

junctions, the concentration of benzene and vehicle counts do not show linear dependence. Amongst the traffic intersections Haji Ali is next to the sea coast, Worli Naka is about 500 m away from coast while Kalbadevi is a congested commercial area and the sampling location is surrounded by tall buildings. Maximum benzene concentration is observed at Kalbadevi traffic junction. The observed concentration is thus not related only to the number of vehicles but also to meteorology, ventilation available, site built-up plan, age and type of vehicles, etc.

A short term (30 min) ambient air quality standard of $30 \mu\text{g}/\text{m}^3$ for benzene has been established in Texas, USA¹¹. If this standard is adopted for comparison of the results of this study, we find that benzene concentration in ambient air has exceeded this limit in all vehicular corridors in Mumbai city.

The high benzene concentration obtained in the present work indicates that though ambient concentrations of benzene may be low in urban settlements, kerbsides, roadside market places (common in Indian urban areas) and traffic intersections are areas of high benzene levels and a large number of people are likely to be exposed to benzene for short and long durations. The concentration of benzene in air cannot be related to only the number of vehicles. The ventilation available, age and type of vehicle and meteorology

also contribute significantly. Concentration of benzene at the traffic corridors should as well be studied along with that in ambient air on a continuous basis to safeguard the health of commuters, shopkeepers and shoppers.

1. USEPA motor vehicle related air toxic study. EPA-420 (R-93-005), US Environmental Protection Agency Office of Mobile Sources, Ann Arbor, Michigan, 1993.
2. Rinsky, R. A., Smith, A. B., Hornung, R., Filloon, T. G., Young, R. J., Okun, A. H. and Landrigan, P. J., *N. Engl. J. Med.*, 1987, **316**, 1044–1050.
3. Yin, S. N. *et al.*, *Br. J. Ind. Med.*, 1987, **44**, 124–128.
4. Ambient water quality criteria document for benzene. Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office (Cincinnati, OH) and Carcinogenic Assessment Group (Washington, DC) and the Environmental Research Labs (Corvalis, OR; Duluth, MN; Gulf, Breeze, FL) for the Office of Water Regulations and Standards, Washington, DC., EPA 440/5-80-018, 1980.
5. Motor vehicle-related air toxics study. Technical Support Branch Emission Planning and Strategies Division Office of Mobile Sources, Office of Air and Radiation, US Environmental Protection Agency, April 1993.
6. Mohanrao, A. M., Pandit, G. G., Sain, P., Sharma, S., Krishnamoorthy, T. M. and Nambi, K. S. V., *Atmos. Environ.*, 1997, **31**, 1077–1085.

7. Srivastava, P. K., Pandit, G. G., Sharma, S. and Mohanrao, A. M., Proceedings of the National Symposium on Environment, 2000, pp. 7–10.
8. Chattopadhyay, G., Samanta, G., Chatterjee, S. and Chakraborti, D., *Environ. Technol.*, 1997, **18**, 211–218.
9. Sirrioughomporn, P., Master's thesis, AIT, Bangkok, 1997.
10. Hussam, A., Alauddin, M., Khan, A. H., Chowdhury, D., Bibi, H., Bhattacharjee, M. and Sultana, S., *J. Environ. Sci. Health, Part A*, 2002, **A37**, 1223–1239.
11. USEPA, EPA-453/R-92-008, National Air Toxics Information Cleaning House, 1992.

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Microbially induced impact on physico-chemical properties of porous lime stones: A case study from Kandhar fort

Since ancient times all types of material have been used by Indians to make artifacts, from simple mono components to complex structures integrating inorganic and organic matter. Such artifacts, even if made with resistant stones and other materials, are influenced by environmental parameters. Historical monuments located specially in tropical wet and dry climates (10 – 20° of the equator) undergo the process of biodeterioration due to environmental factors such as high temperature, high relative humidity and heavy rainfall followed by winter which favours the growth and sustenance of a

variety of living organisms on stone surfaces. All these factors interact synergistically with constitutive stone materials (sandstone, limestone and marbles) and induce changes in their structural and physico-chemical properties^{1–5}.

Researchers have shown that bacteria on stone surfaces produce corrosive organic acids when exposed to pollutants, resulting in significant stone degradation. The scientific understanding of these processes remains limited and because of the major variables involved. It is difficult to assess the relative importance of microbial processes in microbial-induced

stone degradation^{6–9}. Exposure of historical monuments to extremely high concentrations of atmospheric pollutants like carbon dioxide, sulphur oxides, nitrogen oxides, particulate matter, ammonia, ozone, hydrogen fluoride and hydrogen chloride in recent decades has highlighted concern about these issues¹⁰.

Marathwada region of Maharashtra is famous for the caves at Ajanta and Ellora¹¹. There are a few monuments in the region; one of them is the historical fort at Kandhar, famous for its land fort ($180^\circ 50' \text{N}$, 10°E). Its construction is attributed to Rastrakuta Krishna III of

Malkhed in AD 941. Encircling the fort is a ditch 27.4 m in width and 4.6 m in depth filled with water¹². However, this historical monument has been forgotten by the concerned authorities. The influx of workers and visitors brought considerable amount of air pollutants into the fort. The indoor and outdoor climatic environment also created conditions for a massive growth of different microbes. The metabolic activities of these organisms like production of different extracellular polymers, liberation of chelating compounds and of organic/inorganic acids together with the presence of coloured pigments and the mechanical pressure exerted by growing structures or sinking/swelling phenomenon cause different types of damage to this historical monument. The present work focuses on the isolation and characterization of microbes present in the stones and the changes brought about in some of the physico-chemical properties by interaction with these microbes. An attempt has also been made to explore whether the bacterially induced carbonate mineralization could alter these properties, so that, in future, the technique could be applied to the conservation of historical monuments.

Porous limestone samples used as a sculpture monumental stone were collected aseptically during October 2005. The microorganisms were isolated and characterized by standard methods^{13,14}. The principal group of organisms identified qualitatively in the present study is the chemolithoautotrophs and chemoorganoautotrophs.

Chemoautotrophic sulphur oxidizing bacteria attack stones under aerobic conditions by producing inorganic sulphuric acid. Sulphuric acid reacts with the constituents of the stone to form sulphate crusts, precipitated within the pores of the stone, which upon recrystallization exert tremendous stress on the pore walls due to increase in volume, thereby causing damage to the stone^{15,16}.

Autotrophic nitrifying bacteria oxidize ammonia to nitrite and nitrate ions, which may result in nitric acid formation. Heterotrophic bacteria evolve biogenic acids with chelating properties and cause stone dissolution through mobilization of cations like Ca^{2+} , Fe^{3+} , Mn^{2+} , Al^{3+} , Si^{4+} , etc.¹⁷.

Cyanobacteria create variously coloured microbial films on stone surfaces and facilitate adherence of airborne particles of dust, pollen, oil and coal ash, giving rise to hard crust of patinas¹⁸. Respiration and photosynthetic reactions of cyanobacteria also produce acids as by-products causing etching of mineral components and dissolution of binding minerals^{19,20}.

The oxalic and citric acids excreted by various fungi act as a chelating agent thereby leaching the metabolic cations from the stone surface. Oxalic acid causes extensive corrosion of primary minerals and the complete dissolution of ferruginous minerals through formation of iron oxalates and silica gels^{21,22}.

Algae cause deterioration primarily by staining the stone surfaces resulting from different coloured pigments of the algae²³. Algae also produce organic acids which increase solubility of the stone in water, and alter the physico-chemical properties of stone²⁴⁻²⁶. Physico-chemical properties of stone furnish data from which a fair estimate of the durability may be made. The purpose of the study of these properties is to impose on the stone, conditions that in course of a few weeks will approximate the effect produced by actual use during a period of years. The physico-chemical properties of a monumental stone studied in the present investigation are specific gravity, water absorption capacity, aggregate crushing value and aggregate impact value before and after microbial attack. The values of the physico-chemical properties were determined using the relationship suggested by Duggal and Puri²⁷.

About 50 g of stone samples approximately spherical in shape were screened, having circular openings with 1.27 cm diameter. The samples were dried in an oven maintained at 110°C temperature, cooled to room temperature and used to determine the specific gravity. Then 50 g of stone was immersed in water for 24 h and immediately after removal from water, the surface of individual pieces was dried with a blotting paper and weight was recorded. The stones were then placed in a wire basket having ¼ inch mesh and suspended in water. The weight of sample immersed in water was recorded from the weight of water displaced by the sample stones and weight of empty basket suspended in water, the apparent specific gravity was then calculated. Specific gravity is an indicator of hardness and strength of stones.

Water absorption per cubic foot of the stone was determined as follows. About 30 g of spherical stone was dried in an oven for 1 h maintained at 110°C temperature and then cooled in a desiccator to attain the room temperature. The weight of the sample was quickly recorded in air and then in distilled water (25°C). The loss of weight on immersion was recorded and approximate weight of the test sample in water was then calculated by subtracting the predetermined loss of weight just after immersion from the weight in air and at the start. The stones were allowed to remain for 48 h in distilled water. The amount of water absorbed per cubic foot of the samples was determined by standard formula. Stones that have already begun to decompose absorb a much larger quantity of water than dense stones. A low absorption indicates good quality stones, while high absorption is more detrimental to degradation.

The aggregate crushing strength is a relative measure of the resistance or toughness of the stone crushing under a gradually applied compressive load or pressure. Aggregate crushing strength in

Table 1. Changes in physico-chemical properties of stones before and after microbial attack

	Before microbial attack	After microbial attack	After treatment with <i>Myxococcus xanthus</i>	Standard
Specific gravity (g/cm^3)	1.87	1.52	1.80	2.0
Water absorptive capacity (pounds/cubic foot)	4.86	6.33	4.36	7.0
Crushing strength (pounds/sq. inch)	8.63	5.38	3.98	10.66
Aggregate impact value (%)	25	22	23.8	30

pounds per square inch was determined using compression testing machine.

The aggregate impact value of stone is a measure of resistance to sudden impact or shock. The aggregate impact value was determined using Page impact machine. Properly dried (110°C) stones (10–12 mm) were used as test material.

Changes in the physico-chemical properties of stones before and after microbial attack are depicted in Table 1.

The microorganism *Myxococcus xanthus* as a source of calcium carbonate was isolated from sterile dung in contact with soil and characterized by standard methods²⁸. The *M. xanthus* inoculum was prepared according to the method of Gonzalez *et al.*²⁹. Biomineralization experiments were carried out in M-3P media [1% bacto casitone, 1% Ca (CH₃COO)₂, 0.2% K₂CO₃ in a 10 mm phosphate buffer pH 8] by taking 5 g of stone and 100 ml of culture medium in Erlenmeyer flask inoculated with 2 ml of *M. xanthus* inoculum culture. The Erlenmeyer flask was incubated at 28°C with shaking for 21 days. Control experiment identical to that described above was carried out without bacterial inoculation. After three weeks, the samples were rinsed three times with distilled water before drying at 37°C in a dark and dust-protected environment. Weight gain was calculated in terms of difference in weight between fresh and biomineralized stones at the end of incubation (Table 1).

1. Grieken, R. V., Delalienux, F. and Gysels, K., *Pure Appl. Chem.*, 1998, **70**, 1327–1331.
2. Jedrzejewska, H., In *The Conservation of Stone II, Part A* (ed. Manaresi, R. R.), Bologna, 1981, pp. 195–204.
3. Schaffer, R. J., *Chem. Ind.*, 1967, **23**, 1584–1586.
4. Jain, K. K., Mishra, A. K. and Singh, T., *Recent Advances in Biodeterioration and Biodegradation* (eds Garg, K. L., Neelima, G. and Mukerji, K. G.), Naya Prokash, Calcutta, 1993, pp. 323–354.
5. Tiano, P., In *Biodegradation of Cultural Heritage* (eds Garg, K. L., Garg, N. and

- Mukerji, K. G.), Naya Prokash, Calcutta, 1994, pp. 301–321.
6. May, E., Lewis, F. J., Pereira, S., Tayer, S., Seward, M. R. D. and Allsopp, D., *Cab Int.*, 1993, **7**, 109–123.
7. Urzi, C. and Kurmbein, W. E., In *Durability and Change: The Science Responsibility and Cost of Sustaining Cultural Heritage* (eds Krumbein, W. E. *et al.*), John Wiley, New York, 1994, pp. 107–135.
8. Webley, D. M., Henderson, M. E. K. and Taylor, I. F., *J. Soil Sci.*, 1963, **14**, 102–112.
9. Hueck Van Der plas, H. E., *Int. Biodeterior. Bull.*, 1968, **4**, 11–28.
10. Fassina, V., In *Air Pollution and Conservation* (eds Rosvall, J. and Aleby, S.), Elsevier, Amsterdam, 1988, pp. 133–174.
11. Agrawal, O. P., Dhawan, S., Garg, K. L., Shaheen, F., Pathak, N. and Misra, A., *Int. Biodeterior.*, 1988, **24**, 121–129.
12. Anil, K. and Arun, D., *Kandhar – The Capital of Rastrakuta*, Kalpana Prakashan, Nanded, 2005, pp. 183–184.
13. Buchanan, R. E. and Gibbons, N. E., In *Bergey's Manual of Determinative Bacteriology*, Williams and Wilkins Co, Baltimore, USA, 1974, 8th edn.
14. Alexopoulos, C. J., Mims, C. W. and Blackwell, M., In *Introductory Mycology*, John Wiley, New York, 2002, 4th edn.
15. Voute, C., UNESCO, Paris, Document no. 1241/BMF-RD/CLT.
16. Lepidi, A. A. and Schippa, G., In *Colloque International Sur la deterioration des pierres on oeuvre* (ed. Rochelle, L.), Chambéry, France, 1973, pp. 143–148.
17. Jain, K. K., Saxena, V. K. and Singh, T., In *Biodeterioration of Cultural Property* (eds Agrawal, O. P. and Dhawan, S.), McMillan, New Delhi, 1991, pp. 240–249.
18. Wilderer, P. A. and Characklis, W. G., In *Structure and Function of Biofilms*, John Wiley, Chichester, 1989, pp. 5–17.
19. Saiz, J. C., *Soil Biol. Conserv. Biosphere*, 1984, **2**, 757–767.
20. Viles, H. A., *Earth Surface Process Landforms*, 1987, **12**, 319–330.
21. Caneva, G. and Salvadori, O., In *Deterioration and Conservation of Stone* (eds Lazzarini, L. and Piper, R.), UNESCO, Paris, 1998, No. 16, pp. 182–234.
22. Eckhardt, F. E. W., In *5th International Congress on Deterioration and Conservation of Stone* (ed. Felix, G.), Lausanne, Switzerland, 1985, vol. II, pp. 643–652.
23. Saiz, J. C., In *Biodeterioration Research 4: Mycotoxins, Wood Decay, Plant Stress, Biocorrosion, and General Biodeterioration* (eds Llewellyn, G. C., Dashek, W. V. and O'Rear, C. E.), Plenum Press, New York, 1994, pp. 586–604.
24. Griffin, P. S., Indicator, N. and Koestler, R. J., *Int. Biodeterior.*, 1991, **28**, 187–207.
25. Bell, W. H., Lang, J. M. and Mitchell, R., *Limnol. Oceanogr.*, 1974, **19**, 833–839.
26. Garg, K. L., Mishra, A. K., Singh, A. and Jain, K. K., In *Conservation, Preservation and Restoration: Traditional, Trends and Techniques* (eds Kamlakar, G. and Rao, V. P.), Birla Archaeological and Cultural Research Institute, Hyderabad, 1995, pp. 31–38.
27. Duggal, A. K. and Puri, V. P., In *Laboratory Manual in Highway Engineering*, Wiley Eastern Limited, New Delhi, 1991, pp. 1–109.
28. Staley, J. T., Bryant, M. P., Pfennig, N. and Holt, J. G., In *Bergey's Manual of Systematic Bacteriology*, Williams and Wilkins, Baltimore, USA, 1984, pp. 2144–2147.
29. Gonzalez, M. M. T., Chekroun, K. B., Aboud, A. B., Arias, J. M. and Rodriguez, G. M., *J. Sediment. Petrol.*, 2000, **70**, 559–564.

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