Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268	Vol. 13	No. 1	pp. 141-146	2014

Original Research Paper

Biodeterioration of Some Cultural Heritage in Isfahan City, Iran

H. Modaresi, N. Bahador and M. Baserisalehi*
Department of Microbiology, Science and Research Branch, Islamic Azad University, Fars, Iran
*Department of Microbiology, Kazeroun Branch, Islamic Azad University, Kazeroun, Iran

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com
Received: 2-7-2013
Accepted: 12-8-2013

Key Words:
Biodeterioration
Historical places
Isfahan
Molecular technique

ABSTRACT

The aim of this study was to isolate and phenotypical identification of microorganisms involving in biodeterioration of four historical places in Isfahan, Iran. For this purpose two hundred and ten samples were taken from the stones of Chehel Sotoun, Hasht Behesht, Alighapou and Imam mosque. For bacterial isolation blood agar and modified Bristol medium were used and for screening of fungal isolates potato dextrose agar was used. Then the samples were serially diluted and poured on the selected media. For bacterial isolates the plates were incubated at 30°C for 48 hrs and for fungal isolation the plates were kept at room temperature for 4 weeks. The pure colonies were identified using phenotypical and molecular identification. Fungi were characterized using macroscopic characteristics and microscopic arrangements. The results obtained from this study indicated that the dominant isolated bacteria were *Bacillus licheniformis*, *Bacillus thuringiensis* and *Halomonas* sp. In addition the results showed that the fungal isolates belonged to Genus: *Alternaria*, *Cladosporium, Penicillium, Absidia* and *Shaccharomyces*. Furthermore, the most polluted area was Chehel Sotoun which is located in the traffic part of the city. Overall, stone materials of cultural heritage are constantly exposed to biodegradation by microorganisms and presence of organisms including algae, fungi and bacteria are likely to affect or even cause physico-chemical changes.

INTRODUCTION

There are many organisms which are involved in biodeterioration like bacteria, fungi, archaea, algae, and lichens. Presence of these organisms and different types of stones could help the interaction and then make visual and structural damages in historical places (Gadd 2007). Microorganisms within the stones may grow in cracks and pores with different mechanisms (Mc Namara et al. 2006). Indeed, biodeterioration may be defined as any undesirable change in the properties of a material caused by the vital activities of living organisms. In recent years biodeterioration of stone monuments and buildings is a well recognized and studied worldwide (Warscheid 2003, Gordon & Dorn 2005, Nugari et al. 2009). In some tropical regions with high temperatures, relative humidity and heavy rainfall this phenomenon favours the growth of wide variety of living organisms (Gadd 2007). In other words, along with vital activities the initial deterioration of stone exposed to the outdoor environment and action of agents such as rain, wind, sunlight, and pollution may play an important role for physical and chemical processes. When the surface of the monument has undergone this process of alteration, living organisms begin to colonize the area form biofilms and finally cause structural damage (Nuhoglu et al. 2006). Although biofilm formation has an important role in biodeterioration, chemical reactions with the material, physical penetration into the substrate and production of pigments are the other main factors in biodeterioration. Because of photosynthetic nature, the first coloniser of historical places are algae and cyanobacteria; these microorganisms can deteriorate stone either chemically or mechanically. On the other hand, lichens, which are resistant to desiccation and extreme temperatures, are also frequently associated with biodeterioration of stones (Herrera et al. 2004). Hence, The aim of this study was evaluation and characterization of the bacteria and fungi incorporated in biodeterioration of historical places in Isfahan city, Iran.

MATERIALS AND METHODS

The Isfahan City: Isfahan, historically also rendered in English as Ispahan, Sepahan or Hispahan, is the capital of Isfahan Province in Iran, and located about 340 km south of Tehran. Isfahan is located on the main north-south and east-west routes crossing Iran, and was once one of the largest cities in the world. The city is famous for its Islamic architecture, with many beautiful boulevards, covered bridges, palaces, mosques, and minarets. The Naghsh-e Jahan Square in Isfahan is one of the largest city squares in the world and an outstanding example of Iranian and Islamic architecture, which has been designated by UNESCO as a World Heritage Site. The city also has a wide variety of historic monuments and is known for the paintings and history. The city is situated at 1,590 metres (5,217 ft) above sea level on the eastern side of the Zagros Mountains and has an arid climate. Despite its altitude, Isfahan remains very hot during the summer with

maxima typically around 36°C or more (Assari & Mahesh 2011).

Sampling sites: The historical places of Isfahan are well-known examples of various civilizations. The four main historical places identified in Isfahan were chosen for determining the biodeteriorative effects of the microorganisms on stone decay. These places were restored by the Ministry of Culture and Tourism of Islamic Republic of Iran. The characteristics of the historical buildings are given below:

***Imam Mosque*:** Formerly known as Shah Mosque is a mosque in Isfahan, Iran. The mosque is standing in south side of Naghsh-i Jahan Square. The mosque was built during the Safavid period. It is an excellent example of Islamic architecture and regarded as one of the masterpieces of Persian architecture. The Imam Mosque is registered, along with the Naghsh-i Jahan Square, as a UNESCO World Heritage Site. Its construction began in 1611, and its splendor is mainly due to the beauty of its seven-colour mosaic tiles and calligraphic inscriptions.

Âlî Qâpû: It is a grand palace in Isfahan, Iran. It is located on the western side of the Naqsh-e Jahan Square opposite to Sheikh lotf allah mosque, and had been originally designed as a vast portal. It is forty-eight meters high and there are seven floors, each accessible by a difficult spiral staircase. In the sixth floor music room, deep circular niches are found in the walls, having not only aesthetic value, but also acoustic. The name Âlî Qâpû, from Arabic Âlî, "Imperial or Great", and Turkich Qâpû meaning "gate", was given to this place as it was right at the entrance to the Safavid palaces which stretched from the Maidan Naqsh-i-Jahan to the *Chahâr Bâgh Boulevard*.

Chehel Sotoun: It is a pavilion in the middle of a park at the far end of a long pool, in Isfahan, Iran, built by Shah Abbas II to be used for his entertainment and receptions. The name, meaning "Forty Columns" in Persian, was inspired by the twenty slender wooden columns supporting the entrance pavilion, which, when reflected in the waters of the fountain, are said to appear to be forty. The palace contains many frescoes and paintings on ceramic. Many of the ceramic panels have been dispersed and are now in the possession of major museums in the west.

***Hasht Behesht*:** Meaning "Eight Paradises" is a Safavid era palace in Isfahan. It was built in 1669 and is today protected by Iran's Cultural Heritage Organization (Assari & Mahesh 2011, 2012).

Isolation and identification of bacterial isolates: The stone samples were taken from the external walls of the monuments from 3 m height of four historical buildings. They were put into the sterile screw capped tube in January and March in Isfahan to prevent contamination from the soil and ground. Sterile devices were used in the sampling and all of the procedures were done aseptically (Nuhoglu et al. 2006). The microbiological studies were carried out under sterile conditions.

Totally 210 specimens were taken from the stone surface of all the monuments in January and March 2011-2012. During the sampling procedures the daytime and night time average temperature was 20 and -2.5°C in January, and 27 and 9.3°C in March. The first specimen was taken by means of scraping from the stone surface. These samples were used for microbiological incubating and counting. One gram of each sample was squashed with a mortar and weighed. Then, they were put into a 50 mL flask with 10 mL sterile physiological solution (9g/L NaCl), mixed for 15 min and then serially diluted (10^{-1}-10^{-7}) and plated on different microbiological media including: Blood agar and MBM medium for chemolithotrophic bacteria (Vero et al. 1975, Greenberg et al. 1985). Preliminary identification of bacteria was based on phenotypical characteristics and then the isolates were identified using molecular methods.

Isolation and identification of fungi: For isolation of the fungi the samples were cultured on sabouraud dextrose agar containing peptone (10 g), glucose (20 g) and agar (15 g). The medium was supplemented with chloramphenicol and cycloheximide (50 and 500 mg/dl). The cultures were incubated at 26°C and examined twice a week for a total duration of 4 weeks. After that, the isolates were examined macroscopically, and microscopically following staining with lactophenol cotton blue. The identification of yeast was based on their microscopic characteristics as a result of germ tube tests and biochemical tests and the identification of moulds was based on their macroscopic characteristics (growth period, colony morphology, production of pigment on the back of the colony), microscopic arrangements (characteristics hyphae formation, types of conidia, sizes and shapes of the sterigmata, hyphae and organs of reproduction) (Nuhoglu et al. 2006).

Identification of bacteria using PCR technique: For molecular identification single colonies were picked up from solid medium and subjected to release DNA. DNA extraction was carried out using DNA PCR kit (Roche-Germany). Then the purity of the extracted DNA was assessed by absorbance at 260 and 280 nm. The extracted DNA with ratio (260/280 nm) of 1.9 was used for PCR. Amplification of 16SrRNA gene was performed using Forward and Reverse primers with sequence of 5´AGGAGGTGACCAACCGCA3´ and 5´AACTGGAGGAAGGTGGGGA 3´ respectively. The PCR products were run on 1.5% (w/v) agarose gel. PCR products were electrophoresed at 75 V for 20 min

Table 1: Phenotypical identification of isolated bacteria from historical places.

No. of isolates		Phenotypic identification							
	Morphology	Catalase	Oxidase	Gram	Spore	Growth on 7% Salt	Gelatin hydrolysis	Starch hydrolysis	Tyrosine hydrolysis
CH1	Bacillus	+	+	+	+	+	+	+	+
CH2	Bacillus	+	+	+	+	+	+	+	-
CH3	Rod	+	+	-	-	+	-	+	+

CH: Chehel Sotoun

>gb|CP000485.1| Bacillus thuringiensis str. Al Hakam, complete genome

Length=5257091

Score = 658 bits (356), Expect = 0.0

Identities = 358/359 (99%), Gaps = 0/359 (0%)

Strand=Plus/Minus

5'CACCTTAGGCGGCTGGCTCCAAAAGGTTACCCCACCGACTTCGGGTGTTACAAACTCTCG
CACCTTAGGCGGCTGGCTCCAAAAGGTTACCCCACCGACTTCGGGTGTTACAAACTCTCG
TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCG
TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCG
CGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGAA
CGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGAA
CGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGCTCTTTGTACCGTCCATTGTAG
CGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGCTCTTTGTACCGTCCATTGTAG
CACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCC
CACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCC
GGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTAAATGATGGCAACTAAAATCAAG
GGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTAAATGATGGCAACTAAGATCAAG 3'

Fig. 1: Blast alignment for CH1 isolated from Chehel Sotoun.

gb|JN391533.1| *Bacillus licheniformis* strain BB10 16S ribosomal RNA gene, partial sequence

Length=1472

Score = 664 bits (359), Expect = 0.0

Identities = 359/359 (100%), Gaps = 0/359 (0%)

Strand=Plus/Minus

5'CACCTTCGGCGGCTGGCTCCAAAGGTTACCTCACCGACTTCGGGTGTTACAAACTCTCGT
CACCTTCGGCGGCTGGCTCCAAAGGTTACCTCACCGACTTCGGGTGTTACAAACTCTCGT
GGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGC
GGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGC
GATTACTAGCGATTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAAC
GATTACTAGCGATTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAAC
AGATTTGTGGGATTGGCTTAGCCTCGCGGCTTCGCTGCCCTTTGTTCTGCCCATTGTAGC
AGATTTGTGGGATTGGCTTAGCCTCGCGGCTTCGCTGCCCTTTGTTCTGCCCATTGTAGC
ACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCG
ACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCG
GTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGG
GTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGG3'

Fig. 2: Blast alignment for CH2 sample isolated from Chehel Sotoun.

>gb|FJ939560.1| *Halomonas* sp. HTNK1 16S ribosomal RNA gene, complete sequence
Length=1530
Score = 1919 bits (1039), Expect = 0.0
Identities = 1157/1211 (96%), Gaps = 19/1211 (2%)
Strand = Plus/Minus
5' TGGTGTGTGTGGCGACGTGTACAGAGCCCGGAAACCTATACACCGGGACATTCTGATTCA
TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGACATTCTGATTCA
CGATTACTAGCGATTCCGACTTCACGGAGTCGAGTTGCAGACTCCGATCCGGACTGAGAC
CGATTACTAGCGATTCCGACTTCACGGAGTCGAGTTGCAGACTCCGATCCGGACTGAGAC
CGGCTTTTCGGGATTAGCTCACTCTCGCGAGTTGGCAACCCTTTGTACCGGCCATTGTAG
CGGCTTTTCGGGATTAGCTGACTCTCGCGAGCTCGCAACCCTTTGTACCGGCCATTGTAG
CACGTGTGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCC
CACGTGTGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCC
GGTTTGTCACCGGCAGTCTCCTTAGAGTTCCCACCATTACGTGCTGGCAAATAAGGACAA
GGTTTGTCACCGGCAGTCTCCTTAGAGTTCCCGCCATTACGCGCTGGCAAATAAGGACAA
GGGTTGCGCTCGTTACGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCAT
GGGTTGCGCTCGTTACGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCAT
GCAGCACCTGTCTCTGCGTTCCCGAAGGCACCAAGTGATCTCTCACAAGTTCGCAGGATG
GCAGCACCTGTCTCTGCGTTCCCGAAGGCACCAAGTGATCTCTCACAAGTTCGCAGGATG
TCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGT
TCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGT
GCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGA
GCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGA
CTTATCGCGTTAACTTCGCCACAAAGTGCGCTAGGCACCCAACGGCTGGTCGACATCGTT
CTTATCGCGTTAACTTCGCCACAAAGTGCGCTAGGCACCCAACGGCTGGTCGACATCGTT
TACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTACCCACGCTTTCGCACCTCAGT
TACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTACCCACGCTTTCGCACCTCAGT
GTCAGTGTCAGTCCAGAAGGGCGCCTTCGCCACTGGTATTCCTCCCGATCTCTACGCATT
GTCAGTGTCAGTCCAGAAGGCCGCCTTCGCCACTGGTATTCCTCCCGATCTCTACGCATT
TCACCGCTACACCGGGAATTCTACCTTCCTCTCCTGCACTCTAGCTTGACAGTTCCGGAT
TCACCGCTACACCGGGAATTCTACCTTCCTCTCCTGCACTCTAGCTTGACAGTTCCGGAT
GCCGTTCCCAGGTTGAGCCCGGGGCTTTCACAACCGGCTTATCAAGCCACCTACGCGCGC
GCCGTTCCCAGGTTGAGCCCGGGGCTTTCACAACCGGCTTATCAAGCCACCTACGCGCGC
TTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGGAC
TTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCAC
GGAGTTAGCCGGTGCTTCTTCTGCGAGTGATGTCTTTCCTAATGGGGATTAAACACTAGG
GGAGTTAGCCGGTGCTTCTTCTGCGAGTGATGTCTTTCCTAATGGGTATTAACCACTAGG
CGTTCTTCCTCGCTGAAAGTGCTT-ACAACCCGAGAGGCCTTCTTTCACACACGCGGCAT
GGGCTGGATCAGGCTTGCGCCCATTGTCGATAATTCCCCACTGCTGCTTCCCGGTAGGAG
CTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCG-TAGGAG
T-CGG-CC-TGTTCCTCAGTTCCGATGGTGGCGTGATCATCCTCTCAGAACAGCTTACGG
TTCGGGCCGTGT-C-TCAGTCCCGATG-TGGC-TGATCATCCTCTCAGACCAGCT-ACGG
ATCGTTGCCGTGCTGA-CCATAACCTCACCA-CTAGCTAATCGGACTTAG-CTCTATCTA
ATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCGACATAGGCTC-ATCCA
TATAGGCGGGA-ATAG-CGGGA3'

Fig. 3: Blast alignment for CH3 sample isolated from Chehel Sotoun.

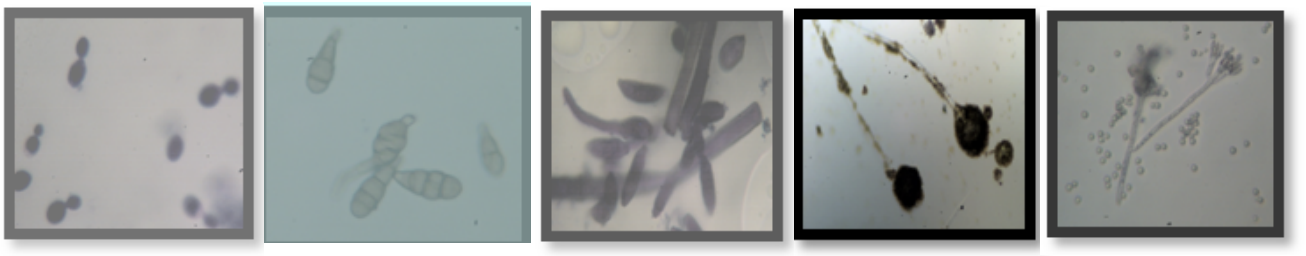

Fig. 4 Fig. 5 Fig. 6 Fig. 7 Fig. 8

Figs. 4-8: Microscopic identification of isolated fungi: *Saccharomyces*, *Alternaria*, *Cladosporium*, *Absidia* and *Penicillium*.

and then DNA bands were virtualized after staining with ethidium bromide. At last the PCR products with pure DNA bands were sent to Macrogen in South Korea (http://www.macrogen.com) for DNA sequencing. The 16SrRNA sequenced data were subjected to BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST) for identifying each respective 16SrRNA gene amplicon.

RESULTS AND DISCUSSIONS

The results obtained from this study indicated that out of 210 samples from different sites gram positive and spore forming bacteria were dominant (Table 1). In addition the Chehel Sotoun were most polluted than the other sites. Furthermore, Figs. 1-3 confirmed that *Bacillus licheniformis* strain BB10, *Bacillus thuringiensis* str. Al Hakam and *Halomonas* HTNK1 are the most frequently detected isolates which have been identified using molecular techniques. In addition, the genus *Halomonas* HTNK1 was for the first time isolated from the sites. On the other hand, fungi were isolated from 80 samples. The colonies were directly examined on agar plates and based on macroscopic and microscopic characterization. Five different morphological types were detected. Fungi genera that were identified in this study, were belonged to Genus, *Cladosporium* sp., *Penicillium, Alternaria, Absidia* and *Saccharomyces*. Although the fungi were isolated from all the sites, the most polluted area was Chehel Sotoun (Figs. 4-8).

The Isfahan city has numerous historical places. These sites are affected by the physical and chemical conditions as well as the dry climate. The assessed chosen sites are located in high traffic routes. Although microorganisms, as a helping factor, can directly or indirectly contribute to stone deterioration by producing biofilm (Nugari et al. 2009), organic and inorganic acids, pigments and several other factors also cause the damage. The impact of fungi on biodeterioration is due to their physical and chemical activities. In addition, the fungi can cause stone deterioration through various mechanisms such as penetration of hyphae into the stone, production of different pigments and production of organic and inorganic acids (Shirakawa et al. 2011).

In fact, scientists believed that several cryptoendolithic fungi may actively bore into the stone and hence physically disrupt its integrity (Gadd 2007). Fungi, unlike the phototrophs, do not require light for growth, and so their boring activity can penetrate to greater depths (Golubic et al. 2005). Indeed, hyphal penetration of materials involves swelling/deflation effects and channelling of water into the substrate which can form cracks, fissures and crevices, extend existing ones and lead to the detachment of crystals (Suihko et al. 2007). Biochemical actions of fungi as well as bacteria can lead to microtopological alterations (Gadd 2007). They are associated with extracellular mucilaginous substances, which contain, amongst many other metabolites, acidic, and metal chelating compounds. Acidic metabolites (oxalic, acetic, citric, other carbonic acids) deteriorate the stone minerals by a solubilizing and chelating effect. Organisms of the genus *Bacillus* have been very frequently identified on stone buildings (Blazquez et al. 2000, Gaylarde & Morton 2002, Kiel & Gaylarde 2006). This is not unexpected, as they are very common in soil and are able to withstand extreme environments because of their spore-forming ability and ease of culture (Qi et al. 2011). Kiel & Gaylarde (2006) found that some strains of *Bacillus* isolates produced acids and surfactants with auto emulsifying activity in the laboratory, indicating that they had the capacity to accelerate stone degradation. In the present study, isolation of this genus from most of the parts indicated that the bacteria with spore could tolerate the arid climate of Isfahan city which in further study using HPLC technique could understand the main mechanisms of the damage.

ACKNOWLEDGEMENT

The authors would like to thank Cultural Heritage Organization of Isfahan city for the formalities and permitting us to get the samples from the selected sites.

REFERENCES

Assari, A. and Mahesh, T.M. 2011. Demographic comparative in heritage texture of Isfahan city. Journal of Geography and Regional Planning, Academic Journals, 4(8): 463-470.

Assari, Ali. and Mahesh, T.M. 2012. Conservation of historic urban core in traditional Islamic culture: Case study of Isfahan city. Indian Journal of Science and Technology, 5(1): 1970-1976.

Blazquez, A.B., Lorenzo, J., Flores, M. and Gomez-Alarcon, G. 2000. Evaluation of the effects of some biocides against organisms isolated from historic monuments. Aerobiologia, 16: 423-428.

Gadd, G.M. 2007. Geomycology: Biogeochemical transformations of rocks, minerals and radionucleotides by fungi, bioweathering and bioremediation. Mycol. Res., 111: 3-49.

Gaylarde, C. and Morton, G. 2002. Biodeterioration of mineral materials. Environmental Microbiology, 1: 516-528.

Gaylarde, P., Englert, G., Ortega-Morales, O. and Gaylarde, C. 2006. Lichen-like colonies of pure *Trentepohlia* on limestone monuments. Int. Biodeterior. Biodegrad., 58: 248-253.

Greenberg, A.E., Trussell, R.H. and Clesceri, L.S. 1985. Standard Methods for the Examination of Water and Wastewater. Sixteenth edition. American Public Health Association, Washington, pp. 860-864, 864-866 and 981-984.

Golubic, S., Radtke, G. and Campion-Alsumard, T. 2005. Endolithic fungi in marine ecosystems. Trends Microbiol., 13: 229-235.

Gordon, S.J. and Dorn, R.I. 2005. Localized weathering: Implications for theoretical and applied studies. Prof. Geogr., 57: 28-43.

Herrera, L.H., Arroyave, C., Guiamet, P., de Saravia, S.G. and Videla, H. 2004. Biodeterioration of peridotite and other constructional materials in a building of the Colombian Cultural Heritage. Int. Biodeterior. Biodegrad., 53: 135-141.

Kiel, G. and Gaylarde, C. 2006. Bacterial diversity in biofilms on external surfaces of historic buildings in Porto Alegre. World J. Microbiol. Biotechnol., 22: 293-297.

McNamara, C.J., Perry, TD., Bearce, KA., Hernandez-Duque, G. and Mitchell, R. 2006. Epilithic and endolithic bacterial communities in limestone from a Maya archaeological site. Microbial. Ecol., 51: 51-64.

Nugari, MP., Pietrini, AM., Caneva, G., Imperi, F. and Visca, P. 2009. Impacts of microbial biofilms in the deterioration of inorganic building materials and their relevance for the conservation practice. Int. Biodeterior. Biodegradation, 63: 705-711.

Nuhoglu, Y., Oguz, E., Uslu, H., Ozbek, A., Ipekoglu, B., Ocak, I. and Hasenekoglu, I. 2006. The accelerating effects of the microorganisms on biodeterioration of stone monuments under air pollution and continental-cold climatic conditions in Erzurum, Turkey. Science of the Total Environment, 364: 272-283.

Qi, W., Guang, Y., Lin, Y. and Xia-Fang, S. 2011. Characterization of bacterial community inhabiting the surfaces of weathered bricks of Nanjing Ming city walls. Science of Total Environment, 409: 756-762.

Shirakawa, M., Loh, K., Silva, M.C. and Gaylarde, C. 2011. Biodeterioration of painted mortar surfaces in tropical urban and coastal situations: Comparison of four paint formulations. International Biodeterioration & Biodegradation, 65: 669-674.

Suihko, M., Alakomi, H., Gorbushina, A., Fortune, I., Marquardt, J. and Saarela, M. 2007. Characterization of aerobic bacterial and fungal microbiota on surfaces of historic Scottish monuments. Systematic and Applied Microbiology, 30: 494-508.

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, pp. 257-266.

Warscheid, T. 2003. The evaluation of biodeterioration processes on cultural objects and approaches for their effective control. In: Art, Biology and Conservation: Biodeterioration of Works of Art (R. J. Koestler, V.H. Koestler, A.E. Charola and F.E. Nieto-Fernandez, Eds.). 7: 14-27.