose Gamma Irradiation Facility of the Nuclear and Energy Research Institute – IPEN-CNEN/SP of the National Nuclear Energy Commission (CNEN) in São Paulo. This facility – IAEA Category IV – is a panoramic wet source storage compact irradiator that means, when it not in use, the radioactive sources are stored and fully shielded in a pool of 7 meters depth deionized water [12], [13]. The current installed activity of the facility is 10.4 PBq (320 kCi) and uses cobalt-60 source pencils (45cm length and 1cm diameter) where the radioactive material was encapsulated in corrosion resistant stainless steel such that gamma radiation can come through but not the radioactive material itself, eliminating the risk of contamination. 64 source pencils were loaded into predetermined positions in source modules and distributing these modules over the source racks over the structure (named racks) that moves from the bottom of the pool to the irradiation chamber level [14], [15].

Samples were packed in paper envelopes and irradiated by gamma rays with absorbed dose of 1 kGy, 6 kGy and 10 kGy. Dose rate was 5-6 kGy.h^{-1}. The absorbed dose, *D*, is the amount of energy absorbed per unit mass of irradiated matter at a point of interest. The International Commission on Radiation Units and Measurements has defined as the mean energy, $d\bar{\varepsilon}$, imparted by ionizing radiation to the matter in a volume element divided by the mass, *dm*, of that volume element [11]:

$$D = \frac{d\bar{\varepsilon}}{dm} \tag{1}$$

The SI unit of absorbed dose is the gray (Gy), and the polymethyl methacrylate PMMA-Harwell Gammachrome and Amber 3042 dosimetry system was used in order to calculate the absorbed dose in the irradiated samples [11], [14].

2.3. Colorimetric Measurements

The color alterations of samples are verified to investigate possible effects of the irradiation on the color of the processed material, which occurs due to the excitation of the electrons. Already, exist several systems to measure the color. The CIELAB system, published in 1976 by the *Commission Internationale d'Eclairage* (CIE), has become the universally accepted colorimetric reference system for quantifying and communicating color.

Color differences can be computed as the relative distance between two reference points within a color space. This difference is typically expressed as delta E (ΔE) and is calculated by comparing reference and sample L*a*b* (L* = Lightness, a* = red to green, b* = yellow to blue) values to pinpoint how far apart two colors reside within a color space. The ΔE calculations will quantify the magnitude of a color difference but do not necessarily indicate the direction of the difference [16], [17].

The ΔECIELAB 1976 uses an equation (2) to calculate the distance between two points of color also this parameter is known as total color.

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \tag{2}$$

The colorimeter PCE-CSM8 is used at three selected points of each biological sample (Table 2 and Fig. 3), firstly at non-irradiated samples. Analysis of samples is performed at least 48 hours after irradiation of the material, when the process of excitation of electrons are already steady.

Table 2: Measurement points description.

Point	Description
A	flower / inflorescence
B	leaf / branches (adaxial surface - top)
C	leaf / branches (abaxial surface - bottom)

Figure 3: Locations of the measurement points in the samples.

Samples are placed on a white tile before measurements with the colorimeter. This piece acts as a reference support to normalize measurements, since the analyzed material is composed of irregular flowers and leaves. Colorimeter calibration was performed using black and white colorimetric standards (SN 283788).

Considering the anatomical characteristics of the leaves, the color measurements is performed on both sides of samples, the adaxial surface (upper part) and the abaxial surface (lower part), covered by the epidermis. Leaves are formed by a dermal system (epidermis), a fundamental system (mesophyll) and a vascular system (vascular bundles). As vascular tissues the leaf forming the ribs, its surface is irregular. Then, tile is marked with a circle and a central rectangle

for accurate positioning of the measure device. In this way, each sample are always observed in the same point for all measures.

The identification of the total color difference (ΔE*) of the unirradiated and irradiated samples with 1 kGy, 6 kGy and 10 kGy and their comparison indicates if this difference is acceptable or not based on an acceptance scale.

2.4. Field-emission Gun Scanning Electron Microscopy (FEGSEM)

Scanning electron microscopy was used to analyze and characterize the non-irradiated (0 kGy) and the effective disinfected (10 kGy) exsiccata samples. Surface topography and elemental analysis of the exsiccata were analyzed by scanning electron microscopy (FEGSEM), using a Jeol JSM-6701F electron microscope with a field emission gun operating at 2kV and 3kV with a coupled Thermo EDS detector.

3. RESULTS AND DISCUSSION

3.1. Colorimetry

The color of samples was measured before and after each cumulative absorbed dose of the irradiation using PCE spectrophotometer and color difference results were compared.

Table 3 shows the criteria proposed by Hardeberg for the interpretation of color difference [17]. Where, the effect can be classified by mean errors of 0-1 as limit of perception, 1-3 as very good quality, 3-6 as good quality, 6-10 as sufficient, and more than 10 as insufficient.

Table 3: Hardeberg Criteria for the practical interpretation of ΔE* measuring the color difference between two color.

ΔE*	Effect
< 3	Hardly perceptible
3 < 6	Perceptible, but acceptable
> 6	Not acceptable

The results of the colorimetric analysis (Table 4) at the three points (A, B and C) of samples SPSF-4021 and SPSF-08821 irradiated with 1 kGy, 6 kGy and 10 kGy indicate that the color difference of the results is predominantly classified as “Hardly perceptible” (Δ**E***<3). Also, color difference is classified as “Perceptible, but acceptable” (3<Δ**E***<6) in only two points.

Table 4: Results of colorimetric analysis of SPSF-4021 e SPSF-08821samples.

Sample	Dose (kGy)	Point A	Point B	Point C
SPSF-4021	1	1.751	0.206	2.152
	6	1.655	0.994	0.009
	10	0.913	4.038	0.095
SPSF-08821	1	0.454	0.052	0.298
	6	1.545	0.583	0.385
	10	3.724	0.639	0.434

Analyzing the results of the SPSF-4021 sample (Fig. 4) in Point A (inflorescence) it is noticed that as the irradiation doses were increased the indicative values of alteration of the coloring suffered a slight decrease. At Point B, branches, the data indicate an increase in the color change. At Point C, abaxial surface, there was a decrease in color change for irradiation between 1 kGy and 6 kGy, nevertheless color remains stable for irradiation of 10 kGy.

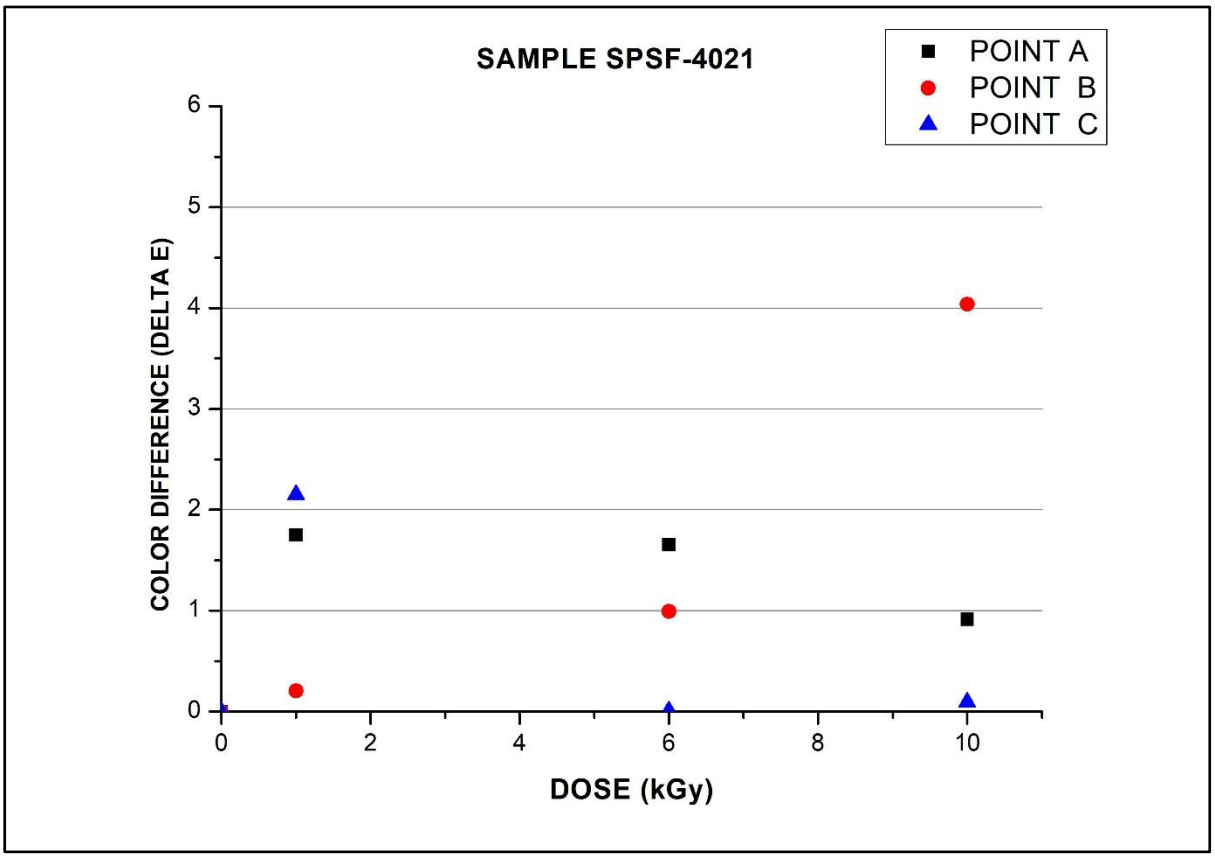

Figure 4: Color changes for the SPSF-4021 sample.

The results of the sample SPSF-08821 (Fig. 5) in Points A (flower), B (adaxial surface) and C (abaxial surface) indicate that as the irradiation doses increases, indicative values of color alteration increase, being more representative in point A. However, measurements remain within acceptable range even though an increased of DeltaE after irradiation.

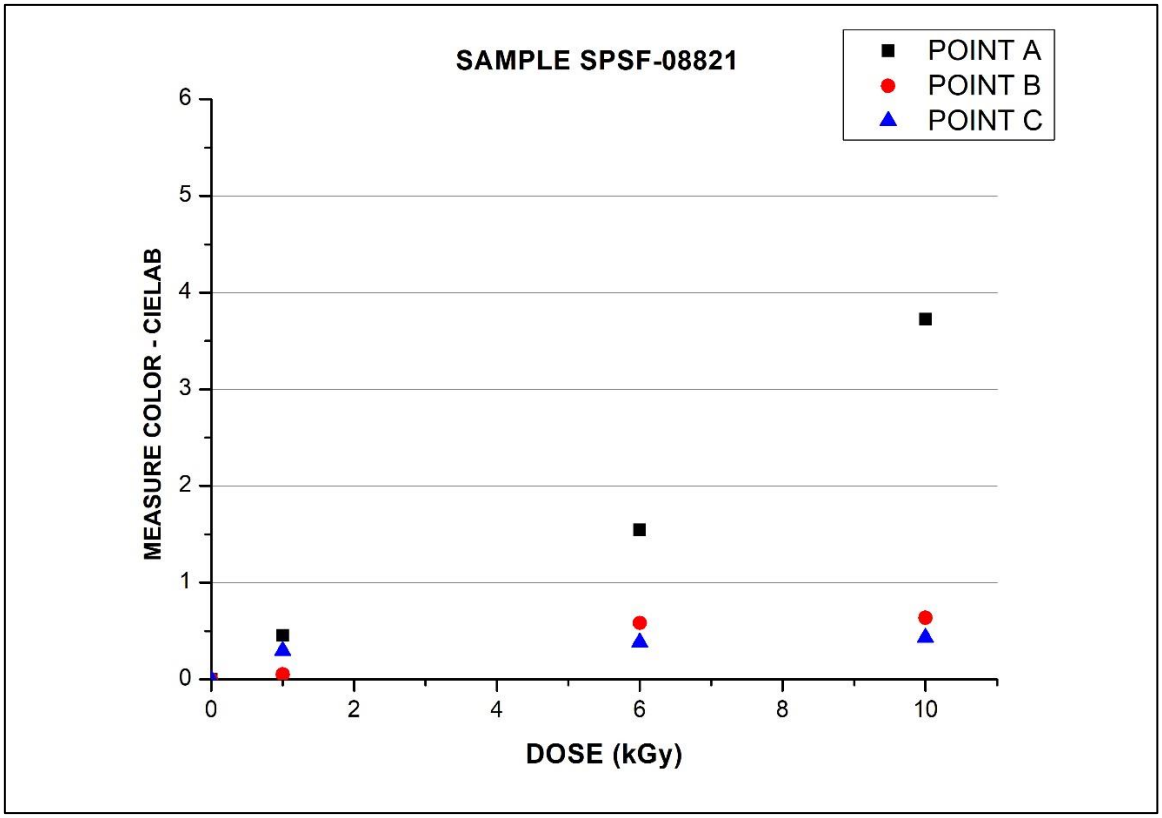

Figure 5: Color changes for the SPSF-08821 sample.

3.2. Scanning electron microscopy analyses

The images show different morphologies for SPSF-4021 and SPSF-08821 exsiccate (Fig. 6 and Fig. 7). Figure 6 (a) and (b) show the flower part of sample SPSF-4021 before and after irradiation (at 10kGy), respectively. Also, the leaf adaxial surface is depicted on figure 6 (c) and (d) before and after irradiation, respectively. Figure 7 shows the leaf adaxial (a,b) and abaxial surfaces (c,d) of samples SPSF-08821 before and after irradiation, where we can observe the presence of the leaf organelles. In both samples, no effect of the irradiation on the structure of the samples can be observed on the FEGSEM images of non-irradiated and gamma irradiated samples.

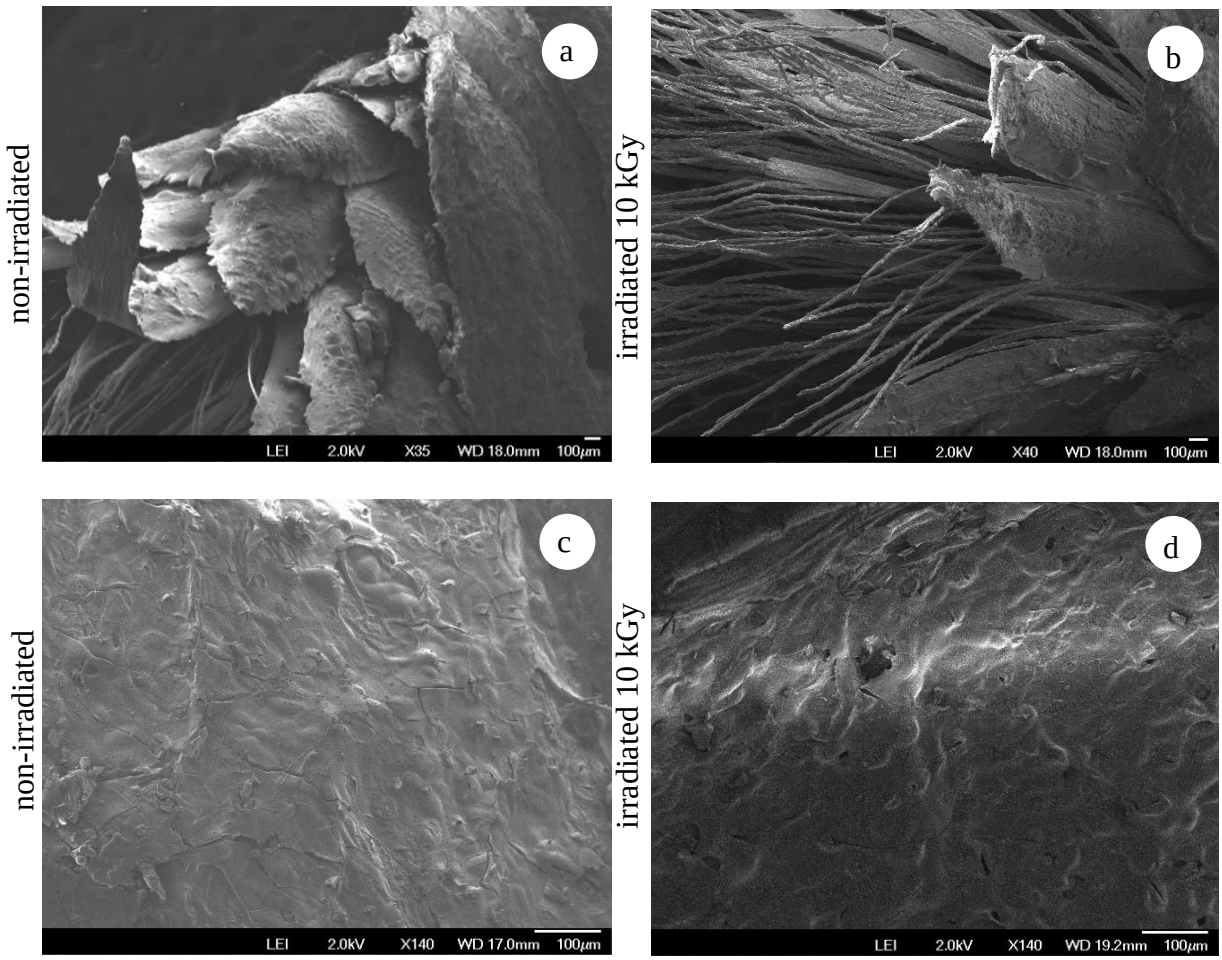

Figure 6: FEGSEM micrographs non–irradiated (0 kGy) and irradiated (10 kGy) of the sample SPSF-4021: (a, b) flower/ inflorescence, (c, d) leaf adaxial surface.

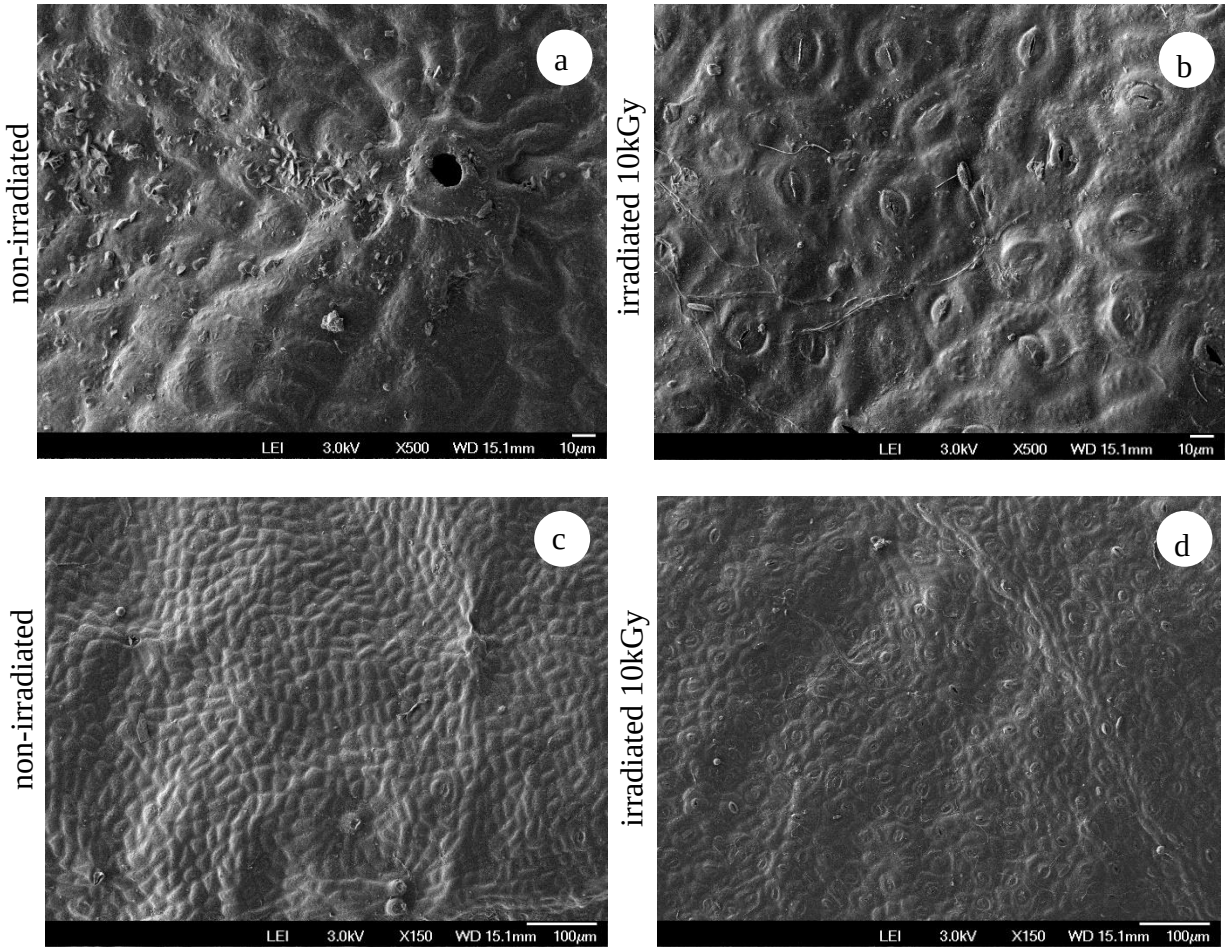

Figure 7: FEGSEM micrographs non–irradiated (0 kGy) and irradiated (10 kGy) of the sample SPSF-08821: (a, b) leaf adaxial surface (c, d) leaf abaxial surface

4. CONCLUSIONS

The results revealed no significant changes on colorimetric morphological properties of then irradiated samples applying absorbed doses of 1 kGy, 6 kGy and 10 kGy. The color changes between the non-irradiated samples and the irradiated sample at the high absorbed dose are perceptible, but acceptable considering the adopted scale. The microscopy images of the non-irradiated and 10 kGy irradiated samples did not show significant differences in the topographic morphology of the exsiccata samples. The results obtained corroborate the studies of the application of gamma radiation to preserve materials of cellulosic origin. That way, the present study demonstrated that the gamma radiation applied to botanical collections as exsiccata for insects and fungi disinfection can be achieved safety applying dose between 1 kGy to 10 kGy with no significant change or modification of color properties of the constitutive materials. Subsequently and for continuity of this research, the other samples will be analyzed.

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