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# Role of fungi in the deterioration of wall paintings

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## Abstract

Wall painting was one of the earliest forms of art all over the world. On account of a variety of factors, these wall paintings undergo deterioration. Growth of fungi over these has been considered a major factor in their decay by many workers and attempts have been made to understand the mechanism of decay as well as to find suitable fungicides for their control. This paper summarizes the present state of knowledge with regard to the role of fungi in the deterioration of wall paintings.

**Keywords:** Wall paintings; Fungal deterioration; Wall paintings; Control of fungal growth

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## 1. Introduction

The tradition of wall painting is very old, dating back to prehistoric times. These wall paintings which are valuable not only as works of art but also as a source of invaluable information about the contemporary society, occupy a place of pride in the repertory of any nation's cultural heritage, and hence hardly need any justification for their preservation. These murals are also an integral part of the structure of the building where these are present (Fig. 1). Not only from the point of view of aesthetics or art history but also from the point of view of conservation these paintings are considered a part of these historic structures (Mora et al., 1984), and as such any decay of the wall paintings or the structure is bound to affect

the aesthetics and stability of the other. Therefore, it becomes imperative to take proper measures for the conservation of wall paintings as well as taking steps for the conservation of the historic buildings.

The wall paintings, generally classified as tempera, secco or fresco depending upon the technique of execution, possess a layered structure consisting of support, ground and the paint layer. These constituents of wall paintings undergo deterioration physically, chemically or biologically (Mora et al., 1984; Subbaraman, 1992). Although generally factors like moisture, salts, atmospheric pollution, etc. have been held responsible for the deterioration of wall paintings in most cases, many workers believe that the growth of biological agencies like fungi is also responsible to a large extent for the decay of murals (Tonolo and Giacobini, 1963; Wazny, 1965; Roznerska, 1967; Ku-

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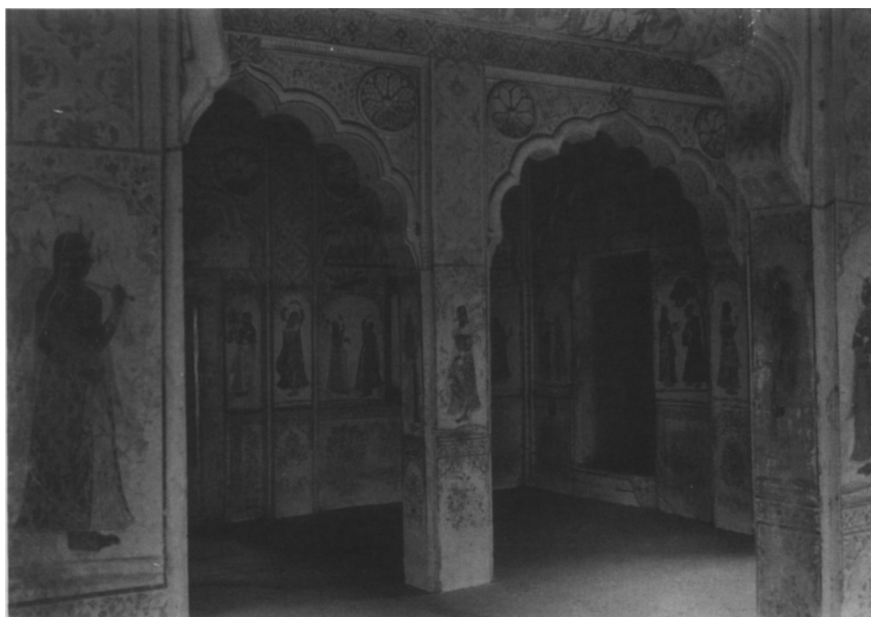


Fig. 1. The wall paintings are an integral part of a historic building as evident in this old fort at Nagaur, India.

ritzyna, 1968; Ionita, 1973; Krumbein and Lange, 1978; Strzelczyk, 1979, 1981; Bianchi et al., 1980; Saiz-Jimenez and Samson, 1981; Jeffries, 1986, 1991; Hirte et al., 1987; Sorlini et al., 1987; Agrawal et al., 1988, 1989, 1991; Sampo and Mosca, 1989; Karpovich-Tate and Rebricova, 1991; Rebricova, 1993; Garg and Dhawan, 1994; Garg et al., 1993). Consequently, several studies done over the years by many workers have not only tried to explain the role played by the fungi in the deterioration of wall paintings but have also endeavoured to find suitable fungicides for their control.

The paper reviews the current state of knowledge in this important area.

## 2. Factors conducive to fungal growth on wall paintings

Relative humidity, temperature and availability of nutrition are the three most important factors which are considered responsible for mould growth over any type of material. The growth of fungi over wall paintings is also governed largely

by these factors. It is well known that microorganisms such as fungi grow rapidly in an environment where relative humidity is more than 65%. In the case of wall paintings, the relative humidity requirements suitable for fungal growth may be fulfilled by atmospheric humidity e.g. in the rainy season when the humidity is generally very high and the growth of fungi is greater (Tilak et al., 1970, 1972; Strzelczyk, 1981; Tilak, 1986, 1991; Agrawal et al., 1988, 1991). In many cases, the dampness in the wall is responsible for meeting the humidity requirements (Fig. 2). According to Strzelczyk (1981), the spores of most of the fungal forms are capable of utilizing condensation moisture. Under conditions of high relative humidity (75–95%), the spores germinate into mycelium. If the humidity conditions remain unchanged, developing hyphae produce further spores within 48–72 h, dominating the surface of paintings.

Generally, a temperature range of 20–35°C is considered optimum for the growth of most species of fungi but there are species which are capable of withstanding a much wider range of temperature.



Fig. 2. The moisture traveling through the walls or roof is an important factor for the growth of fungi. In this extreme case, the plaster containing the paintings has fallen off (Nagaur fort, India).

The nutritional requirements of growing fungal forms are met by the presence of organic compounds either in the ground or the paint layer of the paintings. Since most of the fungi are dependent upon the organic compounds present in the binding media as a source of nutrition, it is believed that the paintings done in the fresco technique where lime is the medium used for the binding of the pigments should not be attacked by fungi. However, there are many examples where fungal growth has been reported from frescoes (Bianchi et al., 1980; Saiz-Jimenez and Samson, 1981; Sorlini et al., 1987; Sampo and Mosca, 1989; Karpovich-Tate and Rebricova, 1991; Rebricova, 1993). It is believed that in the case of frescoes, the nutritional requirements are met by the presence of organic compounds in the dust layer which has accumulated over the paintings. In fact, these dust and dirt particles are not only capable of retaining moisture on the surface of the paintings which in turn helps in the growth of fungal spores but also attract fungal spores (Tilak et al., 1970; Tilak, 1991). Saiz-Jimenez and Samson (1981) believed that bacteria were the first colo-

nizers of moist frescoes and building materials and thus represented the first supply of organic matter there. Karpovich-Tate and Rebricova (1991) subscribed to this hypothesis. In such cases, the death and lysis of such bacteria would promote the growth of fungi. It is already known that some fungi could utilize the cell walls of chemolithotrophic bacteria as the only source of nutrition (Heinen and Lauwers, 1980). Karpovich-Tate and Rebricova (1991) concluded that, although fungi could use organic substances in dust accumulating on the rough surfaces of wall paintings and those in the binding medium of paints and restoration adjunct materials (egg-yolk emulsion) as well as in plaster (cellulose, starch, milk), it is the trophic interrelationship that plays the main role within microbial communities on frescoes, plaster and brick. It has been reported by Sorlini et al. (1987) that materials used in the paintings such as casein or Rabbit's foot gelatin allows abundant fungal growth whereas pigments like limonite, haematite and malachite when added to Rabbit's foot gelatin inhibited the fungal growth totally or partially.

Since fungi are heterotrophic microorganisms, the availability of light is not essential for their growth and hence they are found growing profusely even in dark corners. According to some workers (Bassi and Giacobini, 1973), the growth of fungi is also dependent upon some other factors like the nature of the painting constituents, the adaptability and the life span of microflora as well as the antagonism between fungi of different species. For example, the tolerance of many fungal species to harsh environments is also considered a factor for the survival of certain fungi on wall paintings (Saiz-Jimenez and Samson, 1981). Similarly, there are some fungal species such as *Aureobasidium pullulans* which have been found unable to colonise on the paint films in the absence of other fungal forms like *Aspergillus*, *Mucor*, *Alternaria* and *Cladosporium* (Winters et al., 1976). It is suggested that cellulase from *Aspergillus* and *Alternaria* aids in the initial colonisation of these fungi due to hydrolysis of hydroxy ethyl cellulose present within the paint film. According to Rebricova (1991, 1993), the formation of different microbial communities on the surface of wall paintings depends upon the painting techniques as well as the climatic conditions.

Another factor which plays a vital role in the development of fungi is the dispersal of fungal spores. Mostly it is through air. However, many times the spores may reach the surface through the persons visiting the wall painting sites (Emoto and Emoto, 1974; Agrawal et al., 1988, 1991). In some instances, the materials used for the conservation treatment are the source of fungal growth (Sorlini et al., 1982, 1987; Sampo and Mosca, 1989; Hammer and Lux, 1990; Karpovich-Tate and Rebricova, 1991).

Environmental pollution has also been considered as a contributing factor for fungal growth by Saiz-Jimenez and Samson (1981). According to them, the existence of hydrocarbons and volatile organic compounds in the atmosphere, emitted by the neighbouring oil refineries and cellulose pulp plants can deposit on the paintings and form a layer of organic compounds able to support the growth of microorganisms. Saiz-Jimenez and Samson (1981) are also of the view that saline deposits may provide a suitable habitat for fungi,

especially for the species like *Cladosporium sphaerospermum*, *Engyodontium album* and *Aspergillus versicolor*. Tresner and Hayer (1971) reported that *Penicillium* and *Aspergillus* species were outstandingly more resistant to sodium chloride than other fungal species.

Several workers (Bassi and Chiatante, 1976; Sorlini et al., 1982; Agrawal et al., 1988) have reported the presence of bat and pigeon excreta on wall paintings and these may also provide nutrition for the proliferation of fungi.

### 3. Fungal flora on wall paintings

Since all the conditions required for the growth of fungi are met easily in the case of wall paintings, the problem of fungal growth on wall paintings is widespread as attested by reports from different countries like Italy, Japan, Bulgaria, Spain, Romania, Poland, Switzerland, England, Russia, Germany, India, etc. The fungal species observed by various workers on wall paintings have been reported in Table 1. It may be inferred from this table that although a wide variety of fungal forms may be present on the wall paintings, a few forms namely, species of *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Curvularia*, *Dreschlera*, *Chaetomium*, *Fusarium*, *Trichoderma*, *Gliomastix*, *Aureobasidium*, etc. are more common in their distribution. In temperate climates, fungi like *Aureobasidium pullulans*, *Cladosporium herbarum* and species of *Alternaria*, *Penicillium* and *Aspergillus* have been found to be common on exterior paint surfaces.

### 4. Effects of fungal growth on wall paintings

The growth of fungi on wall paintings manifests itself most commonly by discolouration or deterioration of the surface. The fungi may produce variously coloured superficial stains which may seriously impair the chromatic and aesthetic appeal of the paintings (Figs. 3,4). For example, Emoto and Emoto (1974) reported black, green and red stains on wall paintings from ancient tombs in Japan as a result of fungal growth. Similarly stains of other colours have also been reported by several workers (Gargani, 1968; Ionita, 1973; Bassi and Giacobini, 1973; Arai,

Table 1  
Fungi isolated from deteriorated wall paintings

No.	Fungi	Location	References
1.	<i>Aspergillus</i> sp., <i>Cephalosporium</i> sp., <i>Penicillium</i> sp., <i>Dematiaceous hyphomycetes</i>	Frescoes of ancient Ostia; church of St. Kliment in Rome; frescoes of Sinorelli	Tonolo and Giacobini (1963)
2.	<i>Aspergillus</i> sp., <i>Cephalosporium acremonium</i> , <i>Mucor mucedo</i> , <i>Oospora crustacea</i> , <i>Penicillium</i> sp., <i>Plicaria muralis</i> , <i>Pullularia pullulans</i> , <i>Pyronema domesticum</i> , <i>Rhizopus nigricans</i> , <i>Sporotrichum roseum</i> , <i>Stemphylium</i> sp., <i>Torula murorum</i>	Wall paintings in Polish palaces and Roman catholic churches	Wazny (1965)
3.	<i>Alternaria</i> sp., <i>Aspergillus candidus</i> , <i>Cephalosporium coremioides</i> , <i>Circinella conica</i> , <i>Cladosporium atroseptum</i> , <i>C. epiphyllum</i> , <i>Fusarium</i> sp., <i>Penicillium paxilli</i> , <i>P. puberulum</i> , <i>Sporotrichum bombycinum</i> , <i>Trichoderma roseum</i> , <i>Mycelia sterilia</i>	Wall paintings of temples of Tsminda-Nikolazi and Tsalindzhikha in Georgia, USSR	Voronina (1966)
4.	<i>Cladosporium epiphyllum</i> , <i>Mucor</i> sp., <i>Sporotrichum bombycinum</i> , <i>Mycelia sterilia</i>	Pozhaisky monastery wall paintings in Lithunia	Voronina (1966)
5.	<i>Aspergillus</i> sp., <i>Gliocladium</i> sp., <i>Mucor</i> sp., <i>Penicillium citrinum</i> , <i>P. commune</i> , <i>P. camemberti</i> , <i>P. decumbens</i> , <i>P. frequentans</i> , <i>P. purpurogenum</i> , <i>P. restrictum</i> , <i>Stemphylium</i> sp.	Frescoes in Florence	Gargani (1972)
6.	<i>Aspergillus versicolor</i> , <i>Bispora menzellii</i> , <i>B. pusilla</i> , <i>Cephalosporium acremonium</i> , <i>Cladosporium cladosporioides</i> , <i>C. elatum</i> , <i>C.sphaerospermum</i> , <i>Fusidium viride</i> , <i>Penicillium meleagrimum</i> , <i>Plicaria muralis</i> , <i>Sporotrichum gorlenkoanum</i> , <i>Stemphylium ilicis</i> , <i>Verticillium lateritium</i>	Wall paintings Novogorod, Kostroma, Vladimir, Moscow, USSR	Kuritzyna (1968)
7.	<i>Aspergillus</i> sp., <i>Camposporium</i> sp., <i>Cladosporium</i> sp., <i>Curvularia</i> sp., <i>Haplosporella</i> , sp., <i>Helminthosporium</i> sp., <i>Nigrospora</i> sp., <i>Pithomyces</i> sp., <i>Pseudotorula</i> sp.,	Aeroflora of Ajanta caves, India	Tilak et al. (1970); Tilak (1991)
8.	<i>Alternaria</i> sp., <i>Aspergillus</i> sp., <i>Cladosporium</i> sp., <i>Curvularia</i> sp., <i>Helminthosporium</i> sp., <i>Nigrospora</i> sp., <i>Pithomyces</i> sp.	Aeroflora of Ellora caves, India	Tilak et al. (1970); Tilak (1991)
9.	<i>Alternaria tenuis</i> , <i>Alternaria</i> sp., <i>Aspergillus fumigatus</i> , <i>A. oryzae</i> , <i>A. sydowi</i> , <i>A. versicolor</i> , <i>A. wentii</i> , <i>Aureobasidium pullulans</i> , <i>Cladosporium herbarum</i> , <i>Curvularia</i> sp., <i>Nigrospora sphaerica</i> , <i>Paecilomyces variotii</i> , <i>Papularia sphaerosperma</i> , <i>Papularia</i> sp., <i>Penicillium crustosum</i> , <i>P. frequentans</i> , <i>P. multicolor</i> , <i>P. restrictus</i> , <i>P. rubrum</i> , <i>P. spinulosum</i> , <i>Pestalotia</i> sp., <i>Rhodotorula gelatinis</i> , <i>Sporotrichum</i> sp., <i>Stemphylium</i> sp., <i>Trichoderma viride</i> , <i>Tritiratum album</i> , <i>Zygosporium echinulatum</i> , <i>Z. parasiticum</i>	Polychromed wall painting panels in the main Hall of Horyuji Temple, Japan	Emoto (1971)

Table 1 (Continued)

No.	Fungi	Location	References
10.	<i>Alternaria tenuis</i> , <i>Aspergillus amstelodami</i> , <i>A. niger</i> , <i>A. versicolor</i> , <i>Botryotrichum</i> <i>artogriseum</i> , <i>B. piluliferum</i> , <i>Chaetomium murorum</i> , <i>C. globosum</i> , <i>C. indicum</i> , <i>Circinella sydowi</i> , <i>Cladosporium herbarum</i> , <i>Cunninghamella echinulata</i> , <i>Geotrichum candidum</i> , <i>Mucor spinosus</i> , <i>Penicillium lilacinum</i> , <i>Scopulariopsis brevicaulis</i> , <i>Stachybotris atra</i> , <i>S. cylindrospora</i> , <i>Stemphylium Periforme</i> , <i>Torula herbarum</i> , <i>Trichoderma viride</i>	Paintings from 14–17th centuries in monasteries of Northern Moldavia, Voronet, Sucevita, Humor, Arbore, Patraati, Varatec, Agapia, Putna, the Cozia monastery the princely church at Curtea de Arges	Savulescu and Ionita (1971)
11.	<i>Alternaria</i> sp., <i>Chaetomium</i> sp.	Murals of church of St. Petka Samardzhiyska, St. Panteleymon in village of Boyan, Church of Holy Archangel in village of Arbanasi, Bulgaria	Slavova (1972)
12.	<i>Cladosporium cladosporioides</i> , <i>C. sphaerospermum</i> , <i>Cladosporium</i> sp.	Frescoes in cathedrals, tombs, and burial vaults, Italy	Bassi and Giacobini (1973); Giacobini et al. (1991)
13.	<i>Aspergillus echinulatus</i> , <i>A. niger</i> , <i>A. repens</i> , <i>Chaetomium herbarum</i> , <i>Scopulariopsis brevicaulis</i> , <i>Stemphylium macrosporoideum</i> , <i>S. piriforme</i>	Wall paintings of 14–17th cent. from Northern Moldavia (Humor, Sucevita Voronet, Arbore, Patrauti, Parhauiti, Varatech, Moldavita)	Ionita (1973)
14.	<i>Alternaria alternata</i> , <i>Cladosporium</i> sp., <i>C. herbarum</i> , <i>Epicoccum purpurascens</i> , <i>Gliocladium roseum</i> , <i>G. virens</i> , <i>Gliomastix</i> sp., <i>Pencillium</i> sp., <i>P. citreo-viride</i> , <i>P. citrinum</i> , <i>P. janthinellum</i> , <i>P. oxalicum</i> , <i>P. purpurogenum</i> , <i>Trichoderma viride</i> , <i>Verticillium</i> sp.	Wall paintings of Ozuka tomb (Fukuoka), Chibusan tomb (Kumamoto), Japan	Emoto and Emoto (1974)
15.	<i>Acremonium roseum</i> , <i>Aspergillus echinulatus</i> , <i>Penicillium chrysogenum</i> , <i>Stemphylium piriforme</i>	Wall paintings, Romania	Istudor et al. (1976)
16.	Filamentous fungi	Paintings of Etruscan tombs, Belfry in Arezzo, Italy	Curri (1979)
17.	Micromycetes	Frescoes of the Ragione Palace in Milan, Italy	Sorlini et al. (1979)
18.	<i>Penicillium chermisinum</i> , <i>P. implicatum</i> , <i>Sporotrichum grisellum</i> , <i>Trichoderma viride</i>	Paintings of Roman Catholic Church in Dembna Podgolyanskom, Poland	Smyk (1979)
19.	<i>Cladosporium cladosporioides</i> , <i>Penicillium</i> <i>lanoso-coeruleum</i>	Paintings of burial vault of St. Sebastian in Pavia, Italy	Bianchi et al. (1980)
20.	<i>Alternaria alternata</i> , <i>Acremonium charticola</i> , <i>Arthrinium</i> state of <i>Apiospora montagnei</i> , <i>Aspergillus versicolor</i> , <i>Botrytis cinerea</i> , <i>Chaetomium elatum</i> , <i>Chaetomium</i> sp., <i>Cladosporium</i> <i>sphaerospermum</i> , <i>Cunninghamella echinulata</i> , <i>Engyodontium album</i> , <i>Penicillium brevi-compactum</i> , <i>P. chrysogenum</i> , <i>P. citrinum</i> , <i>P. decumbens</i> , <i>P. frequentans</i> , <i>P. nigricans</i> , <i>P. raciborski</i> , <i>P. verrucosum</i> var. <i>cyclopium</i> , <i>Phoma glomerata</i>	Wall paintings of the monastery of Santa Maria de la Rabida, Huelva, Spain	Saiz-Jimenez and Samson (1981)
21.	Micromycetes	Wall paintings of the church of St. Panteleymon in the village of Boyan, Bulgaria	Hadjivulcheva and Gesheva (1982)

Table 1 (Continued)

No.	Fungi	Location	References
22.	<i>Aspergillus</i> sp., <i>Cephalosporium</i> sp., <i>Cladosporium</i> sp., <i>Epicoccum</i> sp., <i>Penicillium</i> sp., <i>Scopulariopsis</i> sp.	Wall paintings of Palazzo della Ragione in Milan, Italy	Sorlini et al. (1982)
23.	<i>Aspergillus niger</i> , <i>Cladosporium cladosporioides</i> , <i>C. cucumerinum</i> , <i>Epicoccum purpurascens</i> , <i>Fusarium oxysporum</i> , <i>Penicillium frequentans</i> , <i>P. oxalicum</i>	Wall paintings of the Old Churches of Pavia, Italy	Crippa (1983)
24.	<i>Aspergillus glaucus</i>	Wall paintings in the Swiss Baroque monastery church, Switzerland	Raschle (1983)
25.	<i>Cladosporium</i> sp., <i>Doratomyces</i> sp., <i>Fusarium</i> sp., <i>Mucor</i> sp., <i>Penicillium</i> sp., <i>Trichoderma</i> sp., <i>Verticillium</i> sp.	Paintings of Takamatsu- zuka tumulus, Japan	Arai (1984)
26.	<i>Alternaria</i> sp., <i>Aspergillus</i> sp., <i>Cladosporium</i> sp., <i>Penicillium</i> sp., <i>Pestalotia</i> sp., <i>Trichoderma</i> sp.	Mural paintings of Torazuka tumulus, Japan	Arai (1984)
27.	<i>Phialophora</i> sp.	Nakata-Oketsu tumulus, Japan	Arai (1984)
28.	<i>Trichoderma</i> sp.	Hayama-Oketsu tumulus, Japan	Arai (1984)
29.	<i>Beauveria alba</i>	Wall paintings in Canterbury cathedral, England	Jeffries (1986, 1991)
30.	Micromycetes	Wall paintings from the 17th Century in the church of John Bogoslov in Rostov, USSR	Lyalikova and Petushkova (1986)
31.	<i>Alternaria</i> sp., <i>Aschersonia</i> sp., <i>Aspergillus</i> sp., <i>Chaetomium</i> sp., <i>Mucor</i> sp., <i>Penicillium</i> sp., <i>Stemphylium</i> sp., <i>Trichoderma</i> sp.	Wall paintings of Sans Souci Palace, Germany	Hirte et al. (1987)
32.	<i>Alternaria</i> sp., <i>Aureobasidium pullulans</i> , <i>Chaetomium</i> sp., <i>Cladosporium cladosporioides</i> , <i>C. sphaerospermum</i> , <i>Stemphylium botryosum</i>	Wall paintings of the Trinity cathedral in Aleksandrov, USSR	Rebricova et al. (1987); Rebricova (1991, 1993)
33.	<i>Aspergillus repens</i> , <i>A. versicolor</i> , <i>Botrytis</i> <i>cinerea</i> , <i>Cladosporium cladosporioides</i> , <i>Mycelia sterilia</i> , <i>Penicillium chrysogenum</i> , <i>P. verrucosum</i> var. <i>cyclopium</i> , <i>Stemphylium</i> sp., <i>Tritirachium album</i>	Wall paintings of the Pantelimonovsky cathedral in New Athos, USSR	Rebricova et al. (1987); Rebricova (1991, 1993)
34.	<i>Acremonium charticola</i> , <i>Cladosporium</i> <i>sphaerospermum</i> , <i>Sporotrichum</i> sp.	Wall paintings of the George cathedral in Novgorod, USSR	Rebricova et al. (1987); Rebricova (1991, 1993)
35.	<i>Acrothecium</i> sp., <i>Aspergillus niger</i> , <i>Aspergillus</i> sp. (3)	Gambara's Frescoes, Brescia, Italy	Sorlini et al. (1987)
36.	<i>Acremonium indicum</i> , <i>Alternaria alternata</i> , <i>Aspergillus flavus</i> , <i>A. nidulans</i> , <i>A. niger</i> , <i>A. terreus</i> , <i>A. versicolor</i> , <i>Cladosporium</i> <i>cladosporioides</i> , <i>C. herbarum</i> , <i>Curvularia</i> <i>lunata</i> , <i>C. pallens</i> , <i>Chaetomium globosum</i> , <i>Drechslera australiensis</i> , <i>D. hawaiiensis</i> , <i>Emericella nidulans</i> , <i>Epicoccum nigrum</i> , <i>Fusarium oxysporum</i> , <i>F. solani</i> , <i>Macrophomina</i> <i>phaseolina</i> , <i>Memnoniella echinata</i> , <i>Mycelia sterilia</i> , <i>Paecilomyces variotii</i> , <i>Rhizopus nigricans</i> , <i>Stachybotrys atra</i> , <i>Trichoderma harzianum</i>	Wall paintings of Ajanta Caves, India	Agrawal et al. (1988, 1991); Dhawan et al. (1991); Garg and Dhawan (1994) Garg et al. (1991)

Table 1 (Continued)

No.	Fungi	Location	References
37.	<i>Aspergillus versicolor</i> , <i>Beauveria</i> sp., <i>Botrytis cinerea</i> , <i>Chaetomium globosum</i> , <i>Cladosporium sphaerospermum</i> , <i>Epicoccum nigrum</i> , <i>Geomyces pannorum</i> , <i>Penicillium chrysogenum</i> , <i>P. cyclopium</i> , <i>P. verrucosum</i> , <i>Sporotrichum</i> sp., <i>Verticillium lamellicola</i> , <i>V. lecanii</i>	Paintings of the cathedral of Birth of the Holy Virgin in Russia	Rebricova and Karpovich (1988); Rebricova (1991, 1993)
38.	<i>Acremonium charticola</i> , <i>A. terricola</i> , <i>Alternaria</i> sp., <i>Aspergillus sydowi</i> , <i>A. versicolor</i> , <i>Aureobasidium pullulans</i> , <i>Candida</i> sp., <i>Cladosporium cladosporioides</i> , <i>C. sphaerospermum</i> , <i>Hyalodendron</i> sp., <i>Mycelia sterilia</i> , <i>Trichoderma viride</i> , <i>Tritirachium album</i>	Paintings of the cathedral of Birth of Holy Virgin (16th Cent.) in Ferapontovo, Russia	Rebricova (1988, 1991, 1993)
39.	Micromycetes	Paintings of the cathedral of Birth of the Holy Virgin (16th cent.) in Ferapontovo, USSR	Lyalikova and Petushkova (1988); Petushkova et al. (1989)
40.	<i>Aspergillus flavus</i> , <i>A. sydowi</i> , <i>A. versicolor</i> , <i>Aspergillus</i> sp., <i>Botrytis cinerea</i> , <i>Cladosporium cladosporioides</i> , <i>C. sphaerospermum</i> , <i>Coniothyrium cerealis</i> , <i>Engyodontium</i> sp., <i>Oidiodendron cerealis</i> , <i>Penicillium brevicompactum</i> , <i>P. chrysogenum</i> , <i>P. meleagrinum</i> , <i>P. notatum</i> , <i>P. purpurogenum</i> , <i>P. rugulosum</i> , <i>P. variable</i> , <i>Rhodotorula</i> sp., <i>Tilletiopsis</i> sp., <i>Trichoderma harzianum</i> , Mycelium sterile moniliaceum, Mycelium sterile dematiaceum	15th Cent. frescoes of Ognissanty church in Florence, Italy	Sampo and Mosca (1989)
41.	<i>Acrodontium crateriforme</i> , <i>Beauveria bassiana</i> , <i>Cladosporium sphaerospermum</i> , <i>Fusidium viride</i> , <i>Geomyces pannorum</i> , <i>Penicillium</i> sp., <i>Phialophora</i> sp., <i>Tritirachium album</i> , <i>Verticillium lamellicola</i>	Wall paintings of the cathedral of the Nativity of the Virgin in the Pafnutii-Borovskii monastery, USSR	Karpovich-Tate and Rebricova (1991)
42.	<i>Acremonium</i> sp., <i>Aspergillus versicolor</i> , <i>Cladosporium herbarum</i> , <i>C. sphaerospermum</i> , <i>Hyalodendron</i> sp., <i>Penicillium verrucosum</i> var. <i>cyclopium</i> , <i>Tritirachium album</i>	Wall paintings of the church of Precursor in Yaroslav and the church of Assumption of the Holy Virgin in Meletovo, USSR	Rebricova (1991, 1993)

1984, 1985; Agrawal et al., 1988). Fungal growth may also result in serious alterations in the paintings through hyphal penetration or deterioration of the substrate resulting in the detachment of fragments (Giacobini and Lacerna, 1965; Ionita, 1973; Strzelczyk, 1981). According to Gargani (1968), who studied the vigorous attack of fungal growth on the surface of wall paintings in Florence after a flood, the spreading of fungal hyphae was responsible for the powdering of the weakened pigment layer. The growth of fruiting bodies of fungi like perithecia, pycnidia and stroma under the surface has been reported to be

the cause of blisters and craters (Tonolo and Giacobini, 1963; Kuritzyna, 1968). According to Kowalik (1984), who observed the fruiting bodies of fungi *Phoma* sp. and *Aureobasidium* sp. underneath the pigment layer they concluded that such a condition may result in the rupture of the pigment layer in the form of small blisters (Whiteley, 1972). According to Tonolo and Giacobini (1963), abundant sub-surface growth of fungi greatly contributes to loosening of the binding medium between the pigment and its support. Karpovich-Tate and Rebricova (1991) associated the growth of fungi with the formation of a com-



compact yellowish-white coating on the restored frescoes in the Cathedral of Pafnutii-Borovskii. The growth of fungi may also damage the different constituents of the paintings by the chemical action of its various secretions. According to Strzelczyk (1981), the extracellular enzymes, enabling the hyphae to penetrate inside the paintings, cause deterioration of all components of the object.

### 5. Mechanism of decay of wall paintings by fungal action

The decay of wall paintings by fungal action may be both physical as well as chemical in nature. Generally, both these processes occur simultaneously. Although depending upon the kind of the substrate and the fungal form as well as the

environmental conditions, any of the two processes could be predominant and the decay may proceed either from the surface to the substrate or vice versa, depending upon the localization of the fungi.

#### 5.1. Physical decay

This type of decay of the wall paintings is mainly due to the physical action of the growth of fungal hyphae or the fruiting bodies either on or below the surface. According to Wazny and Rudniewsky (1972), the fungal hyphae growing inside the paint layer caused dislodging of the various layers. A similar effect may be brought about by fungal fruiting structures, which are frequently formed under the surface of the painting layer. If the growth is over the surface, it results in the formation of filaments, which on spreading over the surface mask the colour and design of the paintings.

#### 5.2. Chemical decay

Besides physical damage to the paintings, the fungi are also responsible for chemical decay of the wall paintings through their metabolites either by assimilation or dissimilation processes. In the assimilation process, the fungi use the constituents of wall paintings as a carbon source through enzyme production, whereas in the dissimilation process, the decay is mainly by the excretion of waste products or secretion of metabolic intermediates including acids and pigments which can damage, stain or disfigure the surface. The diffusion of metabolic products between cells and the surrounding environment is facilitated rapidly on account of the high surface to volume ratio of fungi. The chemical deterioration of wall paintings on account of fungi may proceed in either of the following ways:

**Acid production.** During metabolic activity, fungi give out acids like gluconic, citric, oxalic, malic, succinic, itaconic, etc. in varying amounts (Gómez-Alarcón and de la Torre, 1994). These acids are capable of reacting with the various wall painting constituents either by solubilization of the cations or by chelation with metal ions present in the pigments. Many a time, the acids given



Fig. 3. In this wall painting at Nagaur fort (India), the fungal growth is visible in the form of black stains.



Fig. 4. The growth of fungi over the paintings is responsible for the covering of painting designs.

out by the fungi produce salts, for example, oxalic acid may react with the calcium ions to produce whewellite and weddellite minerals. The formation of salts like oxalates of calcium, iron, magnesium, etc. was also confirmed by the experiments carried out by Gómez-Alarcón and de la Torre (1994) using acidogenic fungi in conjunction with sandstone, limestone and granite.

The carbon dioxide, produced by all aerobic organisms including fungi as a result of respiration, may change in an aqueous/humid environment to carbonic acid. This carbonic acid is capable of dissolving the calcium and magnesium carbonates of the plaster layer to form calcium and magnesium bicarbonates which are readily soluble in water. In addition to direct attack on wall paintings, the production of acids favours the growth of acidophilic fungal species as secondary growth, thus hastening the decay process.

**Enzyme production.** The fungi, producing different types of enzymes, are also capable of affecting the paintings through the activity of their enzymes (O'Neil, 1986; Rebricova, 1991, 1993). The enzymes, which are protein molecules of high specific activity, perform their catalytic activity by combining with the various painting components.

The extracellular enzymes play an important role in this process. These enzymes transform complex molecules such as protein, cellulose, hemicellulose, lignin, etc. into simple ones that are water soluble. The enzymatic activity results in the loosening, cracking or falling of the paint layer. It also destroys the binder present in the paint layer, plant residues in the plaster as well as restoration materials (Rebricova, 1991, 1993). Extracellular enzymes also enable hyphae to penetrate inside the paint layer.

**Pigment formation.** As a consequence of their metabolic activity, many fungi produce organic pigments of different colours (green, grey, blue, purple, violet, etc.). These pigments, belonging to different classes of compounds like anthraquinones, xanthenes or carotenes, are characteristic of different species but the colour of the stain depends not only upon the chemical composition of the pigment but also on other factors like the composition of painting constituents, presence of other microbial species or environmental conditions. The release of pigments on the substrate or the presence of fungi containing pigments causes the appearance of different coloured stains or patches on wall paintings

(Giacobini and Lacerna, 1965; Gargani, 1968; Ionita, 1973; Bassi and Giacobini, 1973; Emoto and Emoto, 1974; Arai, 1984, 1985; Agrawal et al., 1988). Once formed, these stains are almost impossible to remove from the surface of the paintings.

## 6. Control of fungal growth on wall paintings

As mentioned earlier, the growth of fungi, which has been recognized as an important factor in the deterioration of wall paintings, especially in tropical countries, requires proper preventive and control measures for safeguarding the priceless murals. The control measures include modification of the factors which are responsible for the growth of different fungal forms and the removal of already existing fungal species.

Since moisture is the most vital factor which facilitates fungal growth, the control of biodeterioration of wall paintings could be achieved by proper ventilation of the area and by taking suitable measures to avoid moisture condensation on the surface. Such optimization of temperature and moisture was successfully carried out by Rebricova (1991, 1993) at the Cathedral of Birth of the Holy Virgin in Ferapontovo. In some cases, the use of hydrophobic materials to avoid condensation has also been suggested but in many cases the use of such materials may prove counter-productive, especially when the source of moisture is from the substrate. Air conditioning of the area has also been recommended as a step to control the environmental conditions. In order to eliminate another important source of moisture, i.e. wall humidity, use of epoxy resins as a damp proof course has also been made (Saiz-Jimenez and Samson, 1981; Rebricova, 1991, 1993). However, in many cases, the modification of environmental conditions, which requires regular monitoring, may not be easy besides being a costly proposition. Although modification of climatic conditions may be helpful in reducing further growth of fungi, one of the biggest problems faced by the conservators is to kill the already existing growth. A variety of chemicals, commonly termed biocides (fungicides), have been used for the purpose. The use of biocides for protecting

the wall paintings is considered useful if these chemicals are capable of inhibiting the fungal growth effectively without having a negative effect on the painting materials. Unfortunately, in most cases, the conventional paint fungicides do not always prove effective or they have side effects (Kaplan, 1968; Pauli, 1972). Thus, the search for a better and more suitable fungicide is still continuing.

Another problem generally encountered in the use of fungicides is the method of application without leaving areas of wall paintings untreated, as even a few fungal spores are enough to reproduce new colonies.

The following three methods are commonly used for the application of fungicides on wall paintings:

1. Injection method: This method is used to kill the fungal growth that is located below the surface or in the substrate.
2. Spray method: This method is particularly suitable for covering large areas. However, the penetration of fungicidal solution to deeper areas is difficult to ensure (Pauli, 1972).
3. Brushing method: To ensure a better contact between the affected surface and the fungicide, this method seems to be quite effective. However, the method is time consuming and requires at least two or three treatments.

Generally, the fungicides which are available on the market are aimed to be effective on plant parasites, while their activity on saprophytes such as common fungi has not been tested in most cases. Therefore, Bianchi et al. (1980) suggested that before applying a biocide on wall paintings, it should be first tested *in vitro* on fungi samples obtained from wall paintings. In fact, an ideal fungicide for use on wall paintings should possess the following characteristics (Strzelczyk, 1981): (1) It should have a high fungitoxic value so that it can be used in low concentrations; (2) It should not affect any component of the wall paintings i.e. the fastness of pigments, the binders, the glues in the ground and the plaster; (3) It should have low volatility to ensure a prolonged protective effect on wall paintings; (4) It should not be liable to ageing or associated with the formation of noxious decay products; (5) It should not lose its

fungitoxic properties by combining with constituents of the wall paintings treated; (6) It should have low toxicity to man.

The chemicals that have been used by conservation scientists for the control of fungal growth either in vitro or in situ have been reported in Table 2. However, some workers are of the view that the wall paintings should be first treated with a suitable disinfectant. But only a rather limited choice of suitable disinfectants is available. This is due to their short-term effect. According to Tonolo and Giacobini (1963), the wall paintings should be first disinfected with ethylene oxide and then kept in a nitrogen atmosphere. Saiz-Jimenez and Samson (1981) have reported that ethylene oxide and methyl bromide are effective against selected isolates of fungi in vitro. Hueck-van-der Plas (1966) recommended *p*-chloro-*m*-cresol for disinfecting paints, glues and binders. Payne (1963) found *p*-chloro-*m*-cresol and phenyl mercuric acetate to be effective only for a limited duration as disinfectant but still superior to nystatin (antibiotic used by Gargani (1968) for destroying heterotrophic microorganisms on frescoes in Florence). However, Strzelczyk (1981) recommended the use of 0.3% *p*-chloro-*m*-cresol and 0.1% phenylmercuric acetate in ethanol, for disinfecting mural paintings, based on his laboratory work.

To check the growth of fungi on wall paintings, the disinfecting effect of the fungicide is not sufficient, as a rule, for even if the cause of the growth of fungi was leakage that has been removed, the process of drying of massive guarding structures is sufficiently long. Therefore, fungicides providing protection for a long time are preferable (Rebricova, 1991, 1993). In this connection, the selection of a solvent and method of application of biocide solution is important.

Since most of the fungicides do not have a long-term effect, one of the approaches is to make use of biologically resistant conservation materials. For example, some workers have studied the fungal resistance of materials like gypsum, lime, polyvinyl acetate, carboxymethyl cellulose, casein, gelatin, egg albumin, etc. (Raschle et al., 1989; Rebricova, 1991, 1993; Karpovich-Tate and Rebricova, 1991). It was noted that lime when

present in excess increased the fungal resistance of lime plaster containing organic additives. Another method suggested for the prevention of fungi is the protection of biologically nonresistant materials (Rebricova, 1993). For example, in Poland for the protection of PVS dispersions used in the restoration of wall paintings, sterinol (QAC), tributyl tin oxide (TBTO) and phenylmercurthiomethyl were recommended (Koneczny and Strzelczyk, 1983). Similarly, in Russia natural glues such as hen's egg yolk, sturgeon glue, leather glue and synthetic glues based on vinyl acetate, organo-silicon compounds and acrylic dispersions were used for strengthening the paint layer as synthetic polymers were found to be superior to natural polymers against biological growth (Rebricova, 1991, 1993; Karpovich-Tate and Rebricova, 1991).

## 7. Methodology used for the study of fungi on wall paintings

In order to identify the various fungal forms growing over the surface and to select a proper biocide for conservation treatment, it is important to carry out scientific investigations. The methodology involves not only the identification of damage causing fungal flora but also the effect of various fungicides on these forms as well as painting materials. In addition to the fungal species growing over the surface, it is also necessary to carry out, wherever possible, quantitative analyses in order to ensure correct interpretation of results as there may be many species which though present on the surface of the wall paintings or in the environment, might be dormant and thus not responsible directly for the decay of wall paintings. According to Strzelczyk (1981), out of a wide variety of fungi observed over the wall paintings only a few develop and damage the paintings. He confirmed his findings by comparing the fungal flora identified directly under the microscope with the fungi observed on the culture media inoculated with the samples overgrown with fungi. Some workers have employed non-destructive techniques like cotton swab and paper stick methods for sample collection from murals (Agrawal et al., 1988; Caneva et al., 1991).

Table 2  
Biocides used for the control of fungi on wall paintings

No.	Biocide	Effective concentration	Reference	Remarks
1.	Bavistin (Carbendazim)	100 ppm in distilled water	Bianchi et al. (1980)	Tested in vitro, highly effective; tested in situ also; yearly treatment recommended
2.	Benlate (Benomyl)	100 ppm in 50% ethanol	Bianchi et al. (1980)	As above
3.	Catamine-A	2% soln.	Kuritzyna (1968)	Tested in vitro
4.	Cational-10	2% soln.	Kuritzyna (1968)	As above
5.	Cetyl pyridinium bromide	—	Kuritzyna (1968)	As above
6.	Cetyl pyridinium chloride	1% in ethanol or in distilled water	Kuritzyna (1968); Agrawal et al. (1991)	As above
7.	Dichlorofluanide	—	Raschle (1983)	Tested in situ
8.	Floraslan (Imazalil)	100 ppm in distilled water.	Bianchi et al. (1980)	As in No. 1
9.	Organo-tin compounds	In 70% isopropanol	Raschle (1983)	Tested in situ and found most suitable
10.	Orthophenylphenol	0.02% in ethanol or in distilled	Agrawal et al. (1991); Dhawan et al. (1991)	Tested in vitro
11.	Parachloro- <i>m</i> - -cresol	(1) 0.1–0.3% ethanol	Jeffries (1986, 1991); Hueck-van-der Plas (1966); Strzelczyk (1981)	Disinfections are of limited duration
12.	Parachloro- <i>m</i> - -cresol + Phenyl mercuric acetate	(2) 0.02% in ethanol or in distilled water 0.3% + 0.1% ethanol	Agrawal et al. (1991); Dhawan et al. (1991) Strzelczyk (1981); Jeffries (1986, 1991)	Tested in vitro  No adverse effect on murals
13.	Phenyl mercuric acetate	(1) 0.1% in alcohol	Jeffries (1986, 1991); Strzelczyk (1979, 1981)	Highly fungitoxic. No noxious effect on murals
		(2) 0.001% in alcohol	Agrawal et al. (1991)	Tested in vitro
14.	Preventol R-90 (Benzalkonium -chloride)	0.05% in ethanol or distilled water	Agrawal et al. (1991)	Tested in vitro
15.	Quaternary ammonium compounds	—	Raschle (1983); Kuritzyna (1968)	Tested in situ
16.	Quaternary ammonium compounds + organo-tin compounds	—	Raschle (1983)	Tested in situ
17.	Sodium- pentachloro phenate	0.1% in ethanol or in distilled water	Agrawal et al. (1991)	Tested in vitro
18.	Ethylene oxide	—	Saiz-Jimenez and Samson (1981)	Tested in vitro; effective
19.	Methylbromide Desogen	— 2.5 mg/cm <sup>2</sup> (aqueous spray)	As above Sorlini et al. (1982)	As above In situ spray, highly effective, colourless

Table 2 (Continued)

No.	Biocide	Effective concentration	Reference	Remarks
20.	Organo-tin compound and Methacid	—	Rebricova (1988)	Field trials directly on lower painting layers. Effective up to 2 years
21.	Preventol R-80	1% aqueous solution	Nugari et al. (1991)	Tested in situ on previously mechanically cleaned patches
	Vancide-51	As above	As above	As above
	TBTO (Tributyl-tin oxide)	As above	As above	As above
	Preventol R-80 + TBTO (1:1)	As above	As above	As above
22.	Sodium penta-chlorophenate	1%	Agrawal et al. (1991); Garg et al. (1991); Dhawan et al. (1992)	Tested on replicas. Effective up to 2.5 years
	Phenylmercuric acetate	0.1%	As above	Tested on replicas. Effective up to 1 year
	Zinc DDC	1%	As above	Tested on replicas. Causes whitening and effective up to 1 year
	<i>p</i> -Chloro- <i>m</i> cresol	0.5%	As above	Tested on replicas. Effective only up to 1 month
	Preventol R-90	0.5%	As above	As above
	O-Phenyl phenol	10.5%	As above	As above

The collection of samples, a prerequisite for any type of investigation, varies with the nature of the deterioration, e.g. superficial powder, crusts, flakes, etc. The samples are collected with the help of sterilized tools (scalpels, brushes, swabs, paper sticks, etc.) in sterilized cases. Where it is not possible to analyze the samples immediately, these are preserved at 4°C until such time as they can be analysed in the laboratory. Many workers (Gargani, 1968; Agrawal et al., 1988) have proposed the sampling of fungal growth directly from the wall paintings with the help of sticky tape. The sticky tape directly removes the powdered paint together with fungal fruiting bodies. In this way, direct identification of fungi becomes much easier. These samples are then cultured in the laboratory for further investigations with the help of either a stereomicroscope, transmission electron microscope or scanning electron microscope (Gargani, 1968; Bassi and Giacobini, 1973; Bianchi et al., 1980; Strzelczyk, 1981; Saiz-Jimenez and Samson, 1981; Rebricova, 1984, 1988; Karpovich-Tate and Rebricova, 1991; Giacobini et al.,

1991). However, in order to avoid the discrepancy as a result of the presence of dormant fungal species, many workers consider the method of direct microscopic examination of fungal flora as a better choice.

To carry out a quantitative estimation of fungal contamination of the wall paintings, use is made of a number of quick tests which make it possible to obtain reliable results. For example, fungal contamination is determined by measuring the amount of adenosine triphosphate (ATP) and components of the cellular wall and membrane lipids and also by the activity of the glycolysis ferments. In order to detect viable and metabolically active cells, use is made of fluorescent dyes as well as the indirect immunofluorescence method, scanning electron microscopy and methods based on the variation of electrical resistance of the substrate in the development of fungi (McCarthy, 1989). But these methods are used infrequently as they require a relatively large amount of sample from the priceless wall paintings.

After the identification of fungal flora present

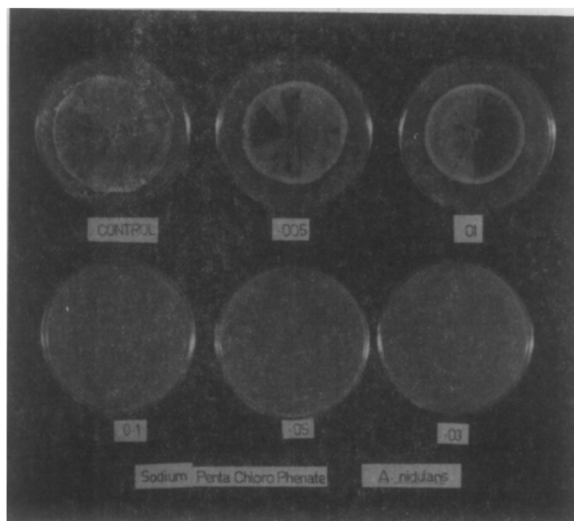


Fig. 5. In vitro testing of the efficacy of fungicide is an important step before carrying out actual treatment.

on the wall paintings, the biocidal testing of fungicides recommended for use on wall paintings is carried out in vitro on fungal samples collected from the site. For the purpose, the fungal spores are grown on Czapek-Dox medium mixed with varying concentrations of the biocide under test and the efficacy determined by observing the fungal growth (Fig. 5) (Kuritzyna, 1968; Bianchi et al., 1980; Agrawal et al., 1991; Dhawan et al., 1991; Garg et al., 1991; Garg and Dhawan, 1994). The fungicide found suitable is then tested on a small portion of wall painting (in situ testing). Some workers have recommended the use of replicas also for biocidal testing after in vitro testing (Garg et al., 1991; Dhawan et al., 1992).

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