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ASSESSING AND FACING THE BIODETERIOGENIC PRESENCE DEVELOPED IN THE ROMAN CATACOMBS OF SANTI MARCO, MARCELLIANO E DAMASO, ITALY

Oana Adriana Cuzman[1*], Deodato Tapete[2], Fabio Fratini[1], Barbara Mazzei[3], Cristiano Riminesi[1] and Piero Tiano[1]

[1]ICVBC-CNR - Istituto per la Conservazione e la Valorizzazione dei Beni Culturali, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy
[2] Durham University, Department of Geography, Durham, UK
[3] Pontifical Commission for Sacred Archaeology, via Napoleone III, 1, 00185 Rome, Italy

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Abstract

Within the huge hypogean complex of San Callisto, Rome, Italy, we are investigating the Roman Catacombs of Santi Marco, Marcelliano e Damaso whose appellation derives from the names of the two most famous Christian martyrs and the pope Damaso buried therein. The cubicles currently studied date back to the second half of the 4th century AD, and contain decorated surfaces, such as the painting depicting the Twelve Apostles over the arcosolium and the peacocks in the niches within the namesake cubicle.
Taking into account the dual perspective ‘heritage conservation and promotion, this work presents the results of the biological investigations carried out at the entrance of these Roman Catacombs and close to a skylight from which the sunlight partially illuminates the corridor. A microclimate monitoring was complementarily performed, based on the evidence that the indoor microclimate is not always necessarily constant and microbial colonies frequently change their appearance throughout the year, thereby suggesting a dependence on the local environmental parameters.
In-situ observations and laboratory characterization of the biodeteriogens revealed the dominance of Basidiomycetes at the entrance, and a compact biofilm mainly consisting of filamentous cyanobacteria and other microorganisms in the lighted areas of the catacombs. Taking into account the specific microclimate, monitored in terms of relative humidity and temperature, preventive solutions were suggested.
The results obtained from testing this monitoring approach encourage further implementation in similar hypogean contexts.

Keywords: hypogean monument, Roman catacombs, microclimate, biodeteriogens, biological colonization

* E-mail: cuzman@icvbc.cnr.it, tel.: +390555225473, fax: +390555225403

1. Introduction

Biological colonization in the hypogean environment is mainly due to the development of microorganisms with low nutrient requirements such as various types of heterotrophic [1-3] and phototrophic microorganisms [1, 4-6]. Very few studies related with the cultural hypogean environments mentioned the presence of macrobiological growth such as the macromycetes [1] or invertebrates [7]. The favourable microclimatic conditions – among which the high level of relative humidity, the almost constant temperature during the year, the low air circulation and the absence or partial presence of light – may easily lead to microbiota development. The biodeterioration is also influenced by the visitors presence [8, 9] or by an inappropriate biocide treatment, as found in the case of the Lascaux caves [10, 11]. However, even in the case of hypogean environments closed to the public, the biological growth may became an outbreak. This is frequently due to the fact that a periodic condition monitoring cannot always be performed, valorisation/preservation of this heritage being postponed or neglected.

With the perspective of a future opening of these Roman Catacombs to the public, strategies of preventive conservation are currently being evaluated by the Pontifical Commission of Sacred Archaeology, with the co-operation of which the present work was carried out.

Recent works revealed in the same huge Christian cemeterial complex only the presence of heterotrophic and/or phototrophic microbiological development [4, 5, 12-14], while our study reports the presence of Basidiomycetes as well. The scope of this paper was to characterize and control the biological development occurred inside the catacombs, taking into account the microclimatic conditions.

2. Experimental

2.1. Historical and environmental setting of Santi Marco, Marcelliano e Damaso Catacombs

Dating back to the second half of 4th century AD, the catacombs are located south of the town centre of Rome, close to the ancient Appian Way, within the broader complex of San Callisto. They run underground for kilometres within the tuff strata of the local geologic substratum, with a succession of cubicles of different size distributed along corridors (Figure 1).

With regard to the historical relevance, one of the three Saints to whom they are entitled is Pope Damaso (305-384 AD), who is remembered for his search for the tombs of the early-Christian martyrs, as well as for his campaign of restorations to make them monumental places open to the devotion of the faithfuls. He also composed more than sixty metrical praises engraved on marble slabs by the calligrapher (and friend) Furio Dionisio Filocalo, and positioned close to the martyrs' graves.

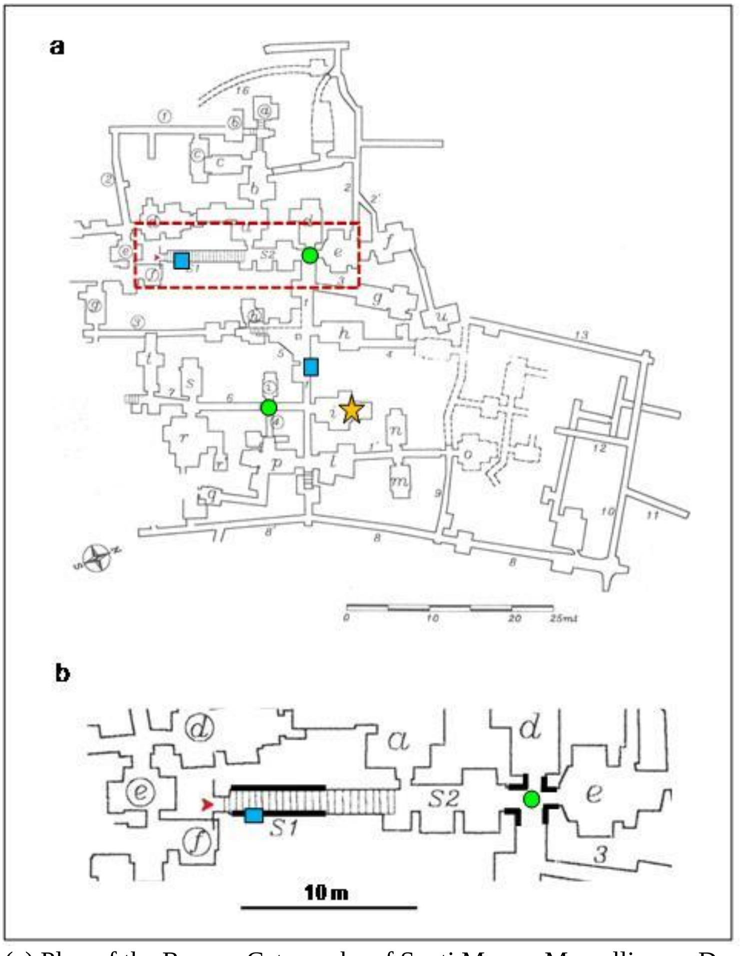

Figure 1. (a) Plan of the Roman Catacombs of Santi Marco, Marcelliano e Damaso, with indication of skylights (green dots), microclimate sensor installed along the corridor (blue square) and the Cubicle of the Twelve Apostles (orange star); (b) detail of the map showing the areas with biological colonization at the entrance S1 along the stairs and near the skylight (black bold lines).

Cultural value is also added by the enormous pictorial heritage preserved in different cubicles. One of the most relevant is the Cubicle of the Twelve Apostles (Figure 1a), so-called from the mural painting in the arcosolium, flanked by two small niches with the representation of peacock as symbol of immortality, spiritual rebirth and resurrection.

Concerning the conservation history, the catacombs of Santi Marco, Marcelliano e Damaso were neglected during the Medieval Age and firstly rediscovered by the Maltese archaeologist Antonio Bosio at the beginning of 17th century. Rigourous research were then conducted by Joseph Wilpert [15] in the first decade of the 20th century. Nowadays the catacombs are closed to the public, a condition which lasts since the execution of the recent archaeological excavations and should (at least ideally) assure a relatively good conservation of the inner surfaces.

Nevertheless, biodeterioration currently affects the entrance S1 to the catacombs (Figure 1), i.e. the inner plaster surfaces beneath the concrete structure (Figure 2a). Several ancient marble fragments and tombstones are hung on the inner walls, the entrance being therefore not only a passage space, but also an exhibition environment.

From the point of view of the environmental relationships between indoor and outdoor, two structural elements should be taken into account: (i) the windows obturared in the entrance (Figure 2a) and (ii) the presence of skylights at some crosspoints between the subterranean corridors (Figure 1, Figure 2c). In particular, we focused on the skylight located about 17 metres from the entrance S1 (Figure 1b), which is actually sealed by a means of a small concrete structure, from which sunligth comes (Figure 2c).

(a) (b) (c)

Figure 2. (a) Outside cement structures which cover the main acceses with the exterior of the catacombs built over the entrance, (b) the skylight, (c) with a detail of the sealed windows present in the entrance structure that keeps only a small round shape air acces.

2.2. Microclimate monitoring

Monitoring of the microclimate (Figure 1) of these catacombs was started since October 2012 within the framework of HYPOGEA project [16, 17], in order to characterize the entrance S1, with regard to that of the outdoor environment, taking into account the following parameters:

- entrance S1: air temperature (Ta) and relative humidity (RH), as well as surface temperature (Ts) close to the biological growth over the left inner wall;
- outdoor: Ta and RH;
- subterranean corridor, between the skylight and the Cubicle of the Twelve Apostles: Ta and RH.

The transducers used for the monitoring of environmental parameters were chosen to ensure operation under extreme conditions of humidity of 100% RH. For these particular environmental conditions it is necessary to observe the particular conditions of implementation of the monitoring network: the electrical connections for the supply of the sensors and for the data connection must be realized by waterproof materials and devices. The water condensation on electrical contacts or other electrical parts can lead faulty operation.

During the installation relevant precautions were dedicated to the position of superficial temperature sensors, in fact, they should adhere at the surface under investigation so as to detect the smaller change in the temperature.

The sampling frequency was fixed every half an hour. This step is sufficiently thick to follow the variations of the environmental parameters.

(a) (b)

Figure 3. (a) The biological colonization at the entrance chamber (entrance door in the background) of the catacomb and (b) on the walls located near the skylight.

2.3. Biological observations

Significant biological presence was observed at the entrance chamber of the catacomb and on the naturally illuminated walls of the corridor. The biological growth in this hypogean monument was monitored for more than one year, in the months of October 2011, January, June and October 2012 and March 2013. The taken samples were analyzed and characterized [18] using a Nikon Eclipse 600 microscope and the images were collected with a Nikon DXM1200F digital CCD camera. Lactophenol cotton blue slide mounts were prepared for microscopic analysis of the fungal mycelium.

3. Results and discussions

3.1. Biological colonization

Two extended types of biological colonization were observed inside of the Catacombs of Santi Marco, Marcelliano e Damaso, (i) at the entrance (Figure 3a) and (ii) in the lighted areas on the walls of the corridor (Figure 3b).

The first type of biological growth (Figure 3a) is made of different white mycelia (Figure 4 and 5), easily removable by hand, which widely cover the stairs and walls of the entrance chamber.

(a) (b) (c)

Figure 4. Types of mycelia developed at the entrance chamber of the catacomb.

The seasonal variation influenced the general aspect of this biological settlement and fruiting bodies formation (Figure 5). Is was observed that during the wet and cold months (January, October and even March) the mycelia was very fluffy and the fruiting bodies developed, while in the summer periods the mycelia were quite dry and the strands were visible.

The strands observed in these catacombs, had a gray-brown colour (the thicker ones), easily removable, with 8 mm maximum diameter, being generally covered by a white mycelium. There were observed white rhizomorphs as well, with a maximum diameter of 2 mm. The strands are colonizing the surface of the walls, covering likewise the stone slabs hanging on it, and were also observed beneath the plaster layer, penetrating the mortar in the masonry (Figure 6). These specialized fungal structures are typical of the *Basidiomycota* phylum [18], the strands containing three types of hyphae: vegetative, skeletal (thin fibre with thick walls), and broad vessel hyphae (Figure 7). According to microscopic and macroscopic observations, it seems that more than one type of strand-forming house-rot fungi are present, such as *Serpula* sp., *Coniophora* sp., and *Antrodia* sp.

The other type of biological growth one is represented by a continuous, felted and shiny biofilm of a dark-blue-green colour (Figure 3b), easily removable, with a white colour underneath where it is attached to the stone material (Figure 8). This biological mat colonized all the walls around the skylight and all the areas reached by the natural light. Some vascular plants (Figure 8) were also observed in the lightest areas, which are near to the skylight and the ground level where the soil is present. Their roots were descending along the walls towards the interior of the catacomb.

The microscopic analysis of this biofilm (Figure 9) revealed the presence of phototrophic microorganisms such as cyanobacteria, green algae and diatoms. There were mainly filamentous cyanobacteria, but fungal hyphae and basidiospores were observed as well, which presented the same morphology as the spores sampled at the entrance in the catacomb.

Figure 5. General aspect of three areas with fungal growth, during monitoring period.

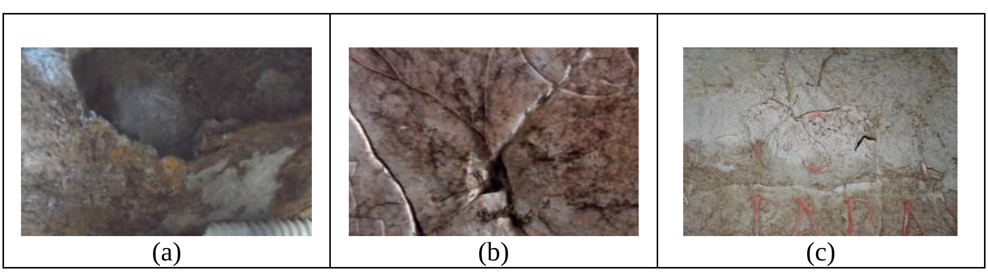

Figure 6. Biodeteriogenic aspects of the fungal development: (a, b) due to the penetration of mycelium and/or strands under the plaster layer or (c) over the painted surfaces.

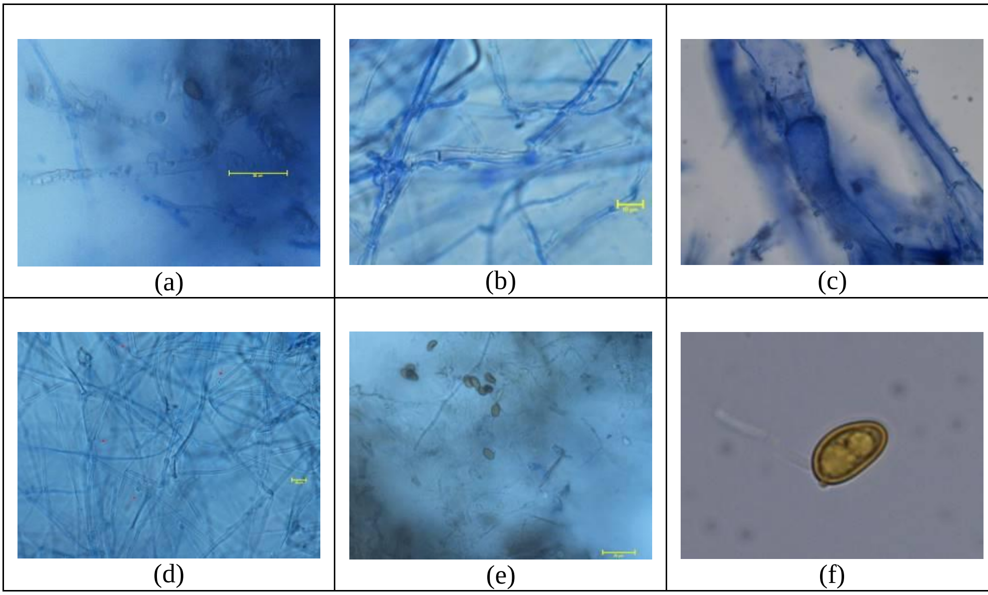

Figure 7. Microscopic aspects of the fungal structures: (a-d) with characteristic hyphae and (e-f) spores for the *Basidiomycetes*, with clamps (white arrows), vessel hypha (c), vegetative and skeletal hyphae (black arrows). Scale bar 10 µm.

Figure 8. (a) The general aspect of skylight where phototrophic biofilm and vascular plants were developed, (b) and a detail of the sample area of the phototrophic mat, where a white layer was observed beneath.

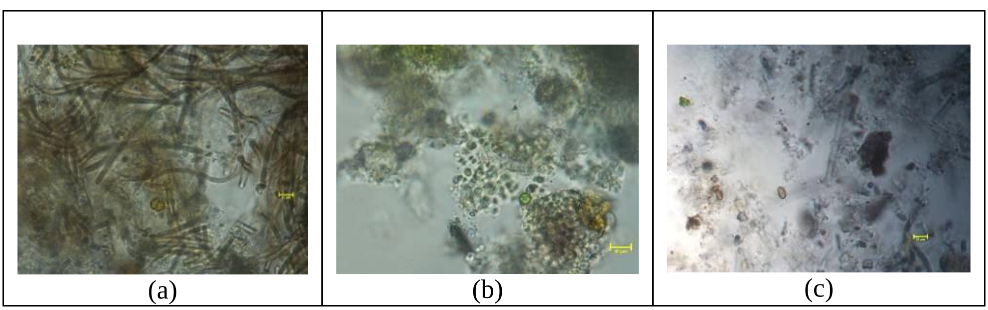

Figure 9. Microbial biodiversity of the phototrophic biofilm with: (a) filamentous and (a, b) coccal cyanobacteria, (b) green algae, (a, c) diatoms and (b, c) basidiospore.

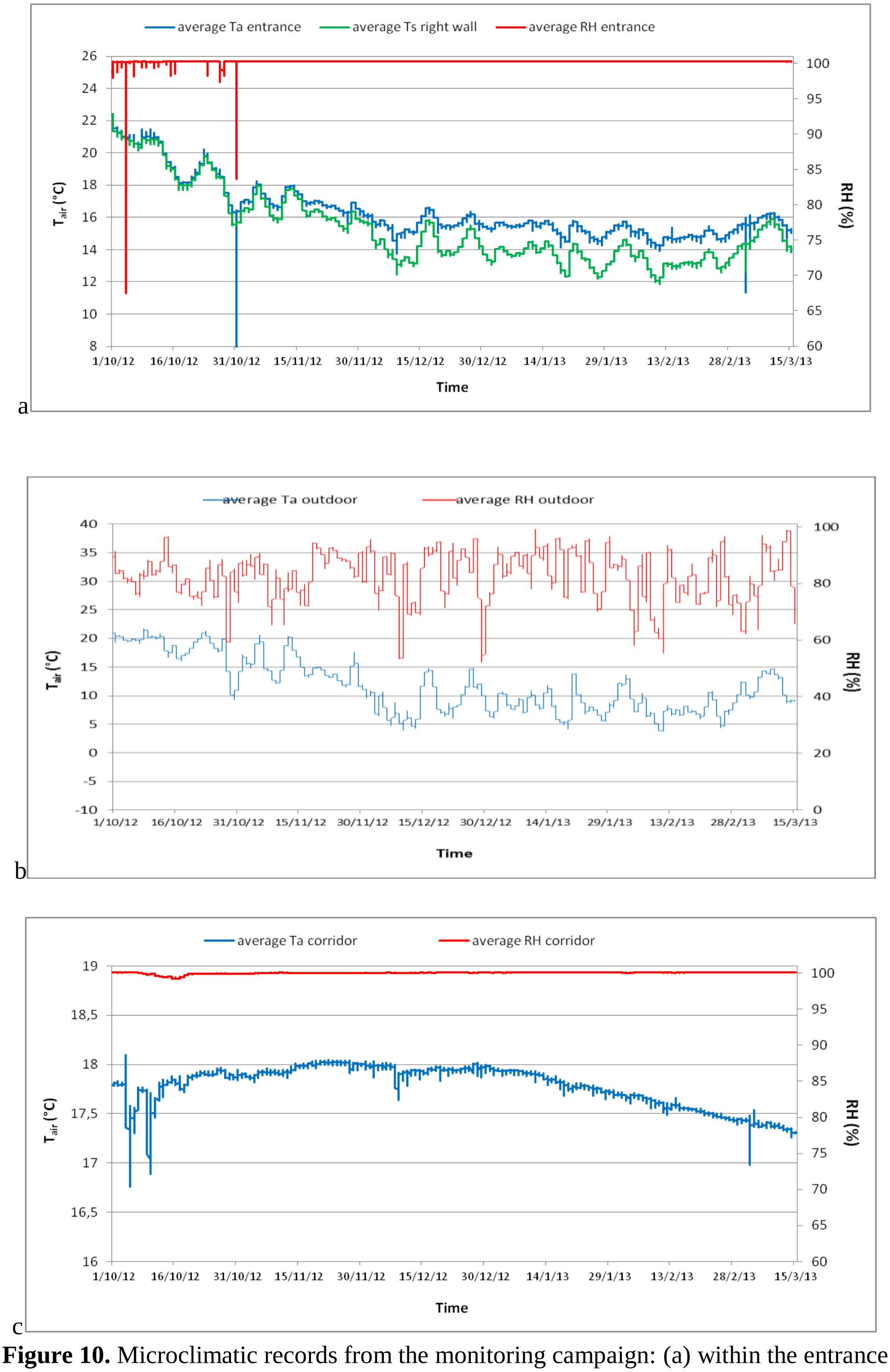

Figure 10. Microclimatic records from the monitoring campaign: (a) within the entrance S1of the catacomb, (b) outdoor of the catacomb - the values represent the daily average of the records and (c) along the corridor inside the catacomb.

3.2. Microclimate characterization and influence on the biological growth

The microclimate records retrieved from October 2012 to 15th of March 2013 showed differential conditions between the entrance S1 and the subterranean corridor (Figure 10), with regard to the outdoor. Environmental monitoring data confirms this difference from the outer to the inner of the Roman Catacombs [17], with particular regard to the indoor microclimate of the cubicles monitored over the same period of time.

The entrance S1 was characterized by an almost constant RH, persistently at 100% and only occasionally oscillating towards lower values, concurrently to the accesses of the maintenance staff (Figure 10a). At the same time, Ta decreased from about 22°C (October 2012) up to 15° (mid December 2012), subsequently oscillating between 16° and 14°C until mid March 2013. The trend of Ts measured close to the biological colonization on the right wall, far from the entrance door of about 1 m, follows the trend of Ta but is a few of °C below (until to 2°C less than Ta) (Figure 10a). These measurements confirm the presence of water condensation on the wall surfaces due to th high level of humidity.

In the subterranean corridor, RH is permanently at saturation (100%), as also observed in the entrance, while Ta tends to remain around 18°C. Nevertheless, a light linear decrease of Ta was recorded starting from the end of December 2012, up to 17.2°C (Figure 10c). These five months and half of monitoring indicates that the fluctuations in temperature in the corridor are less apparent than at the entrance, and the microclimate is warmer in the subterranean environment than the entrance.

The thermal difference between the subterranean corridor and the entrance would further explain the rise of air masses as a sort of humid *föhn* observed after opening of the entrance door, especially during in-situ inspections carried out in March 2013. Anyway, this in-situ impression might be validated through specific air mass movement measurements

Generally speaking, these peculiar conditions are optimal for the fungal growth, including the house-rot fungi, the latter are commonly found on the wood structures [19]. The macromycetes genera observed inside the studied Roman catacombs were often found in masonry as well [20, 21]. These kind of *Basidiomycetes* can survive to a minimal of 20-30% of humidity, a slight acid pH (4.5-5.5), in a wide range of temperatures (5-35°C), using organic (timber, trees) and inorganic nutrients (some metallic elements such as calcium or iron). Their development is stimulated by the air CO_2 concentration up to 10% [19], moisture retention in walls, availability of the innorganic nutrients [20, 21] and the contact with alkalinic substances, such as alkaline sands, ash and urine [19].

In the case of the biological outbreak occurred in these Roman Catacombs, it is not so clear the source of organic nutrient, since no timber is in the cement structures, nor visible infected trees or soil are present in the proximity of the catacomb. These observations led us to suppose that the contamination occured by the spores vehiculated inside the catacomb by water infiltrations. They germinated (Figure 6f) in this favorable microclimate, presumably using organic nutrients coming from soil and/or the phototrophic biofilm. For example, the basidiospora of *Serpula lacrymans* have a germination optimal at 26°C in the presence of humidness [19].

3.3. Controlling strategies

In order to preserve these catacombs for the future generations and having in mind a future perspective of opening to the visitors, the stabilization of an unfavourable microclimate conditions for the micro- and macroorganisms and the biological infestation removal are the first and less invasive remediation measures to be considered.

The curative tactic shoud take into account the following aspects:

- a carefully removal of the fungal structures, phototrophic biofilm and vascular plants in order to not disseminate them, gathering them in paper bags and therefore burning them;
- improving the ventilation and air circulation between the entrance chamber and skylight in order to favour the decrease of the moisture content both in the air and walls;
- reducing/eliminating the penetration of the natural light inside the catacombs;
- a periodically checking of chosen parameters (e.g., air temperature, relative humidity, surface temperature, air mass circulation) in order to monitor and intervene if a new recolonization occurs;
- evaluating of the best biocide treatment (physical or chemical control methods) with respect to the particular microclimate of the enviroment.

Public opening also implies issues related to the logistics and infrastructure necessary to make the catacombs actually accessible to visitors. Among which we can mention here the installation of artificial lights to allow visitors to appreciate the decorations and mural paintings. With this regards, the Pontifical Commission is testing a blue monochromatic lighting system in the neighbouring catacombs of San Callisto [8], which could be a further strategy to prevent biological growth.

4. Conclusions

The biological contamination of the Roman Catacombs of Santi Marco, Marcelliano e Damaso, started has occurred at least 3 years ago, due to the favorable microclimatic conditions. It was observed an extended outbreak of macromycetes at the entrance in the catacomb, while the walls near the skylight were widely covered by a phototrophic biofilm and some vascular plants. These biological colonizations induced mainly mechanical damages due to the hypha development under the plaster layer, and their penetration inside the mortar, beside the plant roots, leading therefore to detachments and loosing of material. The water presence inside the walls and biological structures, can lead to associated damages such as the solubilisation of pigments on the painted surfaces, extraction of metalic ions from stone and plasters due to metabolically-produced acids with chelating action.

The foremost preservation strategies to be straightway considered are related with the biological contamination removal and its control, by creating unfavourable conditions (i.e. reducing the moisture level inside the walls, improving the air circulation, preventing the entrance of natural light) for the biological growth. Monitoring the microclimate and biological development are also essential, before other drastic control strategies might be conceived.

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