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Diversity of Deterioration Causing Fungi at Kamala Raja Post Graduate Autonomous College, Gwalior (M.P.)

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Abstract:

The main aim of this research was to determine the diversity of microorganism on the deteriorated stone under pollution and cold climatic condition. Study is done in the month of January to mid February, in Govt. Kamala raja Post graduate autonomous college. Studies have been carried out on specimens of Main building, Art department, Hindi department, Urdu department aged from 75 years. The result showed that vegetative and reproductive (generative) forms of fungus could develop during the winter months when the day temperature was 18°C and night temperature was 2-3°C. Also the reproductive forms had developed and the whole stone surface was covered with a biofilm caused by microrganism. Various types of pollutants were present on the college building. 12 species of fungi were identified on the deteriorated stone surface even in the coldest month, and identified by staining and morphological appearance. During study it was seen that Aspergillus niger was dominant among all species.

Key words: Biodeterioration, Stone, Pollution, Biofilm, Fungi.

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1. Introduction

KRG college wall, which is made up of stone are continuously affected by physical, chemical and biological agents. The problem of understanding the deterioration of stone is compounded by the large range of types with different mineralogical and physical characteristics and their varying weathering responses under different climatic and environmental condition (Kumar and Kumar, 1999). Microorganisms play major role in destruction of stone. Biodeterioration may be defined as any undesirable change in the properties of a material caused by the vital activities of living organism. The development of specific species on a particular stone surface is determined bythe nature and properties of the stone (mineral constituents, pH, and relative percentage of various minerals, salinity, and moisture content texture). It also depend on certain environmental factors (temperature, relative humidity, light condition, atmospheric pollution level, wind, rainfall).

In other words, the response of living organism to a potentially colonizable surface depends on ecological and physiological requirements of the biological species involved (Kumar and Kumar, 1999). Along with chemical and physical factors, microbial growth plays an important role in deterioration process (Suihko et. al., 2007). The stone ecosystem is subject to harsh environmental changes, especially due to temperature and moisture, exerting extreme selective pressure on any developing microbial community (Warscheid TH & Braams, 2000, May E., 2003).

Mankind contributes to the biodeterioration of exposed stone material as a destroyer of natural balance. Air pollution resulting from anthropogenic sources such as electric utilities, domestic heating and transportation has increased the atmospheric pollution and their deposition on stone surface. The complex physical and chemical interactions of atmospheric

Pollutants with mineral material have accelerated the decay of stones (Warscheid and Braam, 2000, Zanardini et. al., 2000, Tecer and cerit , 2002). Micro biological deterioration, caused by microorganism on the material surfaces, follows the chemical deterioration (Zanardini et. al., 2000). Among the biological agents, microrganism are responsible for cultural heritage (Bock E & Sand W, 1993, Ciferri O & Griffin PS, 1999, Indictor N & Koestler RJ, 1991)

Biodeterioration has usually been considered to be a degradation process following the initial deteriorating effects of inorganic agents. These agents were thought to condition stone surfaces for microbial contamination due to structural changes and the enrichment of organic and inorganic nutrients substrate. The microorganism present on stone wall of building are fungi, bacteria (including actinomycetes), algae (including cyanobacteria), lichens and protozoa (Warscheid and Braams, 2000, Zanardini et. al., 2000, Thomaselli et. al., 2000).

All fungi need some organic source for their growth, which is provided by metabolites of phototropic organisms or airborne deposition. It has been shown that very low nutrient requirement of some rock inhabiting fungi may be fulfilled by remain of polluted air, acid rain or animals remain and secretion (Hoffland E., 2004).The present study was initiated to isolate diversity of deterioration causing fungi for further research. Biodeterioration mechanism is complex and not yet absolutely understood.

Until recently, biodeteriorative colonizers of the stone materials were considered to be autotrophic microorganism, capable of surviving on inorganic substrates, because of their capacity to utilize Carbon di oxide ,and it was considered that the heterotrophic could colonize the stone substrate only later, in accordance to ecological succession utilizing the organic substance released by cellular lysis of autotrophic (Zanardini et. al., 2000). The recent researches shows that numerous heterotrophic microorganism present on the stone work can

utilize for their growth, the airborne organic compound settled on the stone surface. Fungi are among the most harmful organisms associated to biodeterioration of organic and inorganic materials. Their occurrence on the stones is reported to be combined not only with aesthetical spoiling of the monuments, due to color changes and black spots, but also with a strong evidence that these organisms are causing crater shaped lesions, chipping and exfoliation of the rock surface combined with the loss of materials (Urzi et. al., 1995, Urzi et. al., 2000b). They mainly arise from the incomplete combustion of fossil fuel (Saiz Jimenez, 1997, Zanardini et. al., 2000). This evidence indicates that heterotrophic microorganism can act as first colonizers in the areas with a high level of organic pollutants (Zanardini et. al., 2000). The present study was initiated to isolate different variety of deterioration causing fungi which is useful for further research.

2. Material and methods

2.1 Characteristics of Kamala raja post graduate autonomous college

KRG college is situated in Gwalior (Madhya Pradesh) surrounded by densely populated region. This was established in 1937. Around 8000 girls study here. Height of main building of college is around 50-60-feet. Since college is located in densely populated area, so the concentration of pollutants around college is very high.

2.2 Sample collection

Deteriorated stone sample were collected from 4 different locations in KRG college namely Main building, Art department, Hindi department and Urdu department. They were put into petriplates in January to prevent contamination from soil and ground. Sterile devices were used in sampling. During the sampling procedure day temperature was 18°C and

night temperature was 2-3°C. Two different type of specimen were taken from the stone surface of all 4 different locations in the month of January. The first specimen was taken from the stone surface of all the documents by means of scrapping from the stone surface. These samples were used for microbiological incubation. 1gm of each sample is collected in a 50 flask with 10ml sterile physiological solution (9g/l Nacl) mixed for 15min and then serially diluted (10^{-2},10^{-3},……,etc.) (Vero et. al., 1975, greenberg et. al., 1985). The second specimen was collected by air exposure of open poured plates for 10 min in all 4 places.

2.3 Isolation of Fungi

A dilution plate technique and direct exposure of poured media in air for 10 min is used for the isolation of fungi. 100µl portion of the suspension were inoculated on plates containing potato dextrose agar (20% potatao, 2% dextrose and 15% agar w/v) and sabouraud dextrose agar (peptone 10gm, glusose 20gm and agar 15gm).In air exposure method plates which contain PDA or SDA are directly exposed in air for 10 minute cultures were incubated at 28°C for 4-6 days and examined regularly. As soon as colonies appeared, they are subculture on PDA and SDA and incubated. Steps are repeated until pure colonies different fungus is obtained. The isolates were identified morphologically and microscopically following staining with lacto phenol Cotton blue. The identified strains were maintained on PDA slants at low temperature (4-5°C). (Agarwal GP & Hasija SK, 1981)


KRG COLLEGE


KRG COLLEGE MAIN BUILDI

Fig.1.Different deteriorating site of KRG College

3. Results

In the present 12 fungal species isolated from KRG college in the month of January to mid of february.12 different fungus species belongs to 7 genera, out of which the growth of *Aspergillus sp*. are highest. The isolated fungal species are:- *Alternaria sp., Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Candida albicans, Fusarium oxysporum, Penicillium crysogenum, Penicillum digitatum, Rhizopus nigricans, Rhizopus oryzae, Rhizopus stolonifer, Rhodutorula sp.*

TABLE 1 :- Fungi isolated from deteriorated stone material of KRG College

S. No.	Name of species	Main Building		Art Department		Hindi Department		Urdu Department	
		Wall	Air	Wall	Air	Wall	Air	Wall	Air
1	*Alternaria sp.*	+	-	-	+	-	-	-	-
2	*Aspergillus flavus*	+	+	+	+	+	-	+	-
3	*Aspergillus fumigatus*	+	-	-	+	-	-	+	+
4	*Aspergillus niger*	+	+	+	+	+	+	+	+
5	*Candida albicans*	-	+	-	-	+	-	-	-
6	*Fusarium oxysporum*	+	+	+	+	+	+	+	-
7	*Penicillium crysogenum*	+	+	+	-	+	+	+	-
8	*Penicillium digitatum*	+	-	+	-	-	-	-	-
9	*Rhizopus nigricans*	-	-	-	-	+	+	+	-
10	*Rhizopus oryzae*	+	+	+	+	+	-	+	-
11	*Rhizopus stolonifer*	+	+	+	-	+	+	-	-
12	*Rhodutorula sp.*	+	+	-	-	-	-	-	-

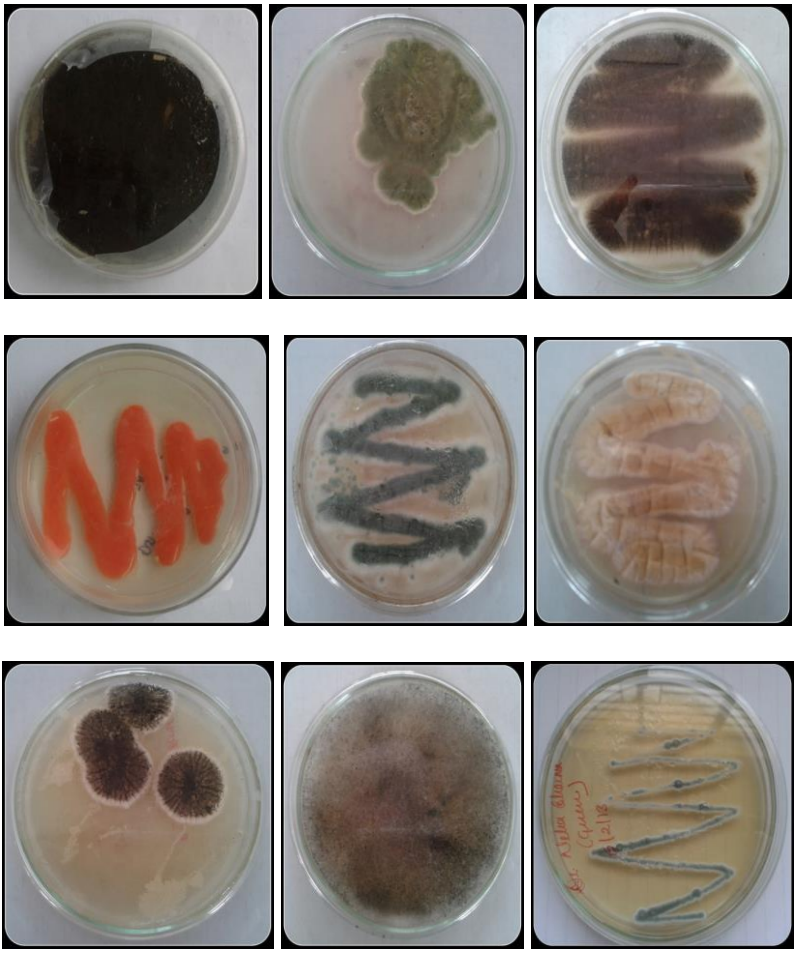

Fig. 2. Variety of isolated fungi from different deteriorating site of KRG College.

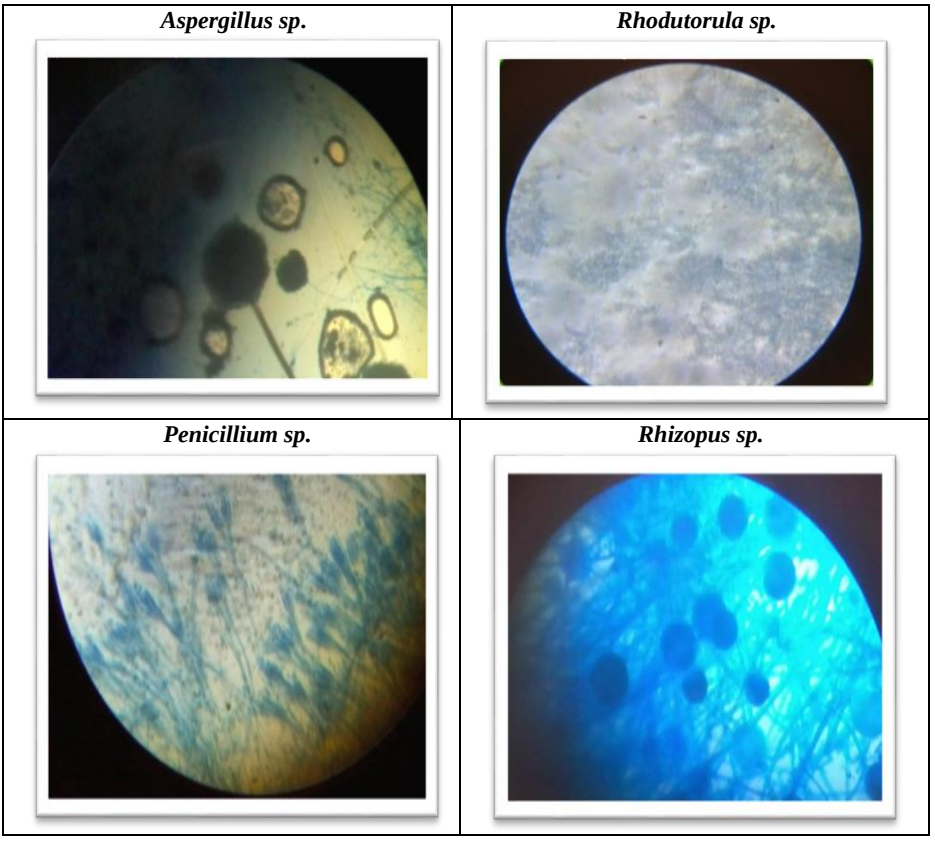

Fig.3. various types of fungi under microscope

3. Discussion

The biodeterioration of ancient buildings and monuments depend upon many factors which includes environmental factors like light, moisture, weather, temperature etc., type of micro organism that is its potential towards the colonization in the surrounding environments, materials of the monuments etc. All these equally contribute the biodeterioration of any monuments (Pocho J. & Jaton C., 1968).

The identified micro fungi cause discoloration as well as

mechanical exfoliation of stone material that was analyzed through mechanical hyphae penetration and production of different pigments (*Alternaria sp.*) and organic acids (some species of genus *Aspergillus* sp., *Alternaria* sp., and *Penicillium* sp.) (Haselwandter K. Mycorrhizal fungi, Silver man MP et. al., 1970) reported that a large number of fungi have great biochemical decay potential. The black fungi such as *Alternaria* have the capacity to settle on the surface of rocks, to attach firmly and to penetrate deeper into the rock. The major way of attack and penetration of this group of rock infecting fungi is physical (Diakumaku et. al., 1995). Acidogenic fungi such as *Aspergillus, Penicillium* produce different organic acids, such as oxalic, gluconic, malic, citric, etc., in low and normal glucose concentrations (De la torre et. al., 1991). These acids form calcium salts or act as chelating agents of mineral cations, favouring the biodeterioration process (Berthelin, 1983). *Aspergillus* species were among dominant microfungi on the mineral substrate (A., Simonoviaova et.al., 2004).

Recently, it has been apparent that the ability of fungi to interact with minerals, metals, metalloids and organic compounds through biomechanical and biochemical processes, makes them ideally suited as biological weathering agents of rock and building stone. Biological and mycological investigations are a very important part of good conservation and cannot be ignored in the modern conservation concept, which includes close collaboration between art and science. This collaboration is the comparative study of the role of microbial colonization on the degradation of historic monuments (Burford P.E. et. al., 2003, Benjamin Otto Ortega-Morales, 2006)

4. Conclusion

KRG College, which is one of the oldest college, whose main building made up of stone are subjected to damage by fungi. Fungi are mainly responsible for deterioration walls of KRG

College. Since these fungi play a major role in nutrientrecycling through biodeterioration, so these fungi cannot be ignored. The possible outcome of this study is that valuable information about the different variety of fungi involved in the deterioration of KRG College will be obtained.

REFERENCES

Agarwal G.P. and Hasija S.K. 1981. *Microorganisms in the laboratory: A laboratory guide for microbiology, mycology and plant pathology.* Print House Lucknow, India.

Berthelin, J. 1983. "Microbial weathering processes." In *Microbial. Geochemistry*, edited by W. E. Krumbein, 223-262. Oxford: Blackwell Scientific Publications.

Bock, E., Sand W. 1993. "The microbiology of masonry biodeterioration." *J. Appl. Bacteriol*. 74: 503-514.

Burford, P.E., Fomina, M., and Gadd, G.M. 2003. "Fungal involvement in bio weathering and biotransformation of rocks and minerals." *Mineralogical Magazine* 67: 1127-1155.

Ciferri, O. 1999. "Microbial degradation of paintings." *Appl. Environ. Microbiol.* 65: 879-885.

De la Torre, M. A., Gomez-Alarcon, G., Melgarejo, P., Saiz-Jimenez, C. 1991. "Fungi in weathered sandstone from Salamanca cathedral (Spain)." *Science of the Total Environment* 107: 159-168.

Greenberg, A.E., Trussell, R.H., Clesceri, L.S. 1985. *Standard methods for the examination of water and wastewater*. Sixteenth edition. Washington American Public Health Association. p. 860-864, 864-866 and 981–984.

Griffin, P.S., Indictor, N., Koestler, R.J. 1991. "The biodeterioration of stone: A review of deterioration

mechanisms, conservation case histories, and treatment." *Int. Biodeterior*. 28: 187-207.

Haselwandter, K. 1995. "Mycorrhizal fungi: siderophore production." *Crit. Rev. Biotechnol.* 15: 287-291.

Hoffland, E. 2004. "The role of fungi in weathering." *Front. Ecol. Environ*. 2: 258-264.

Kumar, R., Kumar, A.V. 1999. In *Biodeterioration of stone in tropical environments*, edited by Agnew Neville, 1 –2. USA7 J. Paul Getty Trust; (0-89236-550-1).

May, E., Lewis, F.J., Pereira, S., Tayler, S., Seaward, M.R.D., Allsopp, D. 1993. "Microbial deterioration of Building stone: A review." *Biodeterioration Abstracts* 7: 109-123.

Ortega-Morales, Benjamin Otto. 2006. "Cyanobacterial diversity and ecology on historic monuments in Latin America." *Micobiologia* 48: 188-195.

Pocho, J. and Jaton, C. 1968. "Biological factors in the alteration of stone." In *Biodeterioration of Materials*, edited by C. Wolters and C. Elphick, 258-268. Elsevier, Amsterdam.

Saiz-Jimenez, C. 1997. "Biodeterioration vs. biodeterioration: the role of microorganisms in the removal of pollutants deposited onto historic buildings." *Int. Biodeterior. Biodegrad*. 40: 225- 32.

Silverman, M.P., Munoz, E.F. 1970. "Fungal attack on rock: Solubilization and altered infrared spectra." *Science* 169: 985-87.

Simonoviaova, A. et al. 2004. "Airborne and soil microfungi as contaminants of stone in a hypogean cemetery." *Int. Biodeterioration Biodegrad*. 54: 7-11.

Suihko, L.M., Alakomi, L.H., Gorbushina, A.A., Fortune, I. and Marquardt, S.M. 2007. "Characterization of aerobic bacterial and fungal microbiota on surfaces of historic Scottish monuments." *Syst. Appl. Microbiol*. 30: 494-508.

Tecer, L. and Cerit, O. 2002. "The effects of air pollution on carbonate stone monuments in urban areas (Sivas,Turkey)." *Fresenius. Environ. Bull.* 11: 505-9.

Tomaselli, L., Lementi, G., Bosco, M., and Tiano, P. 2000. "Biodiversity of photosynthetic micro-organisms dwelling on stone monuments." *Int. Biodeterior. Biodegrad.* 6: 251 – 8.

Urzì, C., Wollenzien, U., Criseo, G., and Krumbein, W. E. 1995. "Biodiversity of the rock inhabiting microflora with special reference to black fungi and black yeasts." In *Microbial Diversity and Ecosystem Function*, edited by Allsopp D., Colwell R. R., Hawksworth D. L., 289-302. Wallingford: CAB International.

Urzì, C., De Leo, F., de Hoog, S., and Sterflinger, K. 2000b. "Recent advances in the molecular biology and ecophysiology of meristematic stone-inhabiting fungi." In *Of Microbes and Art. The role of microbial communities in the degradation and protection of cultural heritage,* edited by Ciferri O., Tiano P., Mastromei G., 3-19. New York: Kluwer Academic.

Vero, L.B., Bianchi, R., Sila, M.M., and Tiano, P. 1975. "Proposal of a method of investigation for the study of the presence of bacteria in exposed works of art in stone." Conservation of stone I International symposium, June 19-21, Bologna; p. 257–66.

Warscheid, T.H., Braams, J. 2000. "Biodeterioration of stone: A review." *Int. Biodeterioration. Biodegrad*. 46: 343-368.

Zanardini, E., Abbruscato, P., Ghedini, N., Realini, M., and Sorlini, C. 2000. "Influence of atmospheric pollutants on biodeterioration of stone." *Int. Biodeterior. Biodegrad.* 45: 35- 42.