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X-ray sterilization of insects and microorganisms for cultural heritage applications

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ABSTRACT

The APAM (Development of Particle Accelerators and Medical Applications) Laboratory of the ENEA Frascati Research Center is engaged in the preservation of cultural heritage as part of the COBRA (Sviluppo e diffusione di metodi, tecnologie e strumenti avanzati per la COnservazione dei Beni culturali, basati sull'applicazione di Radiazioni e di tecnologie Abilitanti) project addressed to the transfer of innovative technologies and methodologies from research to small and medium enterprises involved in the restorative measures. This work aims to demonstrate the effectiveness of ionizing radiation on the disinfection of biodegraded art objects. The conventional methods for the disinfestation of works of art, using chemicals toxic to humans and environment, might cause some damage to the treated material even on micrometric scale (i. e. either cellulose degradation). Ionizing radiations interact with the infesting biological material causing an irreversible DNA degradation. For this reason, they are certainly suitable for removal treatments of both macro organisms and bacterial colonies. A 4.8 MeV electron linear accelerator, normally dedicated to the characterization of dose detectors and radiographies, has been employed to produce Bremsstrahlung X-rays through a lead converter. The spectral fluence of the radiation source has been calculated using the Monte Carlo MCNPX code.

The dosimetric characterization of the radiation field has been made using radiochromic films sensitive in the dose range of our interest (from 50 to 500 Gy) calibrated with a Markus ionization chamber. The irradiation of the artifact prototypes are made within a lead shielded room at a variable distance from the X-rays source. Samples subjected to irradiation consist of a soil bacterium, *Agrobacterium rhizogenes*, and an insect, *Stegobium paniceum*, that are found as wall paintings invasive coloniser and as a pest of books, wood works and paintings, respectively. Tests of irradiation have been performed on pest organisms as well as on woods mock-ups to evaluate potential damage to the material during the sterilization. The growing capacity of the treated bacterial cells re-cultured at the end of the treatment was evaluated on the bacterial sample and resulted to strongly inhibit cell growth during post-irradiation incubation, so that after incubation periods at 28 °C, no significant cell growth was observed. The induced levels of insect mortality and sterility vs absorbed dose and operative conditions have been also evaluated, demonstrating the induction of full sterility since the lower dose and 40% mortality by two days after the higher dose treatment. The experiments proved the ability to efficaciously treat objects of cultural heritage with X-rays in order to prevent the increase of the biodeterioration without damaging the materials: in fact, mechanical tests on both irradiated and not irradiated woods have demonstrated the absence of any induced degradation after the radiation exposition.

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1. Introduction

The research on the use of ionizing radiations applied to cultural heritage goods dates right back to 1960 when the radioresistance of the most significant of microbial stocks was tested [1]. The interest of sterilization treatments by ionizing radiations has risen up during last decades to replace the traditional sterilization/disinfection methods using chemical fumigant gases, mainly the ethylene oxide or methyl bromide, which are seriously harmful for the human health and were recently withdrawn from the market of member states (European Directive CE/1048/2005).

In the framework of the COBRA project, innovative technologies developed by ENEA and involving ionizing and non-ionizing radiations for diagnoses, restorations or preservation of artistic and cultural goods will be transferred to companies involved in the cultural heritage field.

This work presents the preliminary results of the study and the characterization of a linear electron accelerator to effectively treat organisms, both insects and bacterial colonies that can damage cellulosic materials, by using X-ray exposition.

The operative conditions and the field of X-ray expositions have been evaluated by the MCNP6.1 Monte Carlo simulation code. Offline X-ray dose and dose rate measurements have been performed by radiochromic film expositions as well as on-line measurements by a parallel plates Markus ionization chamber. Compression tests of a wood mockup have been done to verify potential cellulose degradation after X-ray exposition. Finally, irradiation treatments on *Stegobium paniceum* and *Agrobacterium rhizogenes* in the dose range 50–500 Gy were performed. The insect *S. paniceum* (Coleoptera: Anobiidae) can feed on a wide range of dried foods and spices as well as leather, books, horn, cheratine and museum specimens such as paintings and furniture. The species is distributed worldwide and, due to the capability to adapt to various climatic conditions and to the extremely wide variety of products this insect can eat, it is considered a very dangerous pest in a museum settings.

Agrobacterium rhizogenes was chosen as a case study of bacterial colonies threat for paper paintings. Gram-negative isolates from *Agrobacterium rhizobium* were, in effect, found as contaminants of Middle Ages wall paintings [2].

2. Experimental setup

The linear electron accelerator (LINAC) in operation at APAM lab in ENEA Frascati is a pulsed (4 μs) S band (3 GHz) standing wave linear accelerator operating in the energy range 3 to 5 MeV and 0.2 A macropulse current, used for irradiation test and studies on the effects of the interaction of electrons or X-rays with matter for scientific, medical and industrial applications [3]. The overview of the setup is shown in Fig. 1 and the operative parameters are summarised in table 1.

Table 1
LINAC operative parameters.

LiNAC operative parameters		
Pick current	0.16	A
Repetition period	54	ms
Repetition frequency	18.52	Hz
Pulse length	3.40	us
Mean current	10.07	uA
Mean energy	4.80	MeV
Mean power	45.33	W

A lead disc of 3 mm in thickness was used as converter to generate Bremsstrahlung X rays. This target has been placed right in front of the exit window of the electrons accelerator, inside a lead collimator.

The irradiation chamber ($400 \times 300 \times 500\ mm^3$), shielded with lead bricks, contains a vertical flat cardboard panel where the samples can be positioned for treatment at various distance from the lead converter.

In order to evaluate the success of the sterilization process the samples consist of a wooden structure (needed to estimate the possible material degradation) with pre-made cavities in which insects and bacteria colonies can be distributed.

Silver fir wood has been cut in square slices ($4 \times 4 \times 1\ cm^3$) with the aim to investigate the X-ray penetration depth by varying the number of slices facing the ionization beam.

In order to study the effectiveness of X-ray irradiations on cultural heritage contaminated with bacteria, an *A. rhizogenes* strain A4 culture was established experimentally in the laboratory and grown bacterial colonies were transferred by a replica plating-like procedure onto paper samples (1 cm × 0.5 cm square-cut pieces of canvas painted with acrylic colours, paper painted with watercolours and non-painted paper, respectively) on *Petri* dishes. Control samples were obtained in the same way and undergone the same manipulations with the only exception of treatment.

Three parameters were taken into account to evaluate X-ray effects on *Stegobium paniceum* by experimenting a 50–400 Gy dose range, with a dose rate of $0.057\ Gy\ s^{-1}$: 1) the dose-dependent mortality induced on treated adults over a 10 days life span; 2) the dose-dependent reduction in egg production (i.e. adult fecundity) and 3) the egg fertility. Larvae were maintained on a dried carrot medium and insects or insect eggs were irradiated inside *Petri* dishes.

3. Results and discussion

In the next paragraphs, the results of irradiation treatments are presented. A preliminary simulation study is made with the MCNP6.1 code of the experimental setup, the fluence spectra and the thickness of the lead target converter. Furthermore, the dose

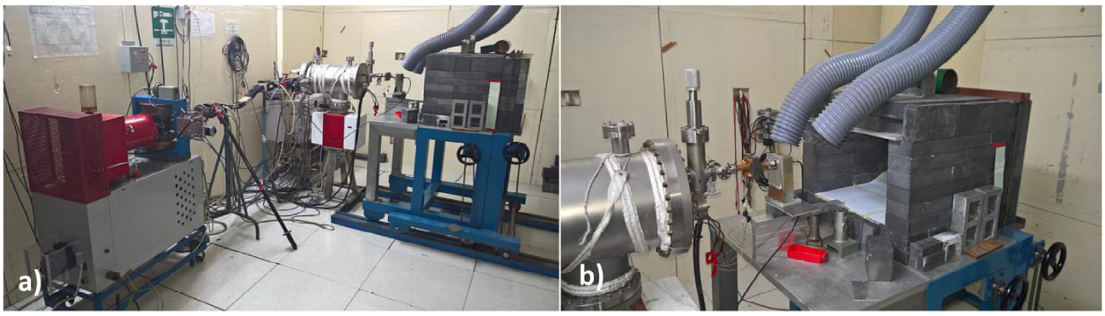

Fig. 1. a) Overview of the 5 MeV LINAC apparatus and b) the lead shielding room in front at the beam exit.

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