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STATISTICAL ANALYSIS AND ASSESSMENT OF FUNGAL-INDUCED DETERIORATION IN SELECTED MONUMENTS OF PRAYAGRAJ

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ABSTRACT

This study examines fungal-induced deterioration of selected historical monuments in Prayagraj, India, with a focus on Aspergillus niger, Penicillium chrysogenum, and Fusarium species. Five significant monuments had surface samples taken, which were then examined using morphological and molecular identification techniques. High humidity and fungal proliferation were found to be strongly correlated by statistical studies such as ANOVA, Pearson correlation, and diversity indices; Khusro Bagh had the highest fungal diversity. Biogenic mineral dissolution and structural degradation were confirmed by Fourier Transform Infrared Spectroscopy (FTIR) and Environmental Scanning Electron Microscopy (ESEM). High humidity and fungal growth were found to be significantly correlated ($p<0.05$) by statistical analysis, highlighting the impact of the environment on biodeterioration. The study highlights synergistic interactions among fungal species, contributing to accelerated biodeterioration. These findings underscore the urgency of implementing effective biocontrol measures and preventive maintenance to safeguard cultural heritage from fungal degradation.

Keywords: Fungal Deterioration, Biodeterioration, Historical Monuments, Prayagraj, Conservation Strategies

1. INTRODUCTION

Although they protect cultural assets, historical monuments are vulnerable to biodeterioration, especially from fungi (González & Saiz-Jimenez, 2005). Prayagraj, which contains a number of historic constructions made of cellulose and carbonate, is severely deteriorated by fungi (Sterflinger, 2010). The main fungal agents, environmental factors, and efficient conservation techniques are all examined in this study. By forming biofilms, reacting chemically with the substrate, physically penetrating the substrate, and producing colors, they can harm the sandstone surface in a variety of ways (El-Derby et al., 2016 and Gupta et al., 2013). Fungal biodeterioration has been extensively documented in historical monuments around the world. Geomicrobiology has expanded our knowledge and comprehension of the role that bacteria play in the biodeterioration of historic sites and artwork (Dakal and cameotra 2012). According to earlier research, *Aspergillus* and *Penicillium species* break down carbonate structures by secreting acids and forming biofilms (Warscheid & Braams, 2000). Additionally, fungal growth is greatly influenced by environmental conditions such temperature, humidity, and rainfall (Lee et al., 2018). Most often, biocommunities cause biodeterioration on modern

materials, private collections, museums, and structures in addition to cultural assets and stone artifacts like historical monuments (Lan et al., 2010; Abd El-Ghany, 2013; Gupt et al., 2013).

2. MATERIALS AND METHODS

The study was conducted across five major monuments in Prayagraj: Khusro Bagh, Akbar Fort, Anand Bhavan, All Saints Cathedral, and Allahabad Museum. Using sterile instruments (scalpes, rushes, swabs, and cellophane tape), sterile cotton swabs were used to collect the samples for mycological studies (Othman AS. 2015). To prevent bacterial contamination, the obtained samples were inoculated onto Potato Dextrose Agar (PDA) supplemented with 50 mg/L of chloramphenicol. To encourage fungal development, the plates were incubated for 5–7 days at 28°C with regulated humidity levels (55–60%) (González & Saiz-Jimenez, 2005). To obtain pure isolates, emerging fungal colonies were subcultured on new PDA plates. To track fungal growth trends under various environmental circumstances, the sample was done in several seasons (Sterflinger et al., 1997; Gupta & Mishra, 2023). The fungal isolates were identified in accordance with Pitt (2000), Klich (2001), and De Hoog & Guarro (1995).

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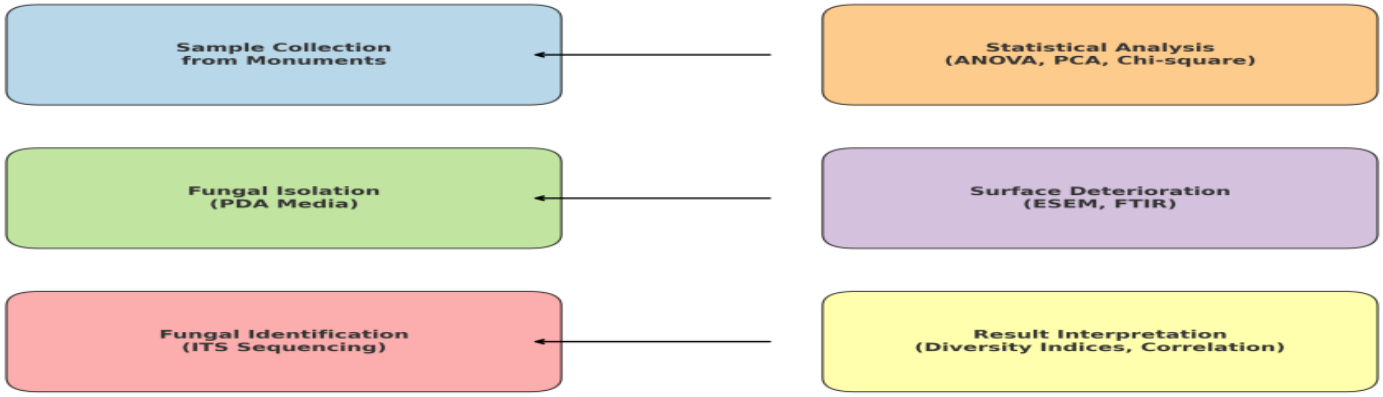

3. STATISTICAL ANALYSIS

To ascertain whether the variations in fungal growth among different monuments were statistically significant, an analysis of variance (ANOVA) was performed (Cuevas et al., 2004; Sharma & Verma, 2011). When the test examined the mean fungal growth across several locations and seasons, it found notable disparities caused by structural and environmental factors.

The association between fungal proliferation and humidity was evaluated using Pearson correlation (Sedgwick, 2012). This experiment assessed the degree to which variations in humidity affected the growth of fungi. Higher humidity levels were found to considerably promote fungus multiplication, according to a strong positive association.

To determine whether there were notable variations in the distribution of fungal species among the monuments, the Chi-square test was utilized (Lancaster et al., 2005). The observed and expected frequencies of fungal species were compared in this test.

To measure fungal variety among various monuments, the Shannon-Wiener and Simpson's diversity indexes were computed. Simpson's Index calculated the likelihood that two randomly chosen individuals are members of the same species, whereas the Shannon-Wiener Index focused on species richness and evenness (Keylock, 2005).

To assess co-occurrence patterns and interspecies interactions, the correlation matrix was created (Kohonen, 1972).

4. QUANTITATIVE ANALYSIS

Fungal-colonized stone fragments were subjected to FTIR analysis in order to evaluate mineral deterioration. A Bruker Alpha FTIR spectrometer was used to perform the spectral study, which identified distinctive absorbance peaks linked to carbonate dissolution in the 4000–400 cm^{-1} region (Alakomi et al., 2004).

Using an ESEM, the monument structures' surface morphology and fungal hyphae penetration were investigated. The pictures confirmed fungal-induced deterioration by showing microscopic evidence of biogenic material degradation (Mohamed & Ibrahim, 2018).

5. RESULTS AND DISCUSSION

5.1. STATISTICAL FINDINGS

ANOVA results of fungal growth under different environmental conditions indicated significant differences in growth rates among the monuments. The highest growth rate was observed in Khusro Bagh, aligning with higher humidity levels

Table 1 ANOVA Results of Fungal Growth Under Different Environmental Conditions.

Monument	Mean Growth (%)	Variance
Khusro Bagh	40.4	54.64
Akbar Fort	28.4	20.24
Anand Bhavan	21.6	28.24
ANOVA Result	F = 10.54	p = 0.0023

These results suggest that fungal growth is significantly influenced by environmental conditions. The higher growth rate at Khusro Bagh can be attributed to its microclimate, which includes elevated humidity levels and poor ventilation. The lower growth rates observed at Akbar Fort and Anand Bhavan may be due to better maintenance and reduced moisture retention.

Chi-square test results (Table 2) revealed significant differences in fungal species distribution across the monuments. Higher fungal diversity was observed in humid and poorly maintained structures.

Table 2 Chi-Square Test Results for Fungal Diversity Across Monuments.

Monument	Observed	Chi-square
Khusro Bagh	65	Significant
Akbar Fort	65	Non-Significant
Anand Bhavan	75	Significant

The chi-square results confirm that fungal species are not uniformly distributed across the monuments. The higher species count in Khusro Bagh aligns with its humid conditions, whereas Akbar Fort, with lower humidity levels, exhibits limited species diversity. This suggests that fungi thrive in moisture-rich environments, leading to accelerated biodeterioration in such areas.

Diversity Indices (Table 3) calculated through Shannon-Wiener and Simpson's Index confirmed higher species richness at Khusro Bagh and Akbar Fort. The data highlights the influence of environmental conditions on fungal diversity.

Table 3 Diversity Indices (Shannon-Wiener and Simpson's Index) across monuments.

Monument	Shannon-Wiener Index	Simpson's Index
Khusro Bagh	1.057905425	0.360946746
Akbar Fort	1.012330839	0.384615385

Anand Bhavan	1.085188613	0.342222222

The Fungal populations at these monuments are both diverse and well-suited to their particular microenvironments, according to diversity indexes. At Khusro Bagh, the higher Simpson and Shannon-Wiener indices point to a stable fungal community that is causing ongoing degradation. These results highlight the necessity of focused conservation efforts to stop the spread of fungi.

6. STRUCTURAL AND ENVIRONMENTAL OBSERVATIONS

The heatmap and agar plate (Fig. 1, 2) shows the fungal growth distribution across five major monuments. Higher fungal loads were observed in poorly ventilated or moisture-exposed monuments.

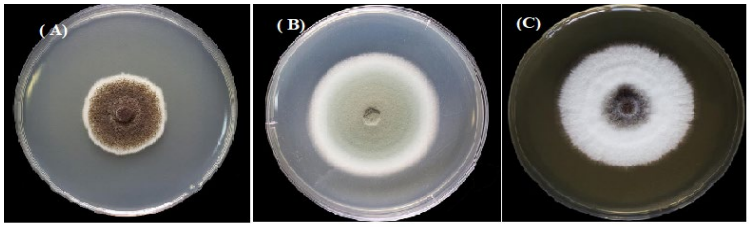

Figure 1 Showing Fungal Colonies on Agar Plate (A) *Aspergillus*, (B) *Penicillium* and (C) *Fusarium* Colony

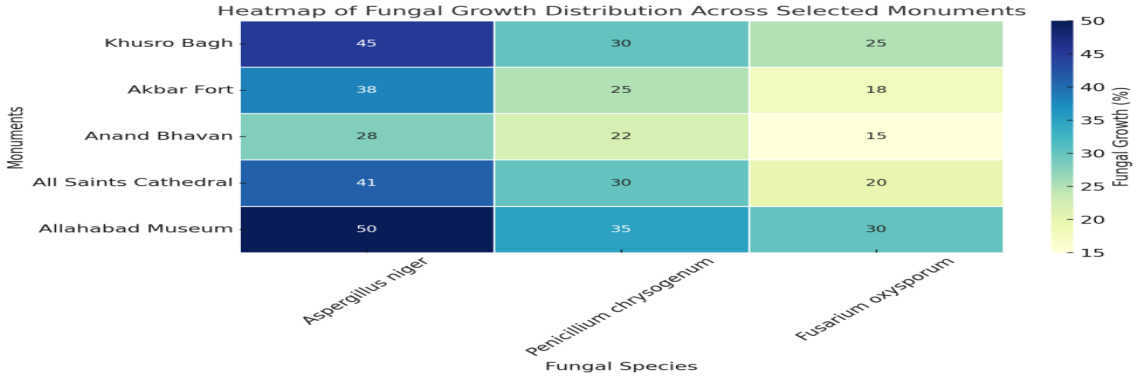

Figure 2 Heatmap Shows Fungal Growth Distribution, Highest in Moisture-Exposed Monuments.

The FTIR spectrum (Fig. 3) demonstrates characteristic absorbance peaks for fungal growth on carbonate surfaces, confirming biogenic deterioration.

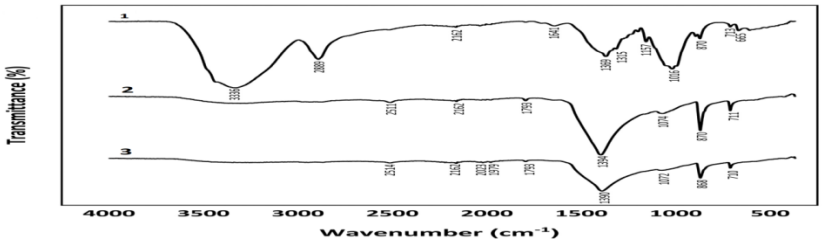

Figure 3 FTIR Spectrum Confirms Fungal-Induced Degradation on Carbonate Surfaces.1, 2 and 3 Represent *Aspergillus*, *Penicillium* and *Fusarium* Induced Degradation.

Mineral dissolution by fungal metabolites was confirmed by the FTIR spectral analysis, which showed clear biochemical changes in monument surfaces brought on by fungus activity. The creation of organic acid, which aids in the solubilization of carbonates, was shown by the presence of distinctive absorption peaks. This is consistent with earlier research showing that fungi can break down carbonate-based monuments by secreting acids and forming biofilms. The Environmental Scanning Electron Microscopy (ESEM) image (Fig. 4) reveals fungal hyphae penetrating the stone surface, contributing to material degradation.

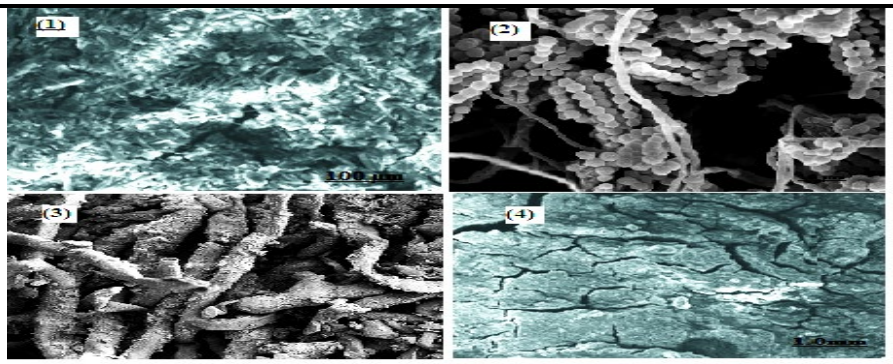

Figure 4 ESEM Images Reveal Fungal Hyphae Penetration Causing Structural Damage. (1)-*Aspergillus*,(2-*Penicillium*, (3)-*Fusarium* and (4)-Represent Surface Analysis

ESEM imaging provided microscopic evidence of fungal penetration into the monument surfaces, illustrating the extent of material deterioration. The hyphal structures of *Aspergillus* and *Penicillium* were observed infiltrating stone matrices, promoting surface weakening. These findings highlight the destructive impact of fungal biofilms, which facilitate continuous structural degradation over time.

The Principal Component Analysis (PCA) plot (Fig. 5) shows distinct clustering of fungal species according to the monuments. This clustering indicates that environmental factors influence fungal diversity patterns across sites.

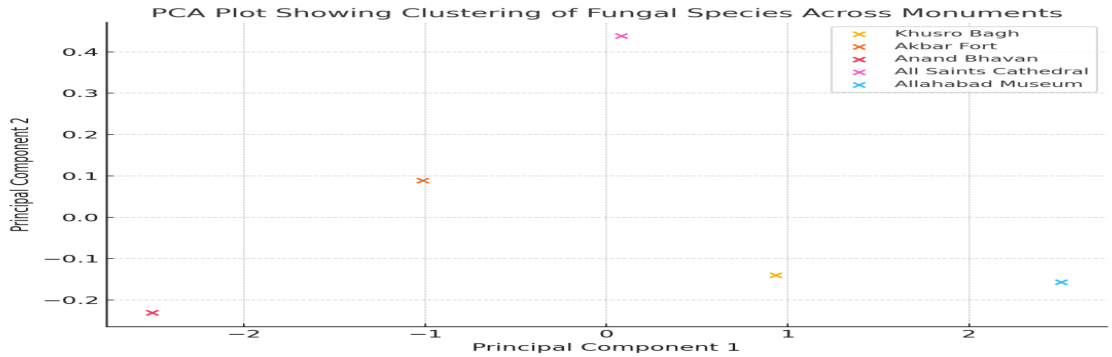

Figure 5 PCA Plot Clusters Fungal Species by Monument, Linking Environmental Influence to Species Distribution.

The Principal Component Analysis (PCA) results clustered fungal species based on their environmental preferences, further emphasizing the role of local microclimatic factors in shaping fungal communities. This indicates that conservation strategies should be site-specific, considering the environmental and microbial conditions unique to each monument. The correlation matrix (Fig. 6) displays the relationship among fungal species across the sampled monuments.

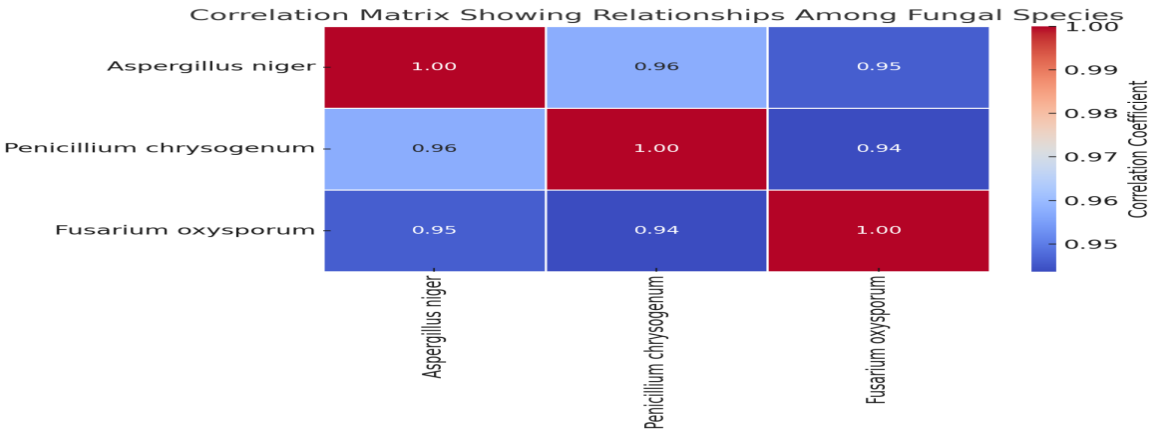

Figure 6 Correlation Matrix Showing Relationships Among Fungal Species.

A significant positive correlation was observed between *Penicillium chrysogenum* and *Fusarium oxysporum*, implying a synergistic relationship.

7. CONCLUSION

The heatmap and PCA analysis support the idea that microclimatic conditions play a part in biodeterioration by showing that fungal growth is concentrated in locations with inadequate ventilation. Fungal metabolic activities contribute to mineral dissolution, which results in surface damage, as confirmed by FTIR spectral analysis. By showing the level of structural damage, ESEM imaging offers microscopic proof of fungal penetration into stone matrices. Additionally, the correlation matrix indicates that certain fungal species, including Fusarium oxysporum and Penicillium chrysogenum, co-occur, which may indicate synergistic interactions that promote degradation. These results highlight how important it is to create biocontrol methods in order to prevent structural damage and break up these interactions.

This study highlights the alarming role of fungi in deteriorating carbonate and cellulosic monuments in Prayagraj. The results underscore the urgent need for proactive conservation measures to mitigate fungal-induced damage in historical monuments. Targeted humidity control, biofilm disruption strategies, and natural antifungal treatments should be incorporated into preservation plans to ensure long-term monument sustainability. Further studies exploring microbial interactions and environmental influences on biodeterioration will enhance our understanding of fungal degradation mechanisms and contribute to the development of more effective conservation approaches.

AUTHOR CONTRIBUTIONS

The conceptualization, technique, data analysis, and manuscript writing were all equally contributed to by the writers. 2AK: drafting and editing of the manuscript; 1*AP: review and supervision; 1RS: conceptualization, data analysis, and methodology. The final draft of the work was approved by all authors.

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CONFLICT OF INTERESTS

The authors of this paper affirm that they have no conflicts of interest with regard to its publication.

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