Screening of Microbial Diversity and Their Role in The Deterioration of City Wall of Jaipur, India

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ABSTRACT

The city wall of Jaipur (INDIA) which surrounded the whole city (old Jaipur) is now comes under UNESCO world heritage site. The Jaipur city wall is the city wall encircling the old Jaipur city in Rajasthan state in India. In this article, discussed about bacterial and fungal diversity and deterioration of the site. Total of 151 bacterial and 125 fungal colonies were identified among them *Klebsiella pneumonia*and *Aspergillus tubingensis* most abundant one. Importance value index (IVI) of bacterial species revealed that the *Klebsiella pneumonia*show maximum IVI value (50.44%) and *Micrococcus sp.* (24.37%) shows the least IVI value. And for fungal species, Importance value index (IVI) discloses *Aspergillus tubingensis* shows maximum IVI value (61.54%) and *Mucor sp.* (30.26%) shows the least IVI value. The surface area of City wall of Jaipur was characterised on the basis of visual inspection method. 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, Temperature, pH, City wall of Jaipur.

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1. INTRODUCTION

Biodeterioration is a complex process it involves various factors including physiological, mechanical and biological. Here the importance of biological factors mainly microbes were discussed. Microbial deterioration is one of the major concerns for cultural heritage. Cultural heritage shows importance in connecting generations, technology, history and science by various means. That's the reason heritage sites should be preserved. The city wall of Jaipur (INDIA) which surrounded the whole city (old Jaipur) is now comes under UNESCO world heritage site. The Jaipur city wall is the city wall encircling the old Jaipur city in Rajasthan state in India. It was built in 1727 when the city was founded by Maharaja Jai Singh II. The wall is six meters high and three meters thick.

It is necessary to conserve this precious heritage artifact. Microbial deterioration casing severe damage to the city wall of Jaipur. 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 (Mohammadi and Krumbein, (2008)). Environmental factors also accelerate the growth of microorganisms. Due to changes of humidity, the expansion and contraction of the cell that supports penetration of the hyphae into the surface of monuments which causes mechanical damage. Endolithic microorganism colonizing to the interior

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part of porous stone (Walker and Pace 2007) and utilize light, moisture, and shelter found inside the stone (Caneva, Gasperini, and Salvadori 2008) and may also modify their surroundings of that shelter (McNamara *et al*. 2006). The role of bacteria in the degradation of the Monument cannot be neglected. Since some of they are phototrophs and require a little amount of light, water, and ions to grow, these bacteria, along with algae, easily colonize the outer surfaces of cultural heritage and develop a biofilm, which, in turn, changes the appearance of monuments and serves as a base for the growth of other bio-deteriogens. The early visible aesthetic signs of biodeterioration such as pigment discolouration and staining are frequently the consequence of both assimilatory and dissimilatory biodeterioration and often result in successive and complex interactions between a community of organisms and the physio-chemical environment provided by these materials in situ (Allsopp *et al*., 2004). Here discussion about city wall of Jaipur deterioration and study of their causative agents mainly bacteria and fungi

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Fig 1:Citty wall of Jaipur.

2. Materials and Methods

2.1 Site Description

City wall of Jaipur is enclosed wall of old City (Jaipur) with gates. which mainly build for protection purpose from invasion. The wall's hight is six meters long and thickness is three meters. In 2019, the walled city of Jaipur comes under UNESCO world heritage site.

2.2 Sample Collection

Samples were collected from the city wall of Jaipur. Ten samples were collected in the month of October from different sampling sites on surfaces. Samples were collectedfrom Chandpole Gate, Surajpole Gate,Ajmeri Gate, New Gate, Sanganeri Gate, Ghat Gate, Samrat Gate, Zorawar Singh Gate, Gangapole Gate and nearby wall byusing sterile swabs. The sampling swabs were immersedin sterile saline (2 mL) in the laboratory tubes, shaken, and spread (0.1 mL) onto media plates.

2.3 Cultivation of Microorganisms

In the cultivation processNutrient agar,Czapek Dox agar and Potato Dextrose Agar (PDA) types of standard agar media were used.Bacteria and fungi were incubated for48h. at 37°C and 7 days at 26°C, respectively. After cultivation, all colonies grown up and pure cultures were isolated by

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repeated sub-culturing method. Further these pure cultures were maintained properly.

2.4 Molecular Characterization of Bacteria and fungi

Genomic DNA of bacteria and fungiwas extracted employing theEXpure Microbial DNA isolation kitfollowingthe manufacturer's protocol. The amplification of the bacterial 16SrRNA gene was carried out with the universal Forward Primer 27F (5' AGAGTTTGATCMTGGCTCAG 3') and Reverse Primer 1492R (5' TACGGYTACCTTGTTACGACTT 3').For the identification of fungal strains, amplification of 18S rRNA was done by using Internal Transcribed Spacer (ITS) primers consisted of ITS-1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). Amplified genomic DNA was purified, by using the Montage PCR Clean up kit (Millipore) and to get partial sequences 16S/18S rRNA gene the purified products were sequenced commercially (Yaaz Xenomics, Coimbatore, India). Then sequence was blast using NCBI (National Centre for Biotechnology Information; http://www.ncbi.nih.gov/) blast similarity search tool. Phylogenetic tree construction was performed (fig.4) using the program Tree Dyn 198.3 (Dereeper *et al*., 2008).

2.5 Assessment of Deterioration of Monument Surface

The basic characterisations of deterioration mechanisms were discussed to understand deterioration process. The physical properties of the surface area of selected sitessimilarly temperature, pH, water absorption, cracks, surface, texture, colour and porosity status of the monuments were observed with the visual inspection method without harming any site.

3. Results

3.1 Isolation of Bacterial and fungal Isolates

From 10 samples several bacterial and fungal colonies were recovered but the most frequent colonies which were present on all the samples were processed for further study. Total of 151 bacterial and 125 fungal colonies were identified from city wall of Jaipur. The dominated bacterial genera were *Klebsiella, Morganella,Escherichia, Pseudomonas, Bacillus,Acidobacter, Micrococcus, Staphylococcus andStreptococcus*and fungal genera*Penicillium, Aspergillus, Fusarium, Rhizopus,Mucor,*and *Cladosporium*. The isolated bacterial species (table-2) were *Klebsiella pneumonia, Morganella morganii, Pseudomonas aeruginosa, Staphylococcus haemolyticus, Escherichia coli,* and *Staphylococcus aureus,* and isolated species of fungi (table-3) were*Fusarium solani, Aspergillus flavus, Aspergillus niger, Aspergillus fumigation,* and*Aspergillus tubingensis*selected on the basis of dominance.

3.2 Molecular Characterization of the Isolates

The dominated isolated nucleotide sequence from City wall of Jaipur was deposited in the GenBank (NCBI database) andaccession numbers regenerated (table-1) and Phylogenetic tree are also generated (fig.-2). These are:

Table1: Accession number of samples sequence collected from city wall of Jaipur site.

SERIAL NO.	SAMPLE ID	MICROORGANISM	ACCESSION NUMBERS
1	CNP10	*Klebsiella pneumonia*	MN490064

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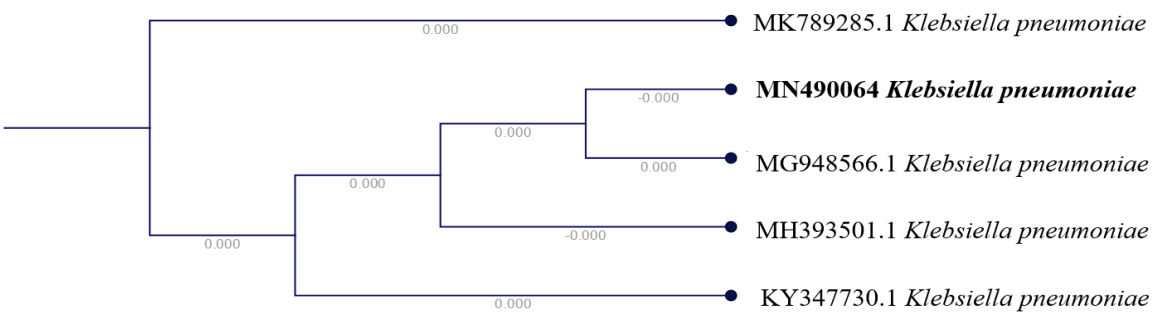

Fig.2: Phylogenetic tree of*Klebsiella pneumonia*bacterial species.

Table 2: RF, RD, RA and IVI of different Bacterial species into city wall of Jaipur.

Isolated Bacteria	Number of Bacteria colonies										RF %	RD %	RA %	IVI
	CNG1	CTG2	CAG3	CCG4	CGG5	CZG6	CSG7	CSG8	CPG9	CNP10				
Klebsiella pneumonia	4	-	3	2	7	-	1	6	1	5	16.32	19.20	14.92	**50.44**
Morganella morganii	-	2	4	6	3	-	4	-	2	1	14.28	14.56	12.93	**41.77**
Pseudomonas aeruginosa	5	3	-	1	2	-	-	3	-	4	12.24	11.92	12.35	**36.51**
Staphylococcus haemolyticus	3	2	-	-	-	4	6	2	4	-	12.24	13.90	14.41	**40.55**
Escherichia coli	6	-	3	4	-	-	5	1	2	3	14.28	15.89	14.11	**44.28**
Bacillus sp.	-	4	-	5	-	2	1	-	-	6	10.20	11.92	14.82	**36.94**
Micrococcus sp.	2	1	-	1	-	3	1	-	1	-	12.24	5.96	6.17	**24.37**
Streptococcus sp.	-	-	3	-	2	-	4	-	-	1	8.16	6.62	10.30	**25.07**

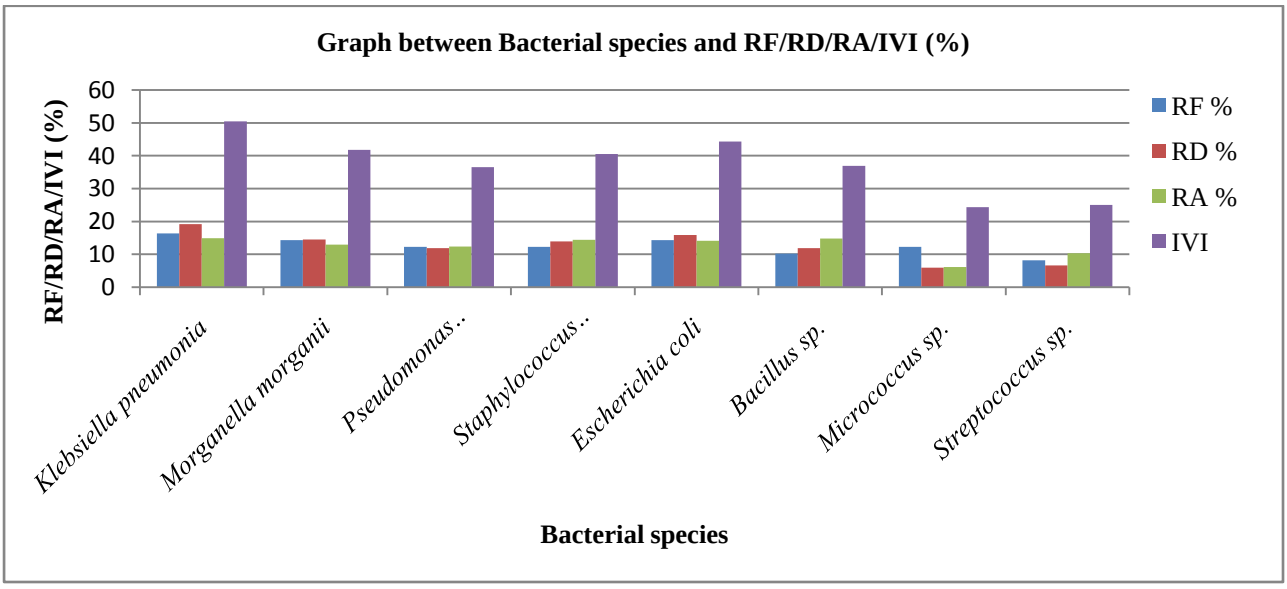

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Fig 3: Graphical representation of bacterial species and RF/RD/RA/IVI (%).

Table 3:RF, RD, RA and IVI of different fungal species into city wall of Jaipur.

Isolated Fungi	Number of Fungal colonies										RF %	RD %	RA %	IVI
	CNG1	CTG2	CAG3	CCG4	CGG5	CZG6	CSG7	CSG8	CPG9	CNP10				
Mucor sp.	-	3	1	2	-	-	1	2	-	1	13.64	8.0	8.62	**30.26**
Aspergillus flavus	-	1	-	-	2	3	-	4	1	-	11.36	8.8	11.36	**31.52**
Rhizopus sp.	5	-	1	3	-	8	4	-	2	3	15.90	20.8	19.15	**55.85**
Fusarium solani	3	4	-	5	-	1	-	2	1	2	15.90	14.4	13.26	**43.56**
Aspergillus niger	4	-	2	1	-	-	5	-	3	1	13.64	12.8	13.78	**40.22**
Aspergillus tubingensis	1	2	1	-	4	5	-	3	8	6	18.18	24.0	19.36	**61.54**
Aspergillus fumigation	2	-	-	-	3	-	7	1	-	1	11.36	11.2	14.45	**37.01**

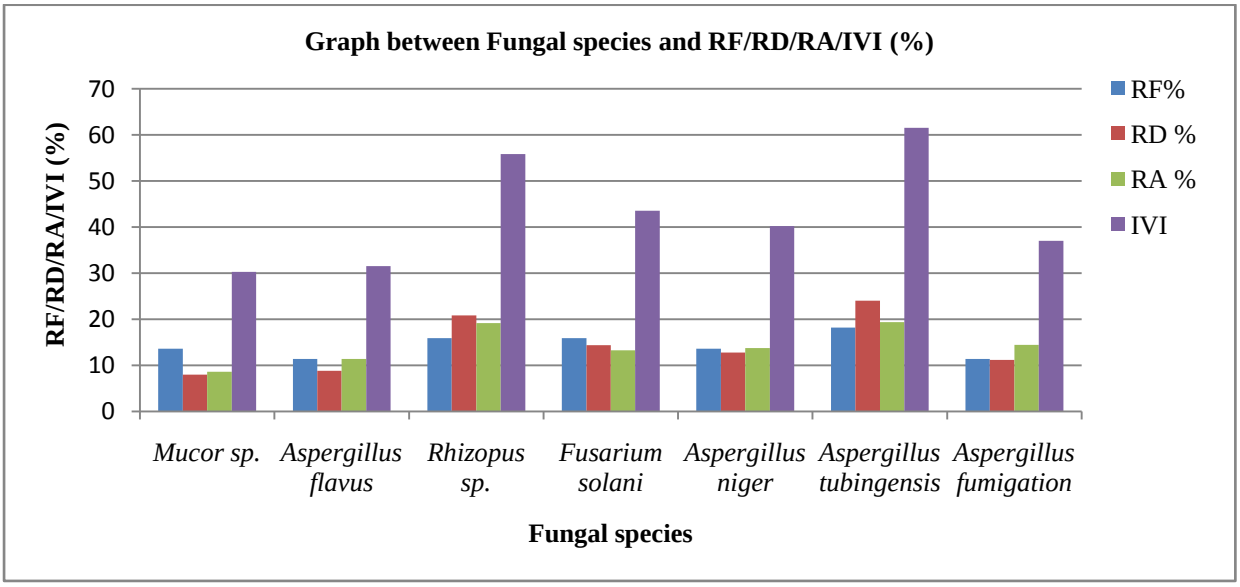

Fig 4: Graphical representation of fungal species and RF/RD/RA/IVI (%).

3.3 Assessment of Deterioration of Monument Surface

The surface area of City wall of Jaipur was characterised on the basis of visual inspection method. The temperature range was from 90.5- 96.4°F, pH range was 6-9, and water absorption was poor to

strong. Further the surface was characterised by porous and non-porous, soft and hard, smooth and rough, presence and absence of cracks. Due to pigmentation the verity of colours was observed.

The Graphsshow (figure-5) the temperature of City wall of Jaipur maximum with 96.4°F and minimum with 90.3°F and with average value of temperature 93.25°F. The maximum pH value of City wall of Jaipur was 9 and minimum was 6 and average value of pH was 7.5. The 60% of City wall of Jaipur's sites were strongly water absorbent and 100% was poorly absorbent. 60% of sites in City wall of Jaipur were porous.

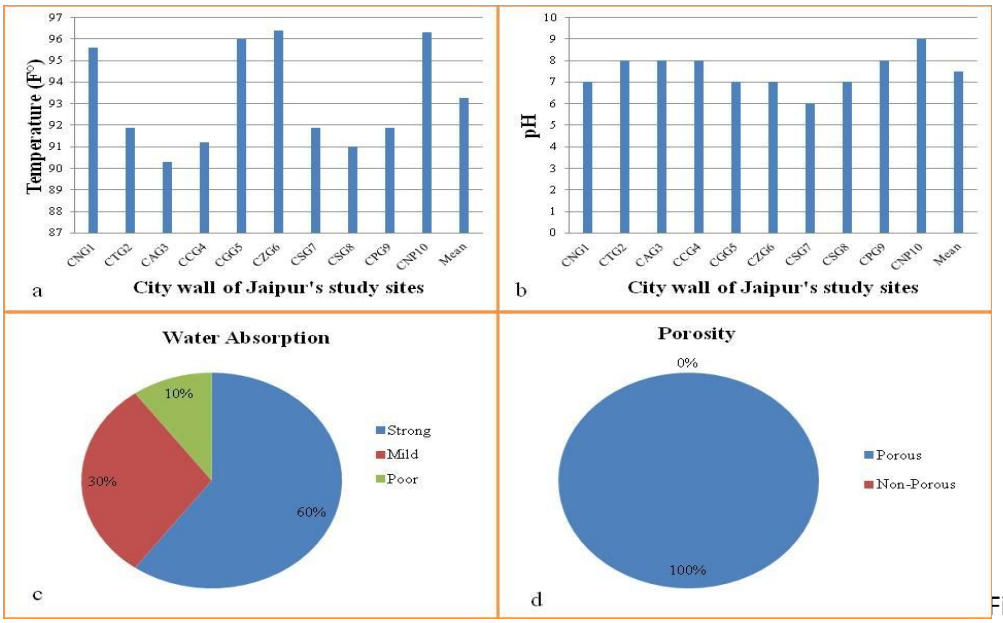

Figure-5

Graphical representation of City wall of Jaipur's sample between (a) Temperature; (b) pH; (c) water absorption; (d) porosity.

The pie graphs describe (figure-6) the 100% hard surface with 70% rough and 30% smooth texture. On the surface 70% cracks was present City wall of Jaipur.

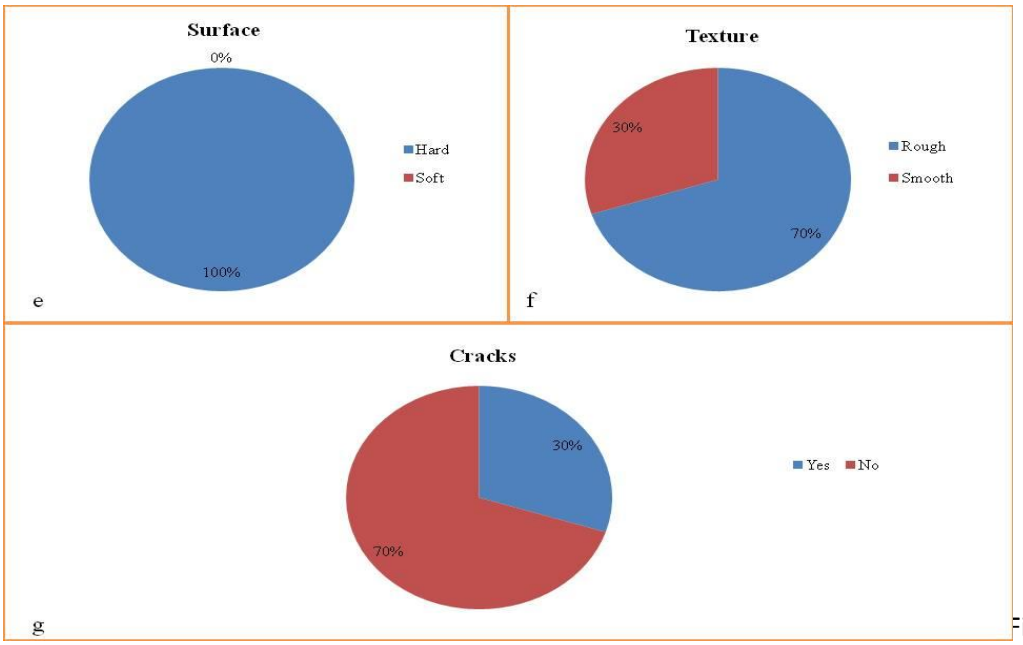

Figure-6

Graphical representation of City wall of Jaipur's sample between e) surface; f) Texture; g) Cracks.

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4. Discussion

During the screening of city wall of Jaipur, a total of 151 bacterial and 125 fungal colonies from which six bacterial species and five 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 *Klebsiella pneumonia*(fig.3) and *Aspergillus tubingensis*(fig.4) respectively due to their high percentage relative values. 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 *Klebsiella pneumonia* show maximum IVI value (50.44%) followed by*Escherichia coli* (44.28%),*Morganella morganii*(41.77%), *Staphylococcus haemolyticus* (40.55%) and *Micrococcus sp.* (24.37%) showed least IVI value; and for fungal species, Importance value index (IVI) disclose (table-3) that *Aspergillus tubingensis*showed maximum IVI value (61.54%) followed by *Rhizopus sp.*(%), *Fusarium solani* (43.56%), *Aspergillus niger*(40.22%) and *Mucor sp.*(30.26%) showed least IVI value.

This research shows that microbes adversely affect monuments sites. The porous surface of sites provides a good environment for growth these microbes (phototrophic microorganisms) help others to grow (heterotrophic microorganisms). These organisms provide huge biomass on sites. Due to pigmentation, the microbe discolours the monument's sites. The water holding capacity increase chances the microbial growth which deteriorates the surface. Physical alterations are the result of an increased mass of microorganisms as they grow and the water-binding capability of microorganisms, by which their volume changes in wet and dry periods and winter frosts because of continual extension and shrinkage of the colony. The release of organic acids exerts a major chemical effect on substrates.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 (Schabereitner-Gurtner, 2000). Due to deterioration of cultural heritage leading effects are stone dissolution, pigmentation or colour alteration, surface alterations, biocorrosion and transformations into smaller sized crystals, etc. (Chand and Cameotra, 2011). Bacteria also used the material components as a substrate for their metabolism. *Aspergillus* was able to solubilize powdered stone and chelate various minerals in a rich glucose medium because they produce organic acids such as gluconic, citric, and oxalic acids (Gupta*et al*.,2013). Fungal hyphae penetrate deeply into the material of heritage site and release extracellular enzymes, resulting in aesthetic deterioration and mechanical disintegration due to material loss, pigmentation, contamination, acid corrosion, and enzymatic degradation (Ettenauer *et al*. 2010; Sterflinger 2010). Jaipur is a cultural hub and tourist place. So, the need of protection and screening of microbial load is required. Jantar- Mantar, Jaipur was also screened and identified microbial load for further conservation strategy (Yadav and Gupta, 2021).Jantar-Mantar is also a UNESCO world heritage site, like that city wall of Jaipur needs to be conserved. Isolated bacteria and fungi from city wall of Jaipur were responsible for health risk and some of them are also pathogenic in nature such as *Klebsiella pneumonia,Morganella morganii,E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus haemolyticus,Mucor sp.*and *Aspergillus flavus*. These cultural heritages attract a bunch of tourists; hence it is an important aspect of health and maintenance of cultural heritage. Due to health and socio-economic regions,

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this is an area of interest for restoring and maintenance (Górny and Dutkiewicz, 2002).

5. Conclusion

Jaipur is an ancient city need to be conserved for preserving their tradition, art, culture and history. It's first time when biodeterioration of city wall of Jaipur was studied. Although some of the parts were painted periodically but it's not enough to maintain because most of the deteriorating bacteria and fungi can be isolated from painted surface. So, need a strategy for removing as well as preventing the growth of these biodeteriogens. With the help of this research a targeted strategy can be formed for conservation purpose.

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