ISPESL
Istituto Superiore per la Prevenzione e la Sicurezza del Lavoro

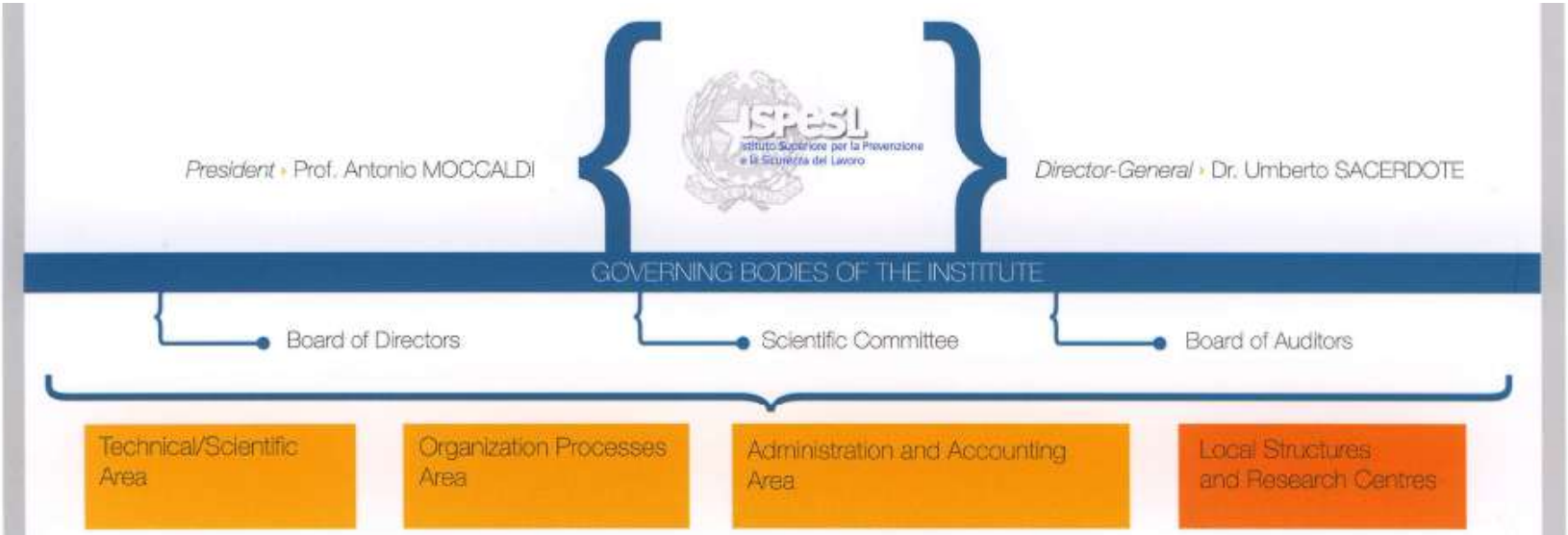
Department of Occupational Hygiene
Laboratory of Biological Agents

"Study on the efficacy of UV-C emitters in the prevention and control of biological contamination in indoor environments"

Dr. Antonella Mansi

Steril-Aire's Conference
Las Vegas, 1st - 2nd October 2009

A Nationwide network



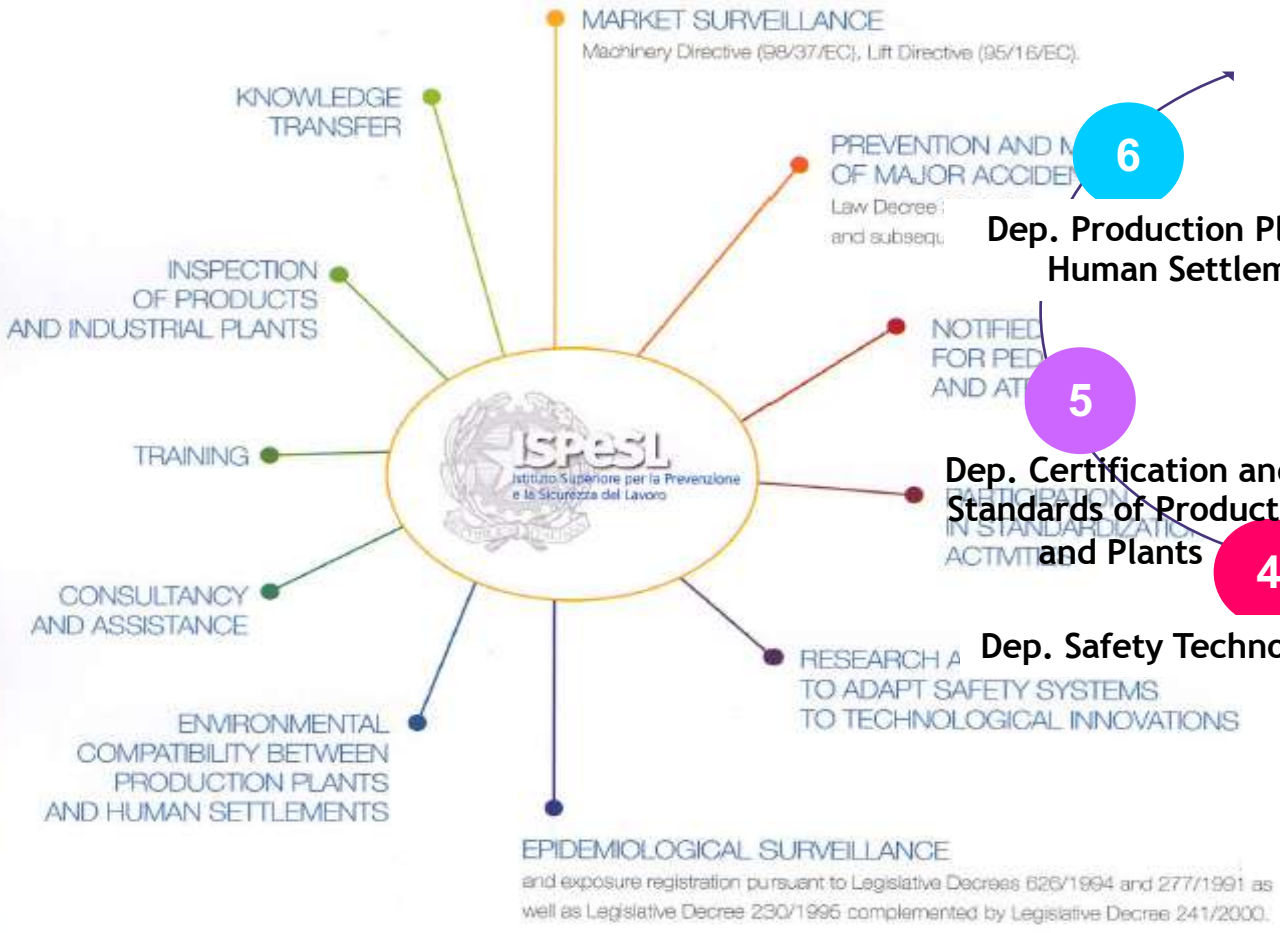
MISSION: occupational safety and health protection in living and working environments.

It is the Italian technical-scientific body of the National Health Service and reports to the Italian Ministry of Health as regards all aspects of occupational safety, health and prevention

STAFF: more than 1200 employees (Researchers, Technicians, Administrative personnel, etc).

ISPESL in Europe and worldwide

Activities



1 Dep. Organization Processes

2 Dep. Occupational Medicine

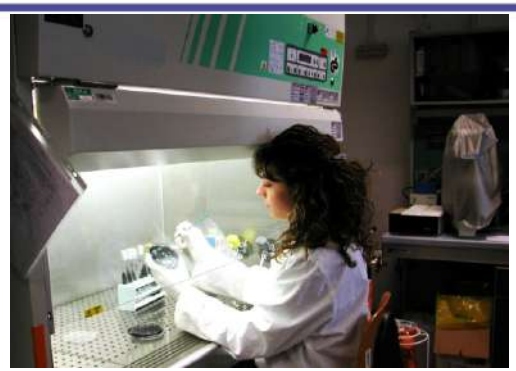
3 Dep. Occupational Hygiene

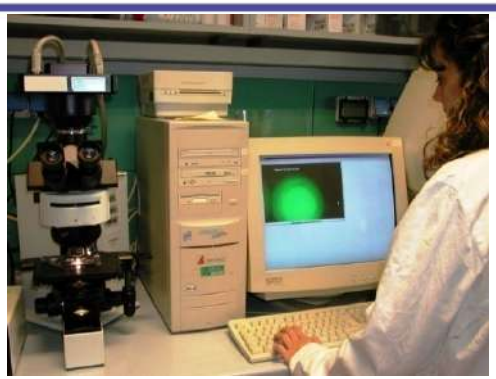
4 Dep. Safety Technology

5 Dep. Certification and Standards of Products and Plants

6 Dep. Production Plants and Human Settlements

Activities of the Laboratory





Research topics: detection, assessment and prevention of biological agents in indoor environments

Development and standardization of technical and scientific methods of measuring and assessment of biological agents

Consultancy: Qualitative and quantitative detection and assessment of biological agents (Legionella spp., Asp.fumigatus, bacterial endotoxins, indoor allergens)

Standardization: Involvement in nationally and internationally working groups for the development of technical standards and guidelines

Training: courses for public and private companies

Italian law on occupational safety and health

Law Decree No. 81/2008 (recently modified and integrated by Decree n.106/2009) has replaced the old legislation on the protection of workers against risk agents (Law No 626 of the 19th of September 1994), introducing severe sanctions for the enterprises breaking safety rules.

Biological Agent Definition (Title X Art. 267)

Micro-organisms, including those which have been genetically modied, cell cultures and human endoparasites, which may be able to provoke any infection, allergy or toxicity

Micro-organism shall mean a microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material;

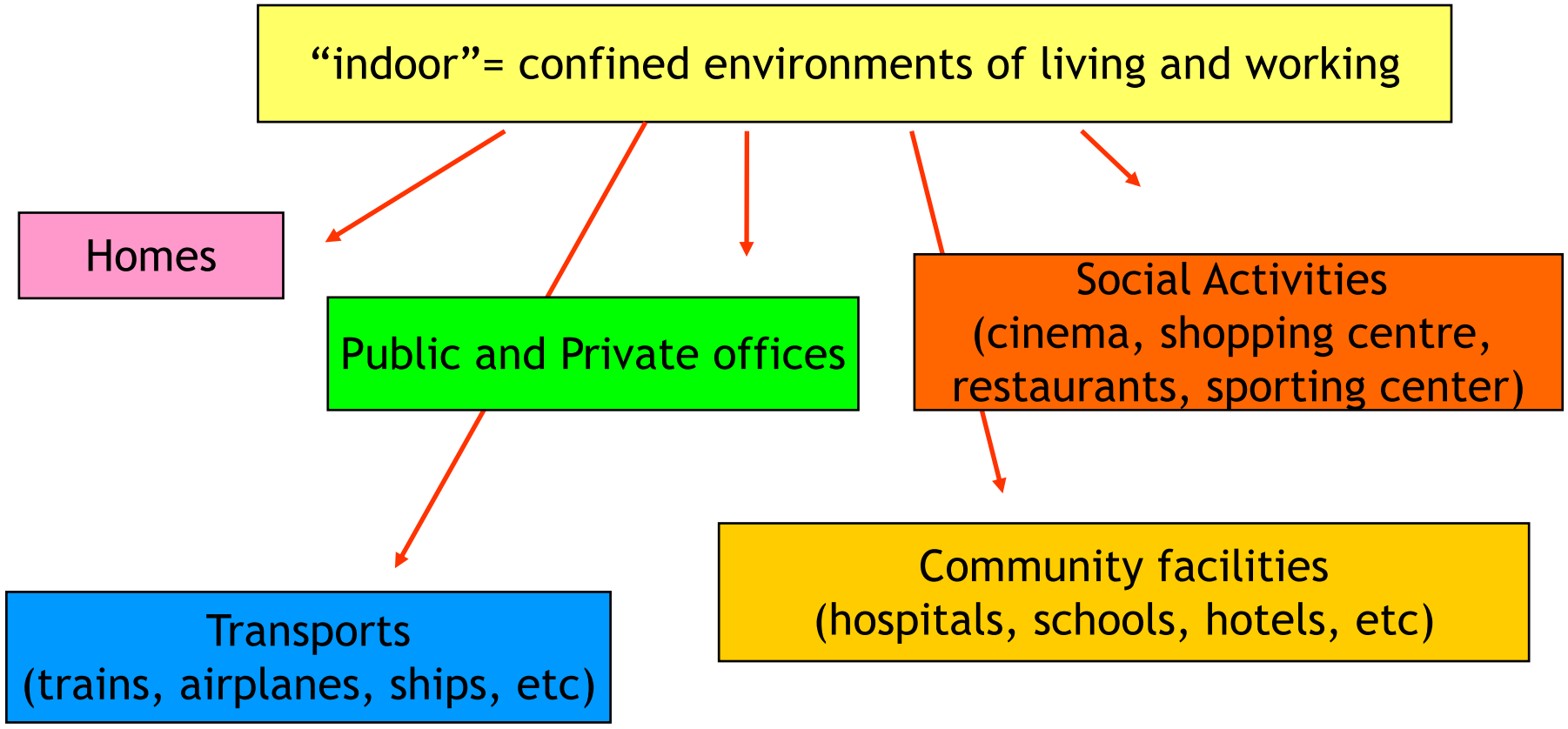
Cell culture shall mean the in-vitro growth of cells derived from multicellular organisms.

Biological Agent Classification (Title X Art. 268)

Four Risk Groups (RG) 1, 2, 3 and 4

Indoor Air and Public Health

"Guidelines for the protection and promotion of health in indoor environments"
issued by Italian Ministry of Health
Supp.ordinario G.U. Serie generale n. 276 del 27 Novembre 2001



Indoor Air Quality (IAQ)

Research Activity Plan 2008-2010

Research Project: "Methodolog
prevention and control of mic
environments"

National Homeland Security Research Center Technology Evaluation Report Biological Inactivation Efficiency by HVAC. 2006. Biological Inactivation Efficiency by HVAC. In-Duct Ultraviolet Light Systems. www.epa.gov

Kujundzic E, Hernandez M. and Miller S.L. 2007. Ultraviolet germicidal irradiation inactivation of airborne fungal spores and bacteria in upper-room air and HVAC in-duct configuration. J Environ Eng Sci, Vol. 6, pp. 1-9.

Levetin E., Shaughnessy R., Rogers CA., Scheir R. 2001. *Effectiveness of Germicidal UV Radiation for Reducing Fungal Contamination within Air-Handling Units*. Appl Env Microbiol, Vol. 67, pp. 3712-3715.

Kujundzic E, Matalkah F. Howard CJ, Hernandez M., Miller SL. 2006. *Ultraviolet germicidal irradiation inactivation of airborne fungal spores and bacteria* [illegible] *and HVAC in-duct configuration*. J [illegible] Vol. 3, pp.536-546.

Ko G., First MW, Burge HA. 2002. The characterization of upper-room ultraviolet germicidal irradiation in inactivating airborne microorganisms. Environ Health Perspect, Vol. 110, N. 1 (Gen.), pp. 95-101.

Andersen B. M., MD, PhD; H. Banrud, DrScient; E. Bøe, BcEcon, MEng; O. Bjordal, MEng; F. Drangsholt, PhD. 2005. Comparison of UVC Light and Chemicals for Disinfection of Surfaces in Hospital Isolation Units. Infect Control Hospital Epidemiol, Vol. 27, pp. 729-734.

Xu P. Kujundzic E, Peccia J, Schafer MP, Moss G, Hernandez M., Miller S. 2005. Impact on environmental factors on efficacy of upper-room air ultraviolet germicidal irradiation for inactivating airborne Mycobacteria . Environ Sci technol, Vol 39, pp.9656-9664.

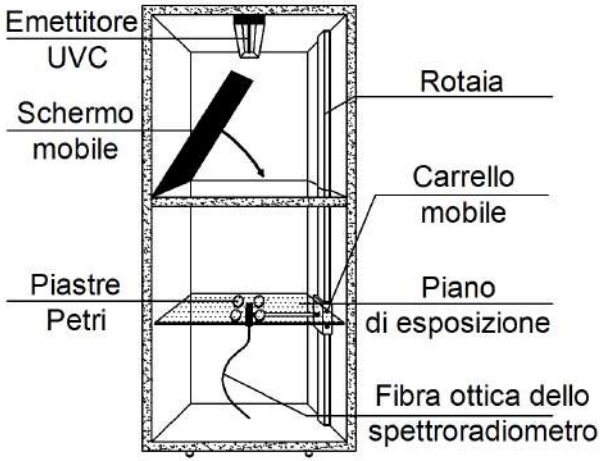
Aim of study

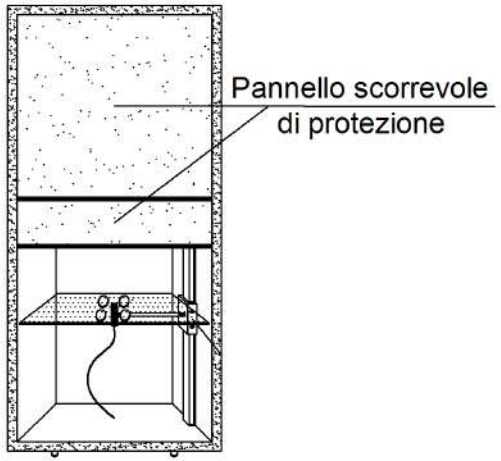
The objectives of the first step of this study were:

-to design and realize an exposure chamber

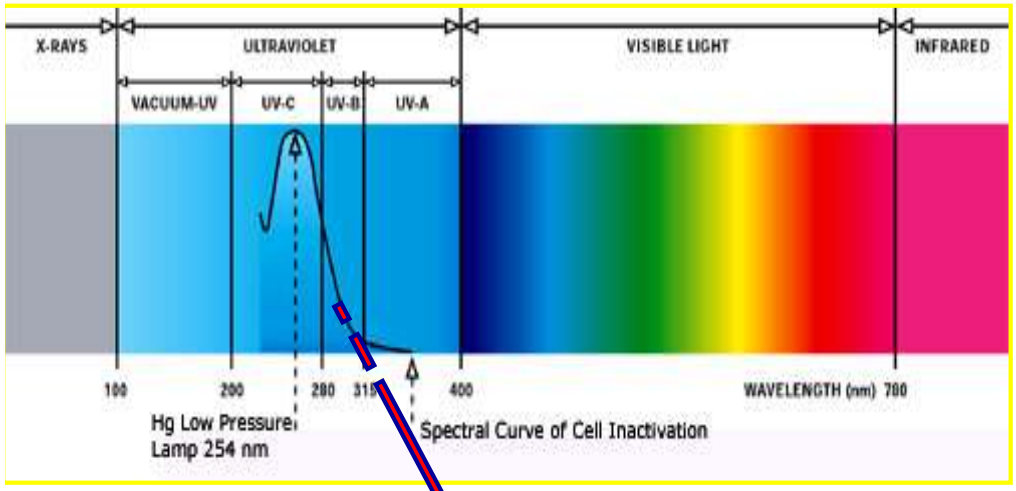
-to determine the germicidal efficacy of an UVC emitter tested on *Aspergillus fumigatus* and *Aspergillus flavus* spores.

-to calculate the reduction (Lethal Dose, DL90) in microbial populations after UVC exposure.



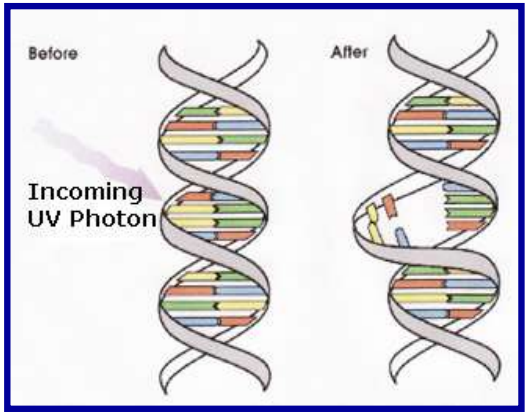


Microorganism susceptibility to UVC



The majority of UV inactivation data for microorganisms are conducted by exposing cells to 254 nm radiation.

λ= 254 nm microbial inactivation



Susceptibility of a microorganism to UVC depends on:

-its ability to repair structural damages

-complexity of the microorganism's **structure** (presence or absence of a cell wall)

Viruses lack a cell wall, so they are more easily inactivated

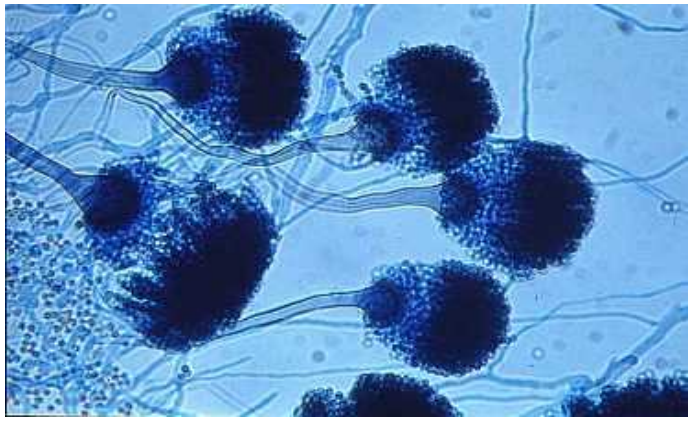
Vegetative bacteria are generally intermediate in susceptibility.

Spores, such as *B. anthracis* or fungal spores are more difficult to penetrate by UVC

Materials and Methods

Reference ATCC strains

Aspergillus fumigatus	KM 8001
Aspergillus flavus	ATCC 10124



Preparation of spores

Fungi were grown on Malt Extract Agar (MEA) plates at 25°C for 5-7 days

Spores were collected by scraping the surface of the agar and then were suspended in sterile phosphate-buffered saline (PBS), pH 7.2

The number of viable spores per milliliter was determined by serially diluting the preparation and spread plating the spore suspension onto triplicate MEA plates.

Purified spores of the strains were diluted in order to obtain a density of 100–150 CFU per ml.

UVC exposure

Purified spores of *Aspergillus spp.* were exposed to ultraviolet irradiation at different exposure times.

Materials and Methods

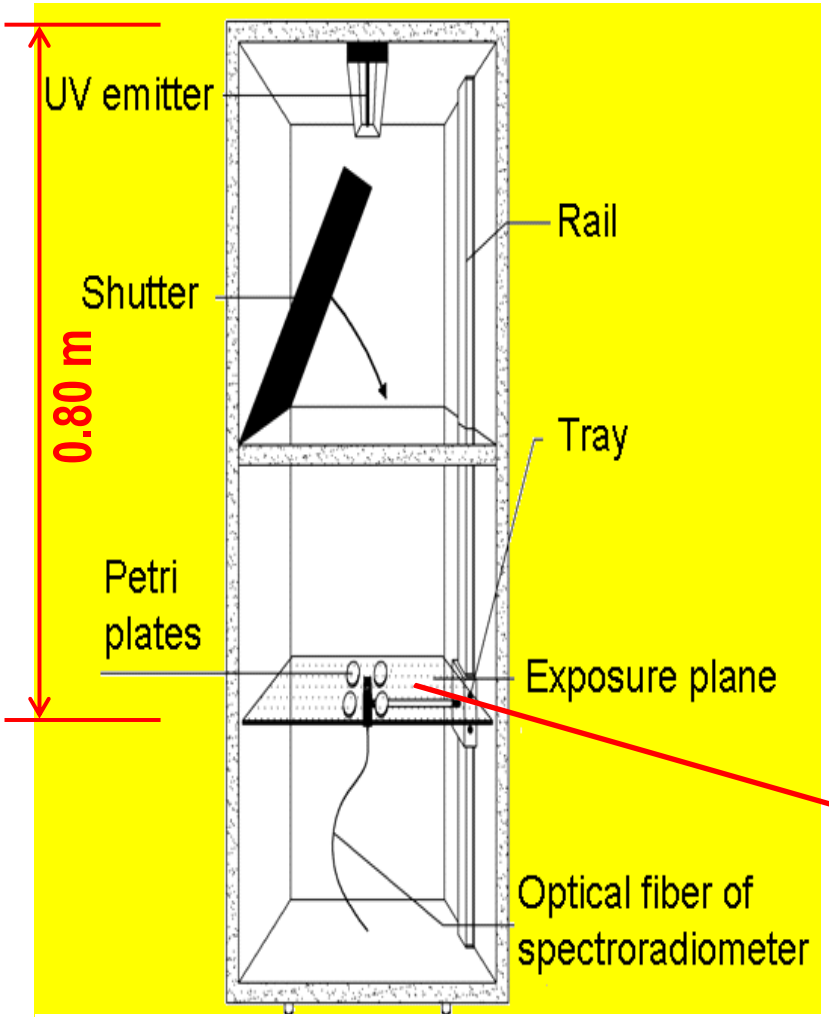


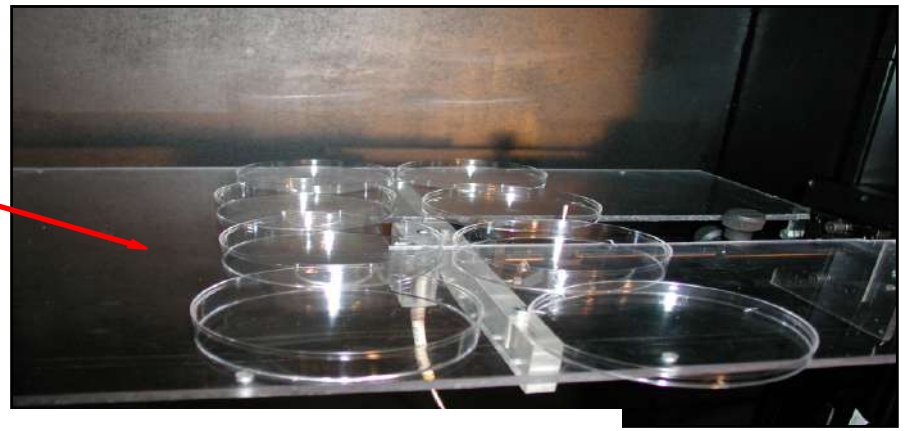
Figure 1 – Scheme of exposure chamber

An UVC emitter (model DE double-ended 0.42 m length) provided by STERILE-AIRE Inc. was installed inside an exposure chamber

The irradiance at the surface of the plates was measured using a spectroradiometer (Ocean Optics model QE 65000)

Irradiance $_{0,8\ m}$ = 1W/m^2

The ozone concentration into the chamber was measured with ozone monitor (Thermo Environmental Instrument 49C Analyzer).



Relative humidity and temperature values inside the chamber were checked during the tests by a calibrated thermo-hygrometer (Velocicalc Plus model 8360-M-GB by TSI Inc.)

Materials and Methods

UVC exposure

Plates were kept at the same distance from the UV source (80 cm) to the aim of maintaining constant the irradiance and varying the absorbed dose by modifying only the exposure time.

Inoculated plates were exposed to the UVC light for different exposure times (Fig.2).

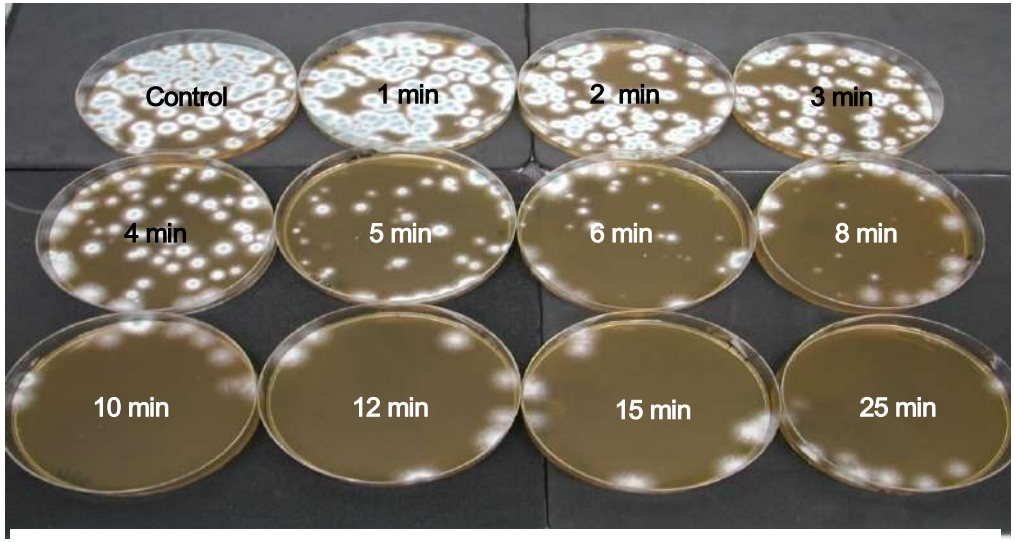


Figure 2. Colony Forming Units for plate (CFUs/plate) in unexposed plates (controls) and in plates at different exposure times (from 1 to 25 minutes).

Plates were placed into an incubator at the appropriate temperature (25°C) until moderate growth was visible (4-5 days), then the colonies forming units per plate (CFU/plate) were counted. Control plates inoculated with the organism were also placed in the incubator to represent the zero exposure time.

UVC dose

UVC dose (J m^{-2}) = Irradiance (W m^{-2}) x exposure time (in seconds)

Results

Assessment of UVC dose

$$\frac{N_t}{N_0} = e^{-K \cdot dose} = e^{-\frac{t}{\tau}} \qquad (1)$$

N_0 is the number of microorganisms at the start, before exposure begins

N_t/N_0 is the fraction of microorganisms surviving at time t

K is a microorganism-dependent rate constant (*Menetrez K.K. et al., 2006*)

in m^2 / W·s

τ is the time at which, at fixed irradiance, the absorbed dose determines the reduction by a factor 1/e of the initial value of the microbial population

It is possible to deduce the number of killed microorganisms at time t:

$$N_k = N_0\left(1 - e^{-\frac{t}{\tau}}\right) \qquad (2)$$

Results

Aspergillus fumigatus

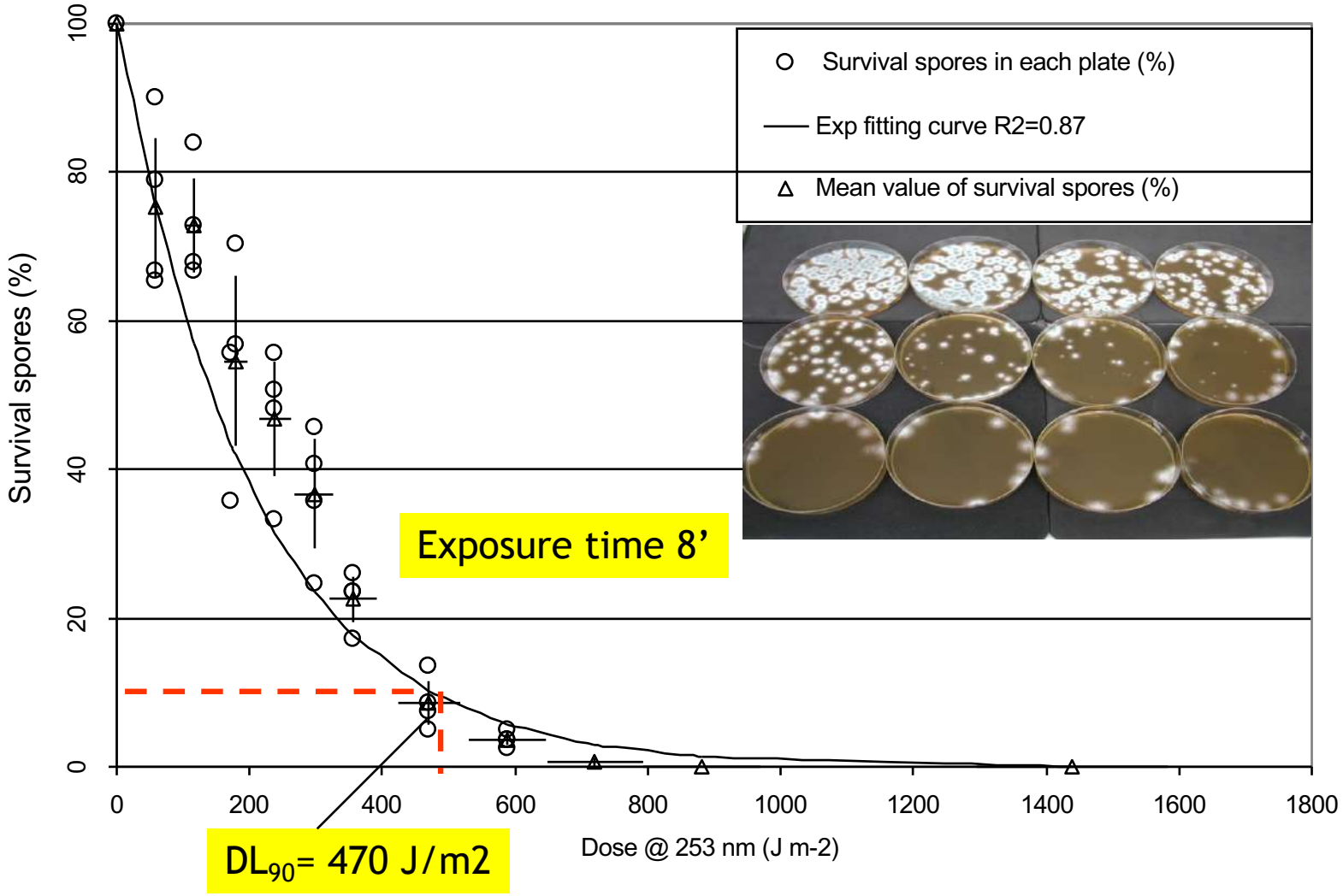


Figure 3. Percentage of survival spores as function of the UV dose (Error bar corresponding to% for the dose and ± s for the mean values of survival spores.)

Results

Aspergillus flavus

Green CF Scarpino PV; Jensen P. Jensen NJ, S.G. Gibbs. Disinfection of selected Aspergillus spp. using ultraviolet germicidal irradiation. Can J Microbiol 2004. Vol 50, pp. 221-224.

DL_{90} *Aspergillus fumigatus* = 540 J/m2

DL_{90} *Aspergillus flavus* = 350 J/m^2

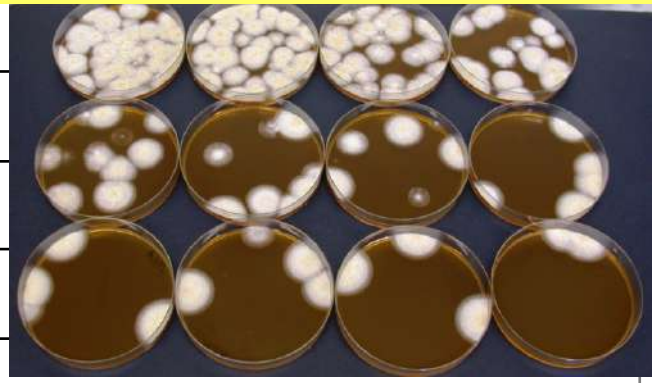


Figure 4. Percentage of survival spores as function of the UV dose (Error bar corresponding to % for the dose and ± s for the mean values of survival spores).

FIRST STEP OF STUDY: CONCLUSIONS

The results show the efficacy of this emitter in inactivating the *A. fumigatus* and *A. flavus* spores to a reproducible degree in the experimental settings.

Development of experimental protocol and working out technical procedures for assessing and comparing the efficacy of inactivation of UV-C emitters

The information generated in this first step of study was used to plan further tests on micro-organisms which play an important role in the biological contamination of indoor environments.