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 monocomponents 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 synergetically with constitutive stone materials (sandstone, limestone and marbles) and induce changes in their structural and physico-chemical properties

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. 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 issues.

Marathwada region of Maharashtra is famous for the caves at Ajanta and Ellora. There are a few monuments in the region; one of them is the historical fort at Kandhar, famous for its land fort (180°50′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 water. 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. The principal group of organisms identified qualitatively in the present study is the chemo lithoautotrophs and chemooorganotrophs.

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.

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.

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 patinas. Respiration and photosynthetic reactions of cyanobacteria also produce acids as by-products causing etching of mineral components and dissolution of binding minerals.

The oxalic and citric acids excreted by various fungi act as a chelating agent thereby leaching the metallic 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.

Algae cause deterioration primarily by staining the stone surfaces resulting from different coloured pigments of the algae. Algae also produce organic acids which increase solubility of the stone in water, and alter the physico-chemical properties of stone. 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 Puri.

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

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

<table>
<thead>
<tr>
<th></th>
<th>Before microbial attack</th>
<th>After microbial attack</th>
<th>After treatment with <em>M. xanthus</em></th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity (g/cm$^3$)</td>
<td>1.87</td>
<td>1.52</td>
<td>1.80</td>
<td>2.0</td>
</tr>
<tr>
<td>Water absorptive capacity</td>
<td>4.86</td>
<td>6.33</td>
<td>4.36</td>
<td>7.0</td>
</tr>
<tr>
<td>(pounds/cubic foot)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Crushing strength (pounds/sq.</td>
<td>8.63</td>
<td>5.38</td>
<td>3.98</td>
<td>10.66</td>
</tr>
<tr>
<td>inch)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggregate impact value (%)</td>
<td>25</td>
<td>22</td>
<td>23.8</td>
<td>30</td>
</tr>
</tbody>
</table>
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 microbiological 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 methods. The *M. xanthus* inoculum was prepared according to the method of Gonzalez et al. Bimineralization 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 bimineralized stones at the end of incubation (Table 1).


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