

Airborne fungal spores colonising marbles exposed in the terrace of Messina Museum, Sicily

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Abstract

Studies were carried out on the air and on Carrara marble blocks located in the terrace of Messina Museum, in order to know the likelihood of airborne fungal spores coming into contact with and colonising tridimensional objects. Our results showed there were not significant differences between airborne fungi circulating in spring and in autumn; *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Cladosporium*, *Ulocladium*, *Aureobasidium*, *Phoma* were the most common isolates. However, only few species were able to settle on the marble surfaces as demonstrated by their isolation after 2 and 6 years of exposition.

1. Introduction

Airborne fungi occur as single units, spores and occasionally as hyphal fragments, conidiophores, associated with inorganic particles or as 'bioaerosol' (Comtois, 1990). Number and type of fungi vary with time of day, weather and seasonal fluctuation, geographical location, and with the presence of a local source of spores (Lacey, 1981).

The transport and ultimate settling on the surfaces is affected by: (i) the physical properties of particles and/or of droplets (size, density and shape); (ii) the environmental parameters (magnitude of air movements, relative humidity, and temperature) and (iii) the bioreceptivity of the surface itself and nutrient availability (Yang and Johanning, 1997; Guillitte, 1995; Urzì and Realini, 1998).

Much of our knowledge on the behaviour of airborne spores comes from various studies on the epidemiology of plants, animals and human diseases, especially infections of the respiratory tract and allergy (Comtois, 1990; Angulo et al., 1996). Very little was reported on the impact of airborne fungi on monuments and rock surfaces in indoor and outdoor environments (Mandrioli and Zenotti Censoni, 1982).

It is well known that once fungi settle on and colonise stone surfaces, they contribute to a great

variety of alterations like black patinas, intergranular growth, marble sugaring, biopitting, etc. Most of the researches have dealt with fungi already settled on the rock surface and/or with 'autochthonous mycoflora' (Saiz-Jimenez, 1995; Urzì et al., 1995; Sterflinger and Krumbein, 1997).

The aim of the present paper was to study the impact of airborne mycoflora on monument surfaces comparing the finding of airborne fungi on a tridimensional object with the one colonising freshly exposed Carrara marble blocks located in the terrace of Messina Museum.

2. Materials and methods

2.1 Sampling and counts of airborne fungi

Airborne fungi were sampled in two different seasons, spring and autumn, using an impact-sedimentation method. In order to simulate the fungal impact with a three-dimensional object, Petri dishes (100 mm) containing three different cultural media (Dichloran Rose Bengal Chloramphenicol, DRBC, Commissione Normal, 1990; Glucose Mineral Agar, GMA, Eckhardt, 1988, plus dichlorane addition; Czapek dox Agar, CZ, Oxoid) were disposed on the 5 faces of a



Figure 1. Exposure table located in the terrace of the Messina Museum on which marble blocks are placed. Polystyrene cube with Petri dishes containing different cultural media (in triplicate): DRBC, Oxoid; Glucose Mineral Agar, GMA, plus dichlorane addition, Czapek dox Agar, CZ, Oxoid.

polystyrene cube (50 cm per side) (Figure 1) in triplicate, and opened for one hour. Incubation was carried out at 28 °C for one week. Counts of total viable fungi and of black fungi were referred to as colony forming units per plate per hour (c.f.u./plate/h).

2.2 Mycological analyses of marble blocks

The presence of fungal colonisation on Carrara marble blocks, exposed in the terrace of Messina Museum (Eureka-Euromarble project EU 496), was checked using different non-destructive methods:

- a) adhesive tape (Gargani et al., 1968) for the direct evidentiatio under microscopy. Adhesive tape stripes (Fungi Tape, DID, S.p.A., Milan, Italy) were placed in contact on each face of the marble probes, and observed under light microscopy (DMLB, Leica) after mounting them on a slide with the addition of a drop of Amman's lactophenol (Fassatiovà, 1986);
- b) agar fingerprint and needle procedure (Wollenzien et al., 1995) for cultural analysis. Each different oriented surface of marble cubes (Figure 1) was sampled after 2 and 6 years of exposure; DRBC medium was used as a cultural medium in both cases. Incubation was carried out at 28 °C for one month to allow for the detection of slowly growing fungi.

Identification of fungi was carried out mainly on the basis of the macroscopic feature of colonies growing on different cultural media (Potato dextrose agar, PDA, Oxoid; Malt extract agar, MA, Oxoid) and on the micromorphology of reproduction structures, according to Fassatiovà (1986), and Ellis (1971, 1976).

3. Results

3.1 Airborne fungi

In Figures 2a–2b are summarised the results of the quantitative analyses carried out in the spring and autumnal sampling.

The number of total c.f.u./plate/h, detected in the sampling campaign carried out in spring was higher than the number obtained in autumn in all oriented surfaces. On DRBC medium, the number of fungi ranged from 40 c.f.u./plate/h in the Top surface up to 83 c.f.u./plate/h in South oriented surface; the higher numbers were found in the West and in South facing surfaces (73 c.f.u./plate/h and 83 c.f.u./plate/h respectively).

No significative differences between orientations and number of fungal colonies were observed in CZ medium; the number of fungi ranged from 35

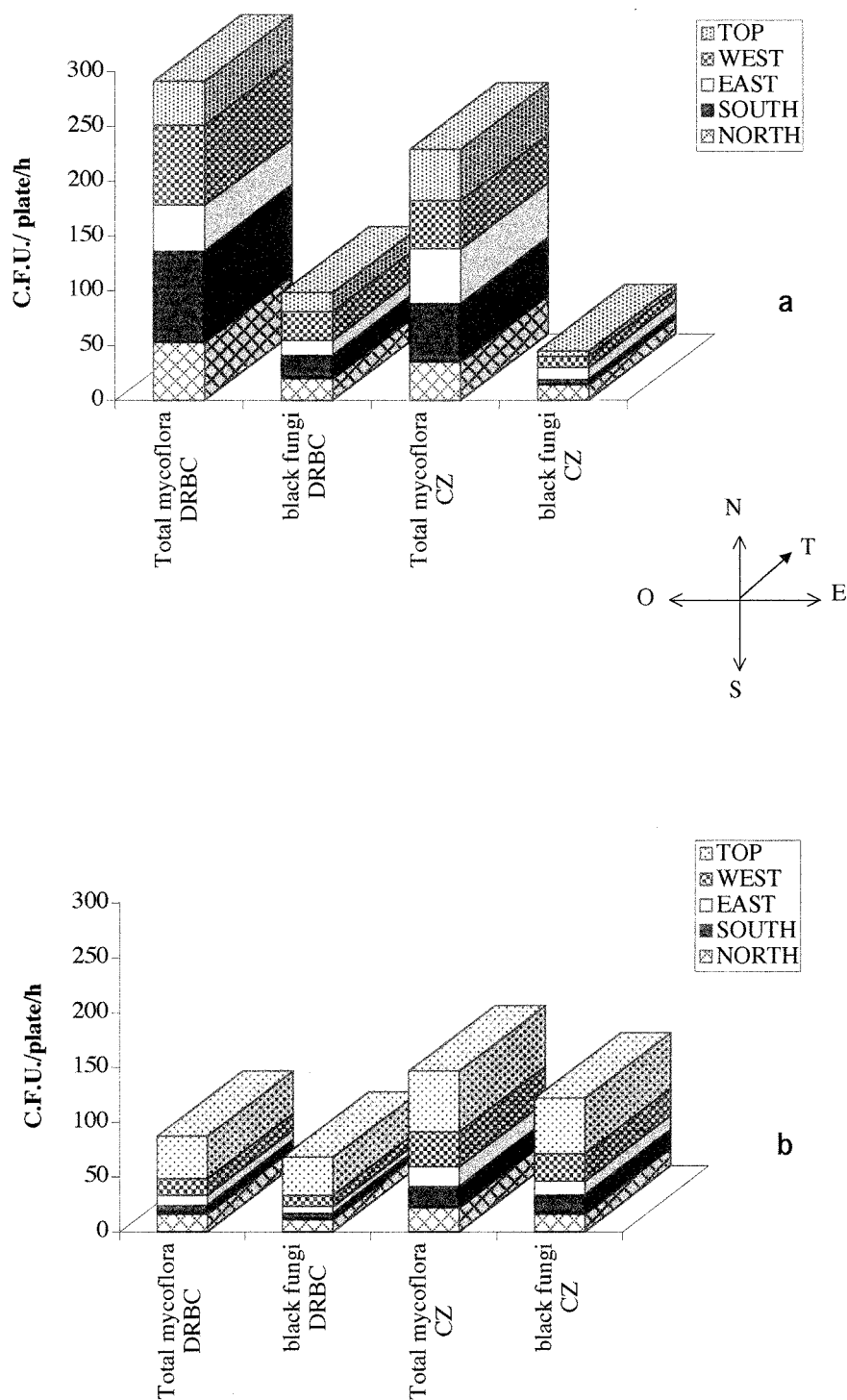


Figure 2. (a) Hystogram representing the number of airborne fungi settled on Petri dishes in spring sampling campaign. (b) Hystogram representing the number of airborne fungi settled on Petri dishes in autumn sampling campaign.

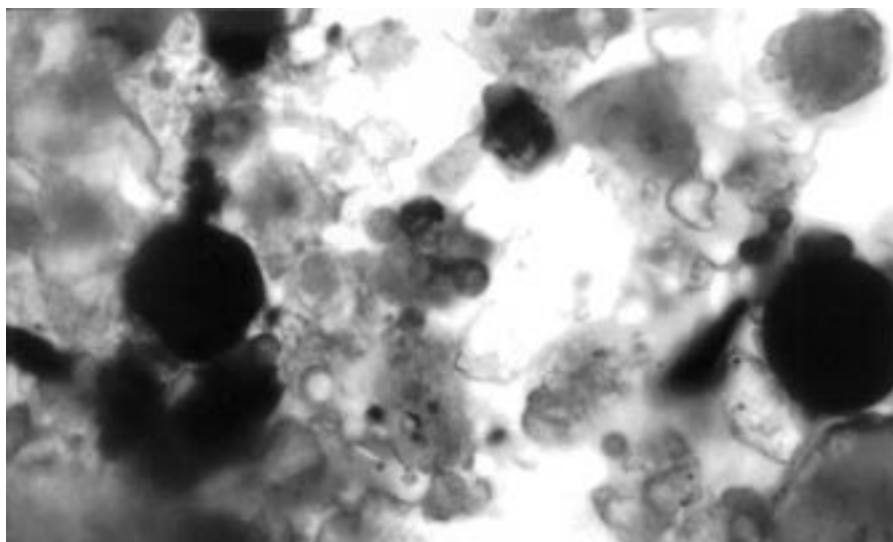


Figure 3. Direct observation of the colonisation of a Carrara marble block by scotch test procedure. Chains or single melanized fungal clamidospore-like cells (Magnification 40×).

Table 1. Fungal species isolated from the air of the Messina Museum in spring and autumn. The different orientations in which Petri dishes were placed is also given

Fungi	Polystyrene block orientation									
	North		South		East		West		Top	
	S	A	S	A	S	A	S	A	S	A
<i>Alternaria</i> spp.	+	+	+	+	+	+	+	+	+	+
<i>Aureobasidium pullulans</i>	-	-	-	-	+	-	-	-	-	+
<i>Aspergillus</i> spp.	+	+	+	+	-	+	+	-	-	-
<i>Aspergillus flavus</i>	-	-	-	-	-	-	-	-	-	+
<i>Aspergillus ochraceus</i>	+	-	-	-	-	+	-	-	-	-
<i>Aspergillus niger</i>	+	+	+	+	+	+	+	+	+	+
<i>Cladosporium</i> spp.	+	+	+	-	+	+	-	-	+	+
<i>Fusarium</i> spp.	-	-	-	-	-	-	-	+	-	-
<i>Phoma</i> spp.	-	+	+	+	+	-	+	-	+	+
<i>Phoma eupyrena</i>	+	-	-	-	-	+	-	-	+	-
<i>Penicillium</i> spp.	-	-	-	+	+	-	+	+	-	-
<i>Stemphylium</i> sp.	-	-	-	-	-	-	-	-	-	+
<i>Trichoderma</i> spp.	+	-	+	-	+	+	-	+	-	-
<i>Ulocladium</i> spp.	+	+	+	+	+	+	+	+	+	+
Black yeasts	+	+	-	+	+	+	+	-	+	-
Yeasts	-	+	+	+	-	-	+	-	+	-

S = Spring; A = Autumn; + = presence; - = absence.

c.f.u./plate/h in the North facing surface up to 53 c.f.u./plate/h in the South facing surface. On GMA the presence of spreading fungi and the over growth of bacteria did not allow for a correct count of colonies;

in addition, a scarce presence of black fungi was noticed.

Black fungi were found in greater amount on DRBC medium, ranging from 14 c.f.u./plate/h up to 26 c.f.u./plate/h, while in CZ medium the number of black fungi ranged from 5 c.f.u./plate/h up to 14 c.f.u./plate/h.

In autumn, a lower number of total mycoflora units were isolated, in all oriented surfaces and media, but the ratio black fungi/total mycoflora resulted higher. The total number of fungi ranged from 18 c.f.u./plate/h up to 39 c.f.u./plate/h on DRBC medium and from 18 c.f.u./plate/h up to 56 c.f.u./plate/h on CZ medium.

Petri dishes positioned on the Top surface supported a higher amount of fungal colonies in both media (39 c.f.u./plate/h on DRBC medium and 56 c.f.u./plate/h on CZ medium).

The list of fungi found is shown in Table 1. No seasonal differences were observed among the most frequent genera isolated. In particular, dematiaceous species belonging to the genera of *Alternaria* and *Ulocladium* and the species of *Aspergillus niger* were found in all orientations and seasons as well as species belonging to the genera of *Cladosporium* and *Phoma*. Black yeasts were found with a minor frequency.

3.2 Mycological analysis of marble blocks

The direct examination of marble surface through scotch test procedure showed, in general, a scarce or no fungal colonisation in the vertical surfaces of

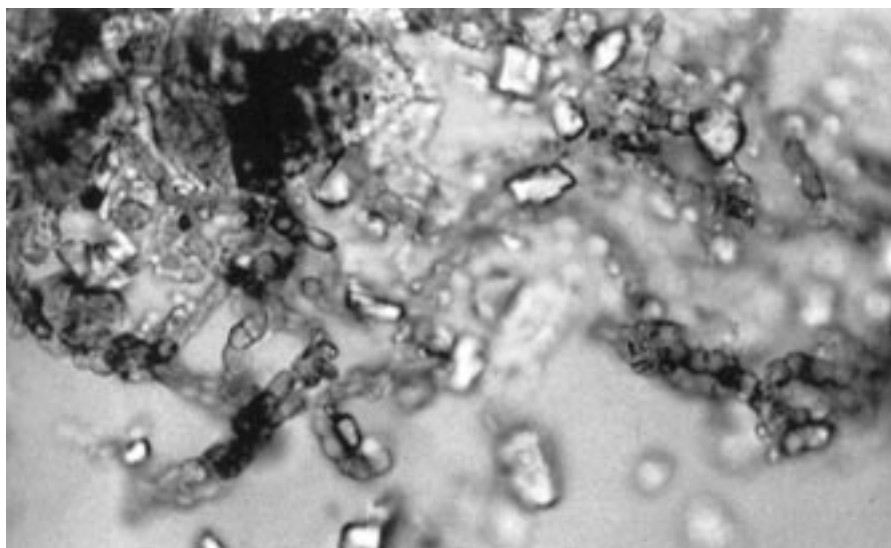


Figure 4. Direct observation of the colonisation of a Carrara marble block by scotch test procedure. Dust particles and black roundish fungal structures on the surface of marbles (Magnification 40×).

the marbles in all oriented faces. Black fungal aggregates together with dust particles were observed in the sheltered parts and on the top surfaces (Figures 3–4).

Cultural analyses carried out with non-destructive techniques confirmed the microscopical observations. In fact, only the top surface of marble blocks resulted colonised (Table 2). After 2 years of exposure the fungi found were of the dematiaceous species as *Alternaria alternata*, *Cladosporium* sp., *Ulocladium* sp., *Aureobasidium pullulans*, and no dematiaceous species such as the genera *Aspergillus* and *Penicillium*. After 6 years of exposure a minor variety of species were found. All of them were dematiaceous species like *A. alternata*, *Cladosporium* sp. and *Aspergillus niger*. No black yeasts were isolated. Results are summarised in Table 2.

4. Discussion

Comtois (1998), talking about pollen behaviour, stressed that Aerobiology is an “impossible science” for two important reasons: one is connected to the size of the particles to be sampled and to the volume of the particles, and the other one is due to the non reproducibility of the sampling itself.

Both reasons create a great variability and affect the possibility of comparison of results obtained from other scientists. These facts should also be taken into

Table 2. Fungal species isolated from the marble blocks after 2 and 6 years of exposition in the Messina Museum

Fungi	Marbles (Top)	
	2Y	6Y
<i>Alternaria</i> spp.	+	+
<i>Aureobasidium pullulans</i>	+	–
<i>Aspergillus niger</i>	–	+
<i>Cladosporium</i> spp.	+	+
<i>Penicillium</i> spp.	–	–
<i>Ulocladium</i> spp.	+	–
<i>Exophiala</i> sp.	+	–

2Y = 2 years of exposition; 6Y = 6 years of exposition.

account when studying airborne mycoflora and its destiny on the surface of monuments.

The impact sedimentation method used in our researches showed that several ‘fungal units’ (Figure 5) have the possibility of coming into contact with both horizontal and vertical surfaces. In addition, it was very useful to reveal the natural impact of fungi on tridimensional items, and it allowed for the isolation of a large amount of fungal species due to the use of different cultural media.

On the contrary, a very restricted diversity of fungal species was found on the marble blocks after 2 and 6 years of exposure and exclusively on the flat top surface. Common species found on marble surfaces

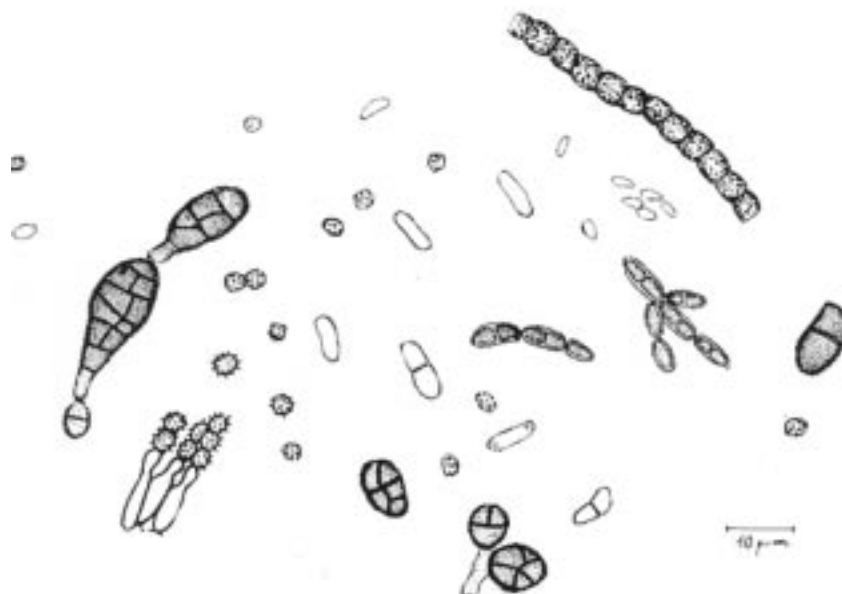


Figure 5. Dimension of most 'fungal units' found in the air of Messina Museum terrace. Bar is 10 μ m.

after 2 and 6 years of exposure were dematiaceous genera of *Alternaria* and *Cladosporium*.

This finding is in agreement with the fact that these genera are among the most diffused conidial airborne fungi. Their frequency can even mask in some periods the presence of other fungi (Lacey, 1981), thus the probability of coming into contact with a receptive surface is higher for them than for other species.

The presence of these genera on the stone is well documented, in fact they are very often isolated from monument surfaces at different stages of deterioration (Urzi et al., 1995; Urzi et al., 1998).

Their ability to colonise the substrate and persistency depend on different factors, among which the most important are bioreceptivity and spatial orientation of artifact surfaces, environmental factors and the presence of peculiar characteristics that allow them to survive in the air and to fit also in the habitat 'marble'.

However the presence of resistance structures (like clamidospores, sclerotia) and their ability to survive in dry conditions or with a low requirement of nutrients and energy, allow them to be less susceptible to seasonal variation. Both genera isolated from marble blocks form resistance structures and can survive under unfavourable conditions. The presence of melanins and, in the case of *Alternaria* species, the capacity to form clamidospore-like structures give to

the strains great resistance against different physico-chemical agents, and facilitate the colonisation of stone surfaces.

5. Conclusions

Our researches have showed that despite the high variety of air-borne fungi circulating in the close environment in which marble items were exposed in Messina, only few species were able to settle and colonise the marble surfaces. This fact can be explained as follows:

- a) in a given habitat microfungi have a very short life cycle: they can appear and disappear as a function of environmental and nutrient changes (Widden, 1981);
- b) only well adapted 'fungal units' can survive on the inorganic surfaces.

Our data, however, partially differ from other researches carried out recently on marble items exposed in Northern climate in the frame of the same Euromarble project (Wien and Munich) as reported by Sterflinger, 1999 and Urzi et al., 2000. In both cases a higher variety of fungi isolated from marble surfaces was reported including black meristematic growing fungi and the colonization of vertical oriented surfaces.

Thus climatic and environmental factors can highly influence the pattern of colonisation of marble surfaces more than the nature of the rock itself.

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