# BACTERIA AND FUNGI: THEIR ROLE IN DETERIORATION OF NAHARGARH FORT

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## Abstract

Nahargarh Fort is situated on Aravali hills and famous for sightseeing. It was built by Maharaja Sawai Jai Singh II in 1734. It is mainly composed of sandstone, lime powder and marble. In this article, discussed about bacterial and fungal diversity and deterioration of the site. Total of 188 bacterial and 145 fungal colonies were identified among them Morganella morganii and Fusarium solani most abundant one. Importance value index (IVI) of bacterial species revealed that the Morganella morganii show maximum IVI value (53.06%) and Bacillus sp. (29.01%) shows the least IVI value. And for fungal species, Importance value index (IVI) discloses Fusarium solani shows maximum IVI value (63.22%) and Aspergillus tubingensis (27.55%) shows the least IVI value. The degenerative potential of isolated bacteria and fungi with the help of FE-SEM images was also analysed. This study helps to find the culturable biodeteriogens mainly bacteria and fungi which excreted most of enzymes, acids and pigments to deteriorate the site and appearance. Identified data helps in providing a strategy for healthy environment and identify indigenous culturable micro-organisms from the ancient fort.

**Key words:** *Biodeterioration, Bacteria, Fungi, FE-SEM, Nahargarh Fort.*

## INTRODUCTION

Nahargarh Fort is situated on Aravalli hills at 26.93ºN, 75.81ºE coordinates. It is famous for sightseeing and breath-taking view of Aravalli Mountain range (Fig.1). It was built by Maharaja Sawai Jai Singh II in 1734. It has 12 identical rooms for queens. Earlier it was named as Sudarshangarh Fort but due to presence of tigers its name changed to Nahargarh fort. It is one of the major tourist attractions of Jaipur. This fort was made for the protection of city and makes a defensive wall around the city with Amer and Jaigarh fort. As it is very important economically as well as culturally, here is the need to protect and maintain this fort and its outer area.

**Fig 1: Nahargarh fort of Jaipur.**

Biological deterioration is a slow and study process. It deeply involved within material or its surface which affects the aesthetic appearance, strength and disintegration. The agents (microorganisms), who affect the biodeterioration, produce different enzymes, pigments organic and inorganic acids. The oxalic acid, carbonic acid and other acids capable of chelating ions such as calcium which were secreted by microbes induce chemical damage (Cariati et al., 2000). The decay may cause the surface to deteriorate over time gradually, leaving a sound surface intact, as well as the possibility of significant pieces of stone chipping away from the surface. The surface of cultural heritage occasionally breaks into blisters; other times, the stone loses all integrity and breaks away completely. The stone may appear to be in fine condition to the human sight, yet it has lost its cohesiveness beneath the surface. In this article, identification of bacteria and fungi which might be responsible for biodeterioration of Nahargarh fort of Jaipur. Identified bacteria and fungi helps to form a conservation plan and maintainace of the fort. Removal of these deteoriating microbial load can be easy after identifying the load and provide healthy environment for tourists.

## MATERIAL AND METHODS

### Sampling

Samples were collected from Nahargarh fort under aseptic conditions (Fig.2), without harming the site in the mid of November month. Jaipur has a semi-arid type of weather conditions. From Nahargarh fort, ten samples (Nahargarh Rani Mahal Wall, Nahargarh Jali of Rani Mahal, Nahargarh Dome of Rani Mahal, Nahargarh Dome's wall of Rani Mahal, Nahargarh Stair's Roof of Rani Mahal, Nahargarh Fort wall, Nahargarh outer wall, Nahargarh Babri wall, Nahargarh Babri Stairs, Nahargarh Jharokha Wall) were collected with the help of sterilized cotton swab (Gorbushina et al., 2004). Collected samples kept in a sterile plastic airtight pouch for its microbial analysis.

The adequate amount of sample was collected aseptically with sterilizing tools.

**Fig-2: Sample collection from Nahargarh fort.**

Bacterial and fungal identification

Swab samples were further cultured on media plates (Nutrient agar for Bacteria and Potato Dextrose Agar for Fungi) and observed morphological characteristics with the help of optical microscope. The isolated bacterium was identified with the help of Bergey's manual of systematic bacteriology (Krieg and Holt 1984). Fungal isolates were identified morphologically according to authentic literature reviews and mycological manuals (Raper and Fennell 1965; Ellis and Ellis 1997; Samson et al. 2004, 2010). Pure isolates were further characterised for molecular identification by using 16S rRNA and 18S rRNA for bacteria and Fungi respectively. By using blast tool at NCBI [National Centre for Biotechnology Information; http://www.ncbi.nih.gov/] site.

Analysis of stone sample deterioration by mixed culture isolates

An experimental set up was prepared to test the degenerative potential of dominant microbial isolates from Nahargarh fort (Yadav and Gupta, 2021). A test stone was taken from the same environment and sterilize that with the help of autoclave (Wiktor et al., 2009; Miller et al., 2008). FE-SEM pictures were also captured to document the test stone's state. With the help of an electron microscope, FE-SEM offers the surface characterization of materials and depth observation of the field at a greater resolution. Mixed inoculums were made by combining pure bacterial and fungal colonies (mainly Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Staphylococcus haemolyticus, Morganella morganii, and Fusarium solani, Aspergillus flavus, Aspergillus niger, Aspergillus fumigation, Aspergillus tubingensis, Rhizopus sp.) with

0.9% saline (0.5 McFarland). The test stone was placed in a pre-sterilized container and sterile water was sprinkled on top to keep it moist. The prepared mixed inoculums were then placed on the stone piece, and the container's lid was closed (Miller et al., 2008). This container was left in a naturalistic environment. Sprinkle sterile water on the test stone aseptically for every 24 hours. A Field emission scanning electron microscope (FE-SEM) was used to determine the test sample's degenerative potential after three years.

## RESULTS

### Isolation of Bacterial and fungal Isolates

Several bacterial and fungal colonies were isolated from ten samples, however the most common and dominating colonies found in all of the samples were processed for further research. Total of 188 bacterial and 145 fungal colonies was identified from which dominated bacterial genera were Morganella, Escherichia, Pseudomonas, Klebsiella, Bacillus, Acinetobactor, Micrococcus, Staphylococcus, Photobacterium, Enterobacter and Streptococcus and fungal genera Fusarium, Aspergillus, Botrytis, Penicillium, Rhizopus, Trichoderma, Candida, Mucor, and Cladosporium. The isolated dominant bacterial species (Fig-5) were Morganella morganii, Streptococcus sp., Pseudomonas aeruginosa, Micrococcus sp., Staphylococcus haemolyticus, Klebsiella pneumonia, Escherichia coli and Bacillus sp. and isolated species of fungi (Fig-6) were Fusarium solani, Rhizopus sp., Aspergillus niger, Aspergillus fumigation, Mucor sp., Aspergillus flavus and Aspergillus tubingensis selected on the basis of dominance.

### Molecular Characterization of the Isolates

The nucleotide sequences which were isolated from Nahargarh fort deposited in the [NCBI] GenBank. After successful submission, unique accession no. for each isolate such as MN490076 for Morganella morganii and MN493125 for Fusarium solani was provided (Table-1). And phylogenetic tree was constructed (Fig-3and Fig-4)

**Table 1: Accession number of samples sequence collected from Nahargarh Fort site.**

| SERIAL NO. | SAMPLE ID | MICROORGANISM | ACCESSION NUMBERS |
|---|---|---|---|
| 1 | NBW8 | *Morganella morganii* | MN490076 |
| 2 | NWR4 | *Fusarium solani* | MN493125 |

**Fig.3: Phylogenetic tree of Morganella morganii.**

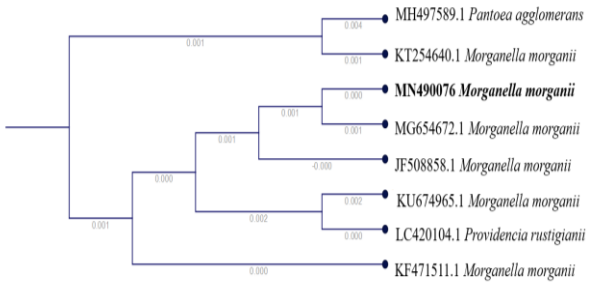



**Fig.4: Phylogenetic tree of Fusarium solani.**

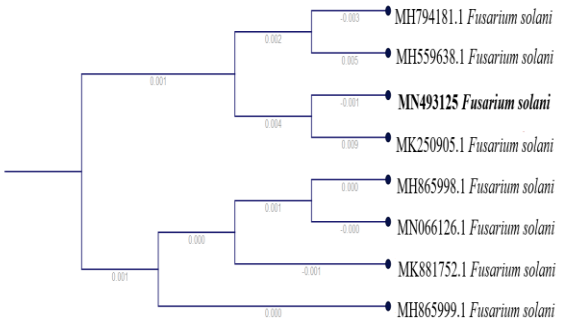


**Table2: RF, RD, RA and IVI of different Bacterial species into Nahargarh fort.**

| Isolated Bacteria | Number of Bacteria colonies | | | | | | | | | | RF % | RD % | RA % | IVI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NRW1 | NJR2 | NDR3 | NWR4 | NRR5 | NFR6 | NOW7 | NBW8 | NBS9 | NJW10 | | | | |
| *Escherichia coli* | 4 | - | 2 | - | 7 | - | 1 | - | 5 | - | 9.43 | 10.11 | 13.11 | **32.65** |
| *Micrococcus sp.* | - | 4 | - | 6 | - | 7 | - | 5 | - | - | 7.54 | 11.70 | 18.98 | **38.22** |
| *Staphylococcus haemolyticus* | 3 | - | 4 | 2 | - | 1 | 6 | - | 3 | 4 | 13.21 | 12.23 | 11.32 | **36.76** |
| *Bacillus sp.* | 5 | 2 | - | 1 | 3 | - | 4 | 1 | - | - | 11.32 | 8.51 | 9.18 | **29.01** |
| *Pseudomonas aeruginosa* | - | 6 | 4 | 2 | 1 | 3 | - | 2 | 5 | 3 | 15.1 | 13.83 | 11.22 | **40.15** |
| *Morganella morganii* | 4 | 3 | 2 | 4 | 6 | 5 | 1 | 4 | 3 | 7 | 18.86 | 20.74 | 13.46 | **53.06** |
| *Klebsiella pneumonia* | 1 | - | 8 | 1 | - | 3 | 2 | - | 4 | - | 11.32 | 16.81 | 10.91 | **36.04** |
| *Streptococcus sp.* | - | 5 | 1 | 3 | 2 | - | 3 | 6 | - | 4 | 13.20 | 18.19 | 11.81 | **43.2** |

**Fig 5: Graphical representation of bacterial species and RF/RD/RA/IVI (%).**

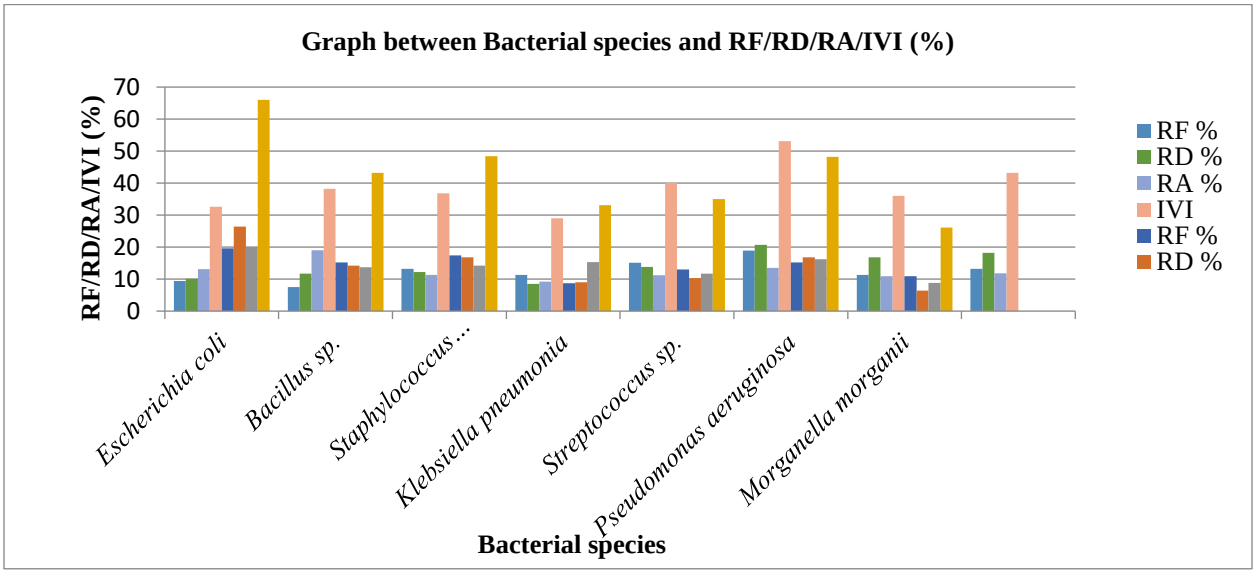
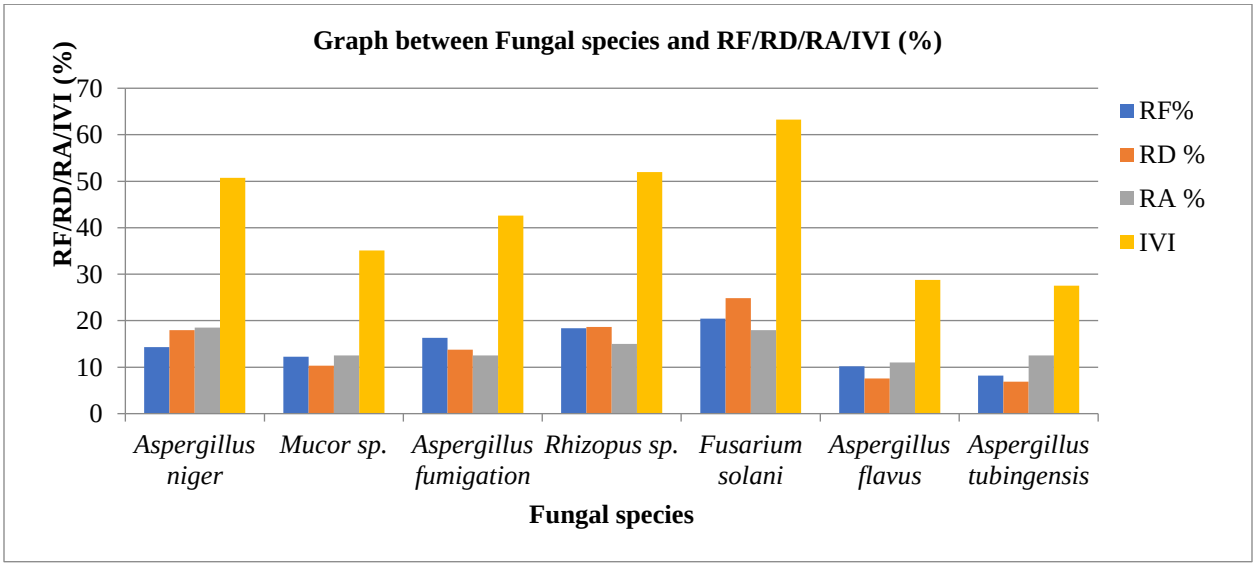


**Table3: RF, RD, RA and IVI of different fungal species into Nahargarh fort.**

| Isolated Fungi | Number of Fungal colonies | | | | | | | | | | RF % | RD % | RA % | IVI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NRW1 | NJR2 | NDR3 | NWR4 | NRR5 | NFR6 | NOW7 | NBW8 | NBS9 | NJW10 | | | | |
| *Aspergillus niger* | 3 | - | 9 | 4 | 2 | - | 1 | 3 | - | 4 | 14.28 | 17.93 | 18.54 | **50.75** |
| *Mucor sp.* | 2 | 4 | - | - | 1 | 2 | - | - | 5 | 1 | 12.24 | 10.34 | 12.49 | **35.07** |
| *Aspergillus fumigation* | 1 | 2 | 5 | 1 | - | 4 | 3 | 2 | - | 2 | 16.33 | 13.79 | 12.49 | **42.61** |
| *Rhizopus sp.* | - | 3 | 2 | 3 | 2 | 1 | 6 | 5 | 4 | 1 | 18.37 | 18.62 | 14.99 | **51.98** |
| *Fusarium solani* | 4 | 3 | 6 | 2 | 1 | 9 | 5 | 2 | 3 | 1 | 20.41 | 24.82 | 17.99 | **63.22** |
| *Aspergillus flavus* | 2 | 1 | - | - | 4 | 1 | - | 3 | - | - | 10.20 | 7.59 | 10.99 | **28.78** |
| *Aspergillus tubingensis* | 4 | - | - | 3 | - | - | 2 | - | 1 | - | 8.16 | 6.90 | 12.49 | **27.55** |

**Fig 6: Graphical representation of fungal species and RF/RD/RA/IVI (%).**

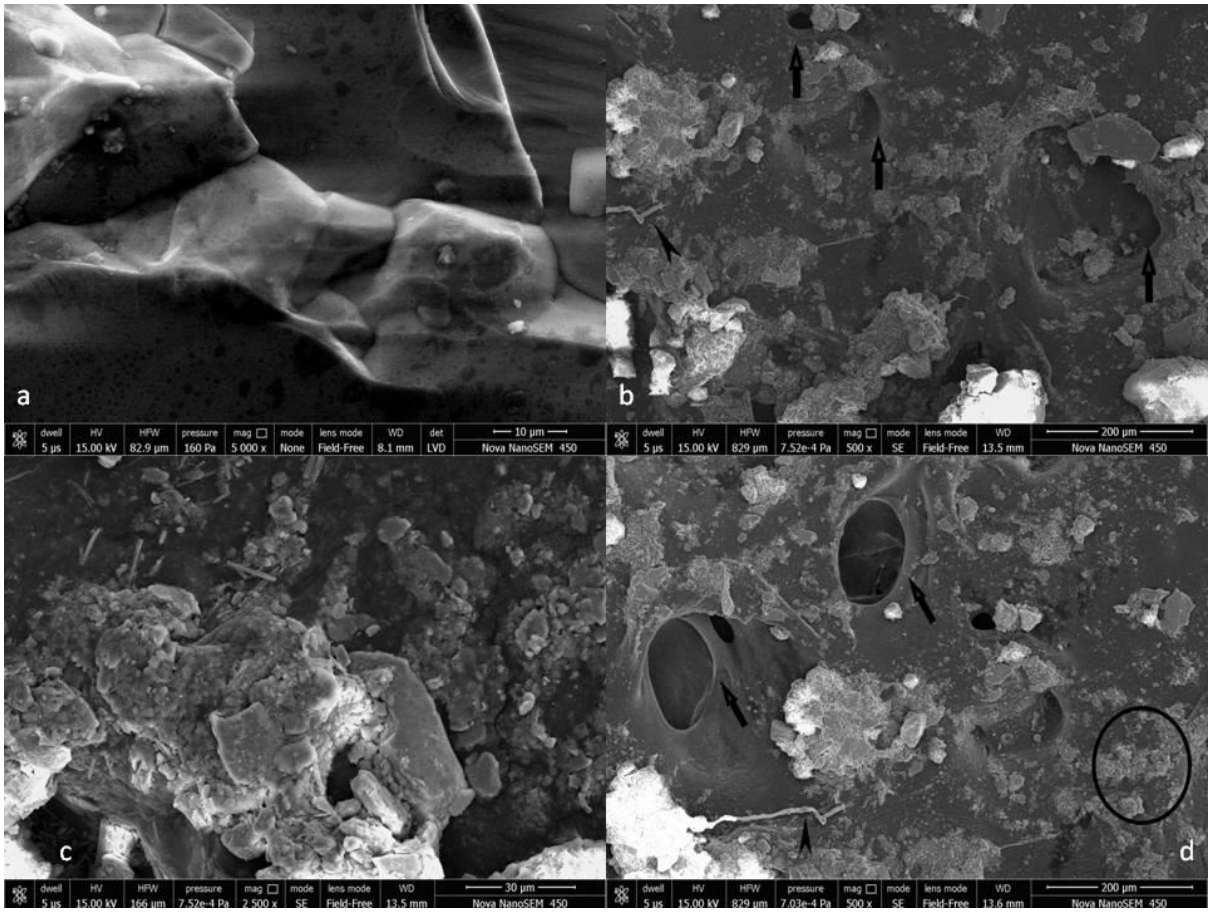



Analysis of stone sample deterioration by FE-SEM

The degenerative potential of the stone piece sample was identified with the help of a Field emission scanning electron microscope (FE-SEM). The difference between the FE-SEM images of the stone piece which were tested for the degenerative potential of isolated bacterial and fungal species (Fig.7). According to the prior FE-SEM image shows, there was no visible deterioration as such but in the following FE-SEM image demonstrate the microbial growth on the stone piece sample which cause damage. Damage can be aesthetical, mechanical and physical. The

results described those bacteria and fungi are capable of degrading the material of heritage sites. Microbial growth in the images (Fig.7b-d) shows, biofilm formation because of Escherichia coli and Pseudomonas aeruginosa are having the potential to form a biofilm (Laverty et al., 2014). Further, they provide the nutrient or perfect growth environment for other heterotrophs so they can cause further deterioration. The following FE-SEM images (Fig.7b-d) reveal the potential mechanical impact of biofilms on the stone piece. These images (Fig.7b-d) showed the pit formation (with arrow), hyphae penetration (with pointed arrow), some rod and cocci colonies of bacteria (circle and oval) and all over the growth of different microbes. Due to microbial adhesion and penetration of the substrate, erosion and the breaking of surface layers happen as physical actions and due to metabolic products and other substances produced by microorganism's dissolution and chelating processes occur as chemical actions (Koestler, 2000; Hirsch, et al., 1995). The chemical and physical changes take place simultaneously (Ascaso et al., 2002).

**Fig 7: FE-SEM image (a-d) of biodeterioration of stone piece by mixed inoculums (bacteria and fungi isolates) prior (a) and following (b-d).**




## DISCUSSION

During the screening of Nahargarh fort, a total of 188 bacterial and 145 fungal colonies from which eight bacterial species and seven fungal dominated species were isolated. The composite results indicate that in all the ten [10-10] samples of bacteria and fungi each was mainly dominated by Morganella morganii and

Fusarium solani respectively due to their high percentage relative values. The microorganism frequency and relative frequency are directly or indirectly correlated with climatic data and weather conditions (Chandel, 1990). Study of importance value index of a species in the community provides idea of the relative importance. Importance value index [IVI] of bacterial species (Table-2) revealed that the Morganella morganii show maximum IVI value (53.06%) followed by Streptococcus sp. (43.20%), Pseudomonas aeruginosa (40.15%), Micrococcus sp. (38.22%), Staphylococcus haemolyticus (36.76%), Klebsiella pneumonia (36.04%), Escherichia coli (32.65%) and Bacillus sp. (29.01%) shows least IVI value. And for fungal species, Importance value index [IVI] disclose (Table-3) that Fusarium solani shows maximum IVI value (63.22%) followed by Rhizopus sp. (51.98%), Aspergillus niger (50.75%), Aspergillus fumigation (42.6), Mucor sp. (35.07%), Aspergillus flavus (28.78%) and Aspergillus tubingensis (27.55%) shows least IVI value. Bacteria can also cause severe problems like the excreted metabolic products such as organic and inorganic acids and exoenzyme such as coagulase, amylase, cellulases, etc. are responsible for the hydrolysis of material (fig.9) (Schabereitner-Gurtner, 2000). Some of the genera have spore production capacities such as Pseudomonas, Aspergillus and Fusarium (Shirakawa et al. 2002). Due to deterioration of cultural heritage leading effects are stone dissolution, pigmentation or colour alteration, surface alterations, bio-corrosion and transformations into smaller sized crystals (Chand and Cameotra, 2011). Microbial metabolic activities proceed to complex biochemical deterioration on the sample. This complex deterioration process involved bio-corrosion whereby organic and inorganic products excreted which can etch and stain the sample site. This can cause weakening the matrix of the material of the sample, leading to more favourable conditions for further attachment and growth and therefore continuing to increase the degradative process (Dakal, and Cameotra, 2012). Bacteria also used the material components as a substrate for their metabolism. Aspergillus produces organic acids such as gluconic, citric, and oxalic acids. In a rich glucose medium, these organic acids able to solubilize powdered stone and chelate various minerals (Lapidi & Schipa, 1973). Fungal hyphae penetrate deeply into the material of heritage site just like in stone piece sample (fig.8c arrow). Aesthetic deterioration and mechanical disintegration caused by extracellular enzymes released by fungi. This results in contamination, material loss, pigmentation, acid corrosion, and enzymatic degradation (Ettenauer et al. 2010; Sterflinger 2010). Polymers such as paints can be hydrolyzed by Pseudomonas genera and lead to degradation (Cappitelli et al. 2007a&b). Isolated bacteria and fungi both were responsible for health risk also some of them are pathogenic in nature such as Morganella morganii, E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Aspergillus sp., Mucor sp. This cultural heritage attracts a bunch of tourists; hence it is an important aspect of health and maintenance of cultural heritage. Due to health and socio-economic regions, this is an area of interest for restoring and maintenance (Górny and Dutkiewicz, 2002).

## CONCLUSION

This study reported the various microbial strains that contribute markedly to the discolouration and biodeterioration of Nahargarh fort, Jaipur. These identified microbial strains show discolouration and deterioration facilitated by their ability to

produce pigments and enzymes. Penetration of hyphae, biofilm formation and discolouration of forts are the reason for aesthetical and physical damage. This study data further helps us in making a strategy to prevent and maintain the cultural heritage of Jaipur.

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