Chapter 23

APPLICATION OF GAMMA RADIATION FOR THE DECONTAMINATION OF EXSICCATAE: SÃO PAULO HERBARIUM CASE STUDY

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23.1. INTRODUCTION

Part of the collection of exsiccatae (dried plant specimens) of the Maria Eneyda P. Kaufmann Fidalgo Herbarium (Herbarium SP) of the Instituto de Pesquisas Ambientais in the State of São Paulo was contaminated by microorganisms in 2020 due to water infiltration in the temporary storage site of the collection. The situation was evaluated, taking into consideration the large volume of material and the nature of the emergency and with the goal of minimizing the deterioration of the collection. Based on the literature, research and experience with the use of ionizing radiation at the Multipurpose Gamma Irradiation Facility at IPEN, the collection was submitted for treatment by this method. About 100 000 specimens of exsiccatae were decontaminated with a 10 kGy dose of radiation.

Herbarium SP, which was established in 1917, is managed by the Environmental Research Institute of the Secretariat for Infrastructure and Environment of the State of São Paulo. It is the third largest herbarium in Brazil, with a collection of some 550 000 specimens of Brazilian flora and representative collections of all cryptogams and phanerogams and all Brazilian ecosystems.

Herbarium SP carries out scientific research on vascular plant systematics. It undertakes floristic studies and makes floristic inventories to support studies on the structures and dynamics of plant communities in order to generate knowledge about the functioning, history, distribution and environmental relationships of plant communities. Herbarium SP serves specialists and students from different regions in Brazil and abroad.

The procedures for preserving, conserving and controlling the biodeterioration of exsiccatae collections under the custody of herbaria follow the policies and guidelines of the institution in question and are generally defined in collaboration with the curators. Continuous and permanent monitoring of collections is required, and many decontamination and disinfestation methods are used, including the application of chemical substances and physical and biological control methods.

In 2019 and 2020, structural problems in the herbarium building of Herbarium SP led to the collection being relocated until a renovation of the building was completed. Part of the collection was temporarily stored in rooms located in other buildings and in two refrigerated steel containers positioned close to the Herbarium SP building (Figs 23.1 and 23.2). A large number of stacked cardboard boxes were stored in the containers, which contained approximately 100 000 specimens of Orchidaceae, Poaceae, Fabaceae and Bromeliaceae.

In 2020, rainwater infiltrated the containers, which caused high humidity inside and allowed the growth of fungi and some insects in the exsiccatae. Exsiccatae are rich in nutrients that favour the development of microorganisms that feed on cellulose, chlorides, salts and adhesives. The microorganisms also excrete organic acids and toxins that can damage the collection (Fig. 23.3).

There was concern about the degradation of the exsiccatae and about the presence of fungi that could cause allergic reactions among people who may handle the collection, since mould and insect excrement irritate people's respiratory systems [23.1].

FIG. 23.1. Container used for temporary storage of the collection during renovation of the Herbarium SP building.

FIG. 23.2. Internal view of the container with storage boxes of exsiccatae.

FIG. 23.3. Exsiccatae with mould and microorganism residues.

In this context, an evaluation was carried out to define which method of disinfestation and disinfection of the material would be the most appropriate to use, taking the following into consideration:

(a) The need for an immediate intervention;
(b) The large amount of material;
(c) Contamination by both insects and fungi;
(d) The need to avoid causing damage to the exsiccatae and harm to people who may handle the collection;

(e) The impracticability of applying conventional methods (gas chambers, anoxia, fumigation with chemicals);
(f) The unavailability of financial resources from the state government in the required time frame.

It is common practice for herbaria to use toxic fumigants, such as methyl bromide and phosphine, in the mass treatment of contaminated materials. However, these fumigants have carcinogenic properties and are toxic to the environment.

23.2. GAMMA RADIATION TREATMENT

The analysis and comparison of traditional methods showed that ionizing radiation was the best alternative in this case. It is an effective treatment for disinfecting a collection contaminated by insects and fungi. Studies on the effects of gamma radiation on cellulose based materials have been carried out on diverse cultural heritage artefacts, such as books, documents, wooden objects, ethnographic collections, sculptures, paintings, photographs and film [23.2–23.11]. However, no information was found in the literature on the irradiation of exsiccatae.

Early results of research that was being carried out at the time on botanical material were used as a reference for the procedure. The research carried out on samples of different species and ages showed that radiation disinfection of botanical collections could be safely achieved by applying a radiation dose of between 1 and 10 kGy without significant alteration or modification of the optical, morphological or surface characteristics of the constitutive materials [23.12]. Information about each step of the process is given below.

23.3. PROCEDURES FOR THE DECONTAMINATION OF THE COLLECTION

23.3.1. Diagnosis

Initially, a diagnosis and examination of the contamination (xylophagous insects and fungal attack) was carried out as a collaboration between technicians from the herbarium and irradiation specialists. This analysis considered the state of conservation and fragility of the exsiccatae and the types and levels of the contamination (Fig. 23.4).

FIG. 23.4. Damaged and mouldy specimens. (a) Mouldy Meliaceae fruit. (b) Mouldy Menispermaceae fruit. (c) and (d) Several exsiccatae showing leaves severely affected by mould.

Mycology experts collected the contaminated material for microbiological analysis. Analyses of samples of the greenish mass collected from the material were carried out. About 30 agar plates were prepared with several random samples on each plate. The plates were incubated for a week and then checked for the development of colonies of microorganisms. Slides were prepared with fragments of these colonies to observe the microscopic features of taxonomic relevance. Upon examination of the slides under a microscope, structures such as conidiophores and conidia were analysed, along with their morphological characteristics. This verified the presence of species of the genera *Trichoderma* and *Penicillium*.

23.3.2. Pretreatment procedures: Basic guidelines and Nuclear and Energy Research Institute support

The contaminated material was immediately separated. All exsiccatae were removed from the packages that were affected by the rainwater infiltration in order to verify the state of each one. The exsiccatae were dried naturally in a room, but those that were especially damp were dried in ovens (Fig. 23.5), since it is necessary for the materials to be as dry as possible for the irradiation.

The exsiccatae were packed in cardboard boxes measuring 36 cm × 36 cm × 50 cm. They were fixed inside the boxes to ensure stability during transport and handling. Each box was packed so that the weight was approximately 15–20 kg to facilitate handling during placement in the irradiation chamber and to prevent damage during the stacking of the material inside the chamber. The boxes were closed with adhesive tape.

The collection to be treated was packed in 670 boxes, which were divided into groups of 32 boxes, matching the processing capacity of the irradiator. All boxes were labelled with an identification of the type and quantity of the material, the name of the institution or herbarium, the place of origin and the name and telephone number of the person in charge (Fig. 23.6).

The transport of the boxes was carried out using Herbarium SP's fleet. Care was taken to ensure that closed vehicles were used and that the vehicles were adequately cleaned after each journey in order to avoid cross-contamination during transport. Herbarium SP's team was responsible for moving the boxes both to and from IPEN, which, like Herbarium SP, is in the city of São Paulo.

FIG. 23.5. Samples drying naturally and in an oven before processing in the irradiator.

FIG. 23.6. Packing the exsiccatae in sturdy cardboard boxes with an identification label. In the image on the right, the orange sticker on the label indicates that the material has been irradiated.

23.3.3. Receipt and storage of materials to be decontaminated at the Nuclear and Energy Research Institute

Deliveries to and retrievals from IPEN were carried out between 12 and 26 August 2020 and between 2 and 11 December 2020. Upon receipt of the boxes at IPEN, the number of boxes and the condition of the packages were carefully checked and a delivery order was issued. The boxes were stored in a safe place, which made it possible to separate the treated material from that yet to be processed in the irradiator.

23.3.4. Disinfection at the Multipurpose Gamma Irradiation Facility

The material was treated with gamma radiation from ^{60}Co sources at IPEN. The irradiation phase started with the positioning of the boxes inside the IPEN irradiation chamber. Owing to the high level of contamination, a dose of 10 kGy was used at a dose rate of 5–6 kGy/h. To reach this dose, the processing time for each batch of 32 boxes was 3 hours and 20 minutes at room temperature. The boxes were rotated halfway through the irradiation. The dose rate was determined by taking into consideration the position in relation to the ^{60}Co source and the density of the material.

Studies to determine the ideal dose to eliminate microbiological contamination of cultural heritage objects showed that an absorbed dose of between 0.5 and 3 kGy is sufficient to stop insect metamorphosis. Fungi and spores are eliminated by an absorbed dose of between 6 and 10 kGy [23.6].

FIG. 23.7. Storage of exsiccatae at Herbarium SP after processing by ionizing radiation and before the procedure for removing residue.

Dosimetric calculations were verified by dosimetry measurements with instruments calibrated and validated according to national and international standards [23.13]. The polymethyl methacrylate dosimetry system, with calibration traceable through the International Dose Assurance Service of the IAEA, was fixed at strategic points on the boxes, and measurements were made using an ultraviolet–visible spectrophotometer at the conclusion of the radiation processing to verify that the recommended dose had been applied, taking into consideration the uncertainties in the measurement of between 2% and 2.5%.

A treatment certificate was issued by IPEN providing information on the radiation dose, the dosimetry system used and the name of the person responsible for performing the services.

23.3.5. Procedures after irradiation

It is important to highlight that irradiation does not render the material sterile, nor does it prevent the collection from being contaminated again by a new infestation. Hence, when considering treatment, the maximum radiation dose that the exsiccatae can withstand must be established, particularly if they are to undergo multiple treatments, as the effects of radiation treatments accumulate [23.14].

After the treatment of the exsiccatae, the fungi and insects were eliminated. The same materials that were sampled to identify the fungi were analysed again, following the same methodology, and the absence of live fungi and viable spores was confirmed. Residue of the microorganisms remained on each exsiccata, which needed to be removed before the collection was returned to the collection's shelves (Fig. 23.7).

As a last step, the treated collection was stored on steel shelves in the main room of the herbarium in an air-conditioned environment with the relative humidity set at around 50% and the temperature set at 15–18°C.

IPEN recommended that information on the irradiation be included in the collection's records, showing the date of processing, the place of the irradiation and the absorbed dose.

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