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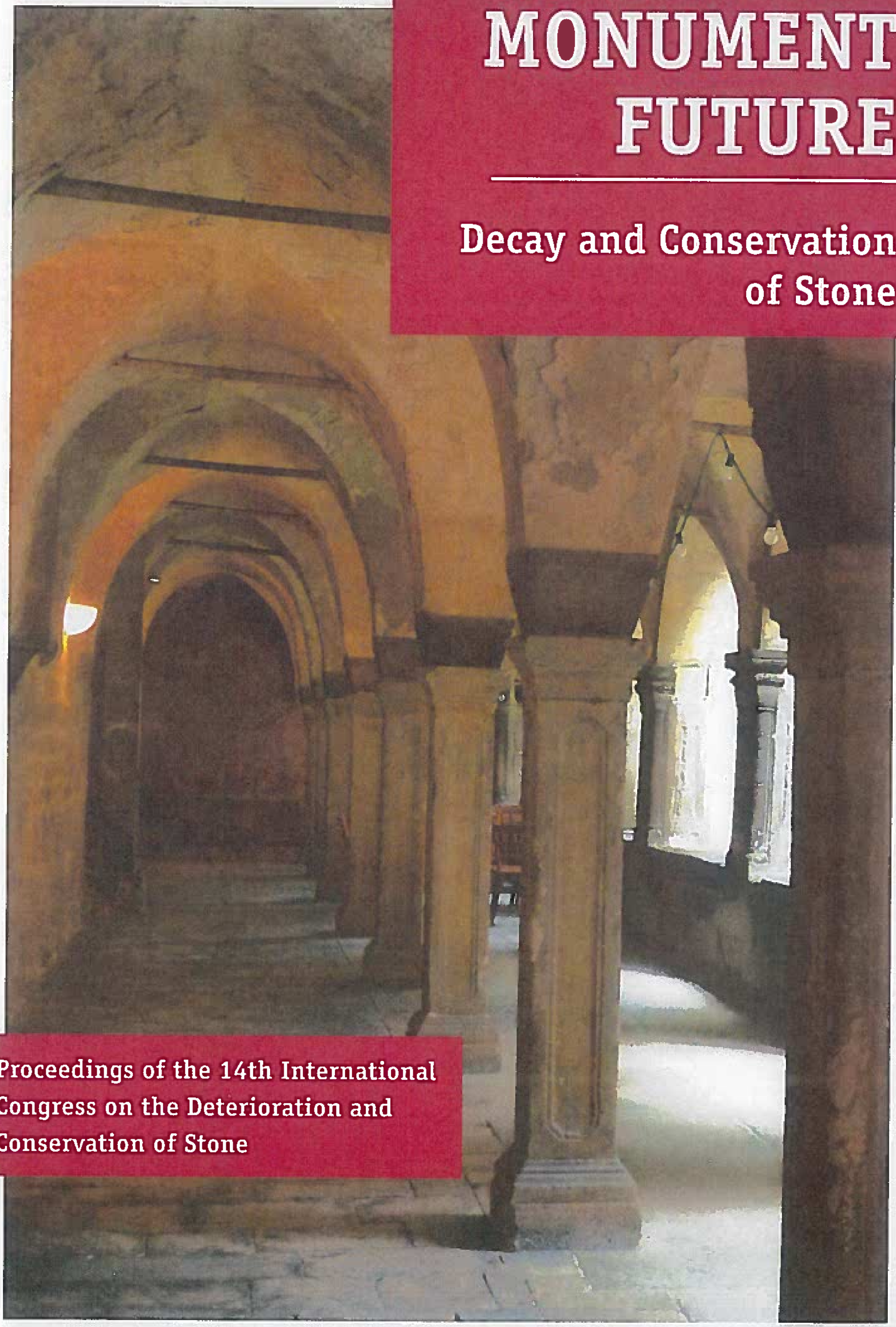
MONUMENT FUTURE

Decay and Conservation of Stone



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LIVE AND LET DIE? BIOFILMS ON STONE: POSSIBILITIES, LIMITS AND RISKS OF COMBATING

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Abstract

Biogenic growth on and in rock is one of the most important weathering phenomena. Algae, fungi, lichens and bacteria cause visual impairments as well as serious substantial damage to the rock. Treatments with toxins, surface coatings or hydrophobic treatments are not always effective and can even aggravate the damage. While superficial biogenic crusts are relatively easy to remove mechanically and by common rock cleaning techniques, deeper settlements are still an unsolved problem. It is therefore essential to understand how far microorganisms can penetrate different types of rock and how long it takes them to do so. In 2004, test specimens from different monumental rocks were therefore exposed on the grounds of the Charterhouse Mauerbach (AT) and the colonization by microorganisms was examined after 13 years of exposition.

Introduction

Microbes are successful invaders on all types of stone as well as on modern and historical buildings (Scheerer et al., 2009; Pinar & Sterflinger, 2009). Microbial decay processes cause both structural and aesthetic damage to stone. The formation of pigmented biofilms, biomineralization, dissolution of metals by acids and chelating agents, degradation of organic components and strengthening agents are some of the harmful phenomena caused by microbial growth. Bacteria and fungi produce a variety of pigments ranging from light yellow, orange, deep purple and green to dark brown and black. However, the formation of biofilms on and in building materials is not only an aesthetic problem for monuments. Bacteria and fungi are capable of producing a wide range of organic acids that attack carbonate and dissolve metal ions, resulting in significant loss of material or diagenesis of minerals. Not only limestone but also granite, feldspar, aluminosilicates and silicates, quartz and glass are attacked in their organic acids (Sterflinger, 2000).

While the massive influence of biology on the weathering of stones has been neglected for a long time, a reverse trend has emerged in the last three decades: Natural stone facades and stone sculptures are treated with biocide solutions as a matter of course along with restoration and maintenance measures, but also in the belief that they have a preventive effect; lichens and other biogenic crusts are mechanically removed and considerable material losses are often accepted. In the context of the presentation, these cleaning and poisoning measures, which are now almost standardized in monument conservation, should be critically questioned based on the following considerations: (1) How quickly do different natural stones develop biogenic colonisation in the Central European climate, which cleaning intervals have to be considered? (2) Is the use of biocides sensible and necessary and, if so, under what conditions and with what means? (3) Is the removal of biogenic crusts, in particular lichens, always necessary or do they only cause abrasive cleaning cycles? (4) What influence do surface coatings – sludge, frames – have on biogenic weathering? In order to achieve a first approximation of the answers to these questions a long-term experiment was planned on natural stones that have been exposed on the grounds of the Mauerbach Carthusian monastery (AT) since 2004.

Materials and Methods

In 2004, four test specimens made of stone – affectionately known to us as "Steinmännlein" – were erected in cooperation with the Federal Office for the Protection of Monuments in the courtyard of the Charterhouse in Mauerbach (Lower Austria) (Fig. 1): (1) Lindabrunn conglomerate, (2) Aflenz sandstone, (3) St. Margarethen sandstone, (4) Laas marble and (5) Carrara Marble. The selected lithotypes are important building- and decorative-stones in Vienna, which have a characteristic lithological composition with well-recorded petrophysical properties (table 1) (Rohatsch et al., 2014; Rohatsch, 2005). All lithotypes belong to the group of carbonates and consist mainly of the minerals calcite and subordinate dolomite. However, they differ not only in terms of their origin and their physical properties, but also in terms of the primary causes of damage. The cause of damage to the conglomerate from Lindabrunn is the frost sensitivity of the fine-grained sandy layers. The primary cause of damage to the porous calcareous arenas from St. Margarethen and Aflenz is the moisture circulating and lingering in the pore space. The primary cause of damage to the marbles is thermal alternating stress, which leads to the first loosening of the texture due to the anisotropic expansion behavior of calcite (e. g. Koch, Siegesmund 2004). Detailed physical properties of the investigated

Figure 1: Stone figures at the time of exposition in 2004.

lithotypes were described by Ban et al. 2016; Ban et al. 2019; Moshammer et al. 2015; Unterwurzacher, Mirwald 2008; Rohatsch 2005.

In Mauerbach the climate is moderate. Even in the driest month a lot of rain falls. The climate is classified as Cfb, according to the Köppen-Geiger classification. The average temperature in Mauerbach is 9.1 °C per year. Over a year the precipitation adds up to 658 mm. After 13 years of expostion samples were taken for microbiological investigation of (1) depth of colonization, (b) type of colonization.

The samples were taken with hammer and sterile chisel and then packed in sterile bags for transport and storage. Two samples were taken from each statue, one from the front and one from the back of the edge of the main plate. To determine the CFU (colony forming units/g rock), 1 g of sample was ground in a mortar. The sample was then transferred to 10 mL 0.001 % Tween80 solution and applied to culture media: 2 % MEA and rose bengal agar for fungi, lysogeny broth (LB) for heterotrophic bacteria. Molecular identification of fungi and bacteria was carried out based on sequencing of 18S and 16S DNA respectively according to Sterflinger et al. (2018). Databank search was carried out using the BLAST tool (Altschul et al., 1997). In order to visualize the the penetration depth of the biofilm a stainig with Peridic Acid Schiffs Reagent (PAS) was carried out. The technique spcifically stains carbonhydrates in biofilm resulting in a purple color change.

Results and Discussion

Microorganisms – including lichens, fungi, bacteria and algae – colonise natural stone to a depth of several mm. Once colonisation has taken place down to the pore space and the structure, complete removal of the infestation is no longer possible or would involve considerable loss of material. If the biofilms are removed on the surface, this often leads to the rapid formation of new crusts and coatings, as the organisms remaining in the stone – this is particularly the case with fungi and lichens – grow out of the rock again (Fig. 2) . This results in renewed cleaning cycles, which in turn can be associated with loss of material. Accompanying measures, such as consolidating or slurrying the surfaces, can aggravate the situation when biomass is trapped in the pore space, although the slurry is actually intended to protect the natural stone. The lower the water vapour diffusion and the higher the proportion of organic additives in slurries (e. g. cellulose, methyl cellulose, silicone), the more the microbiological colonisation in the pore space is favoured (Fig. 3). The use of biocides may be appropriate where colonisation in the pore space is present, but (a) it must be ensured that the penetration depth of the biocide is sufficient and (b) the biocide used is effective and does not exert selective pressure on the microbial community.

Deep settlement in natural stone is therefore an unsolved problem and – apart from the fact that many objects already have such a problem – such settlement could only be limited if cleaning measures were taken in time. At the same time, however, interventions in the object should be kept as low as possible in order to protect the material; a thoroughly tricky situation. For the reasonable scheduling of cleaning cycles it is therefore necessary to know how quickly microbiology penetrates the rock from above. This requires field studies over long periods of time.

After 13 years of exposure in the open-air site of the Kartause Mauerbach, all rock types showed a significant colonization with biofilms (Fig. 4). colonization was particularly pronounced on the horizontal and sloping surfaces. There, the water availability is higher than on the vertical surfaces, where the water quickly runs off, and thus the growth of organisms – including mosses – is favored. Strongest visible colonization was observed on those rock types with high porosity: Aflenzer and St. Margarethener sandstone.

Concerning the depth of colonization the stone types showed significant differences: Whereas on both marble types the colonization was limited to the surface (Fig. 5), on both sandstones the colonization after 13 years of exposition reached a depth of up to 16 mm (Fig. 6). In the Lindabrunner Konglomerate the biofilm follows the contact zones along the inclusions (Table 1).

The colony forming units/g of stone confirmed the results of the PAS stain. Both bacteria and fungi showed high numbers of CFU in the sandstones whereas the numbers on both marble types were 3–4 powers of ten lower than on porous sandstones and conglomerate. (Table 2). The extermely low cell counts in Carrara marble can be explained by the lack of pores in the marble. Carrara does not allow the easy penetration my microbes as porous stones do. Due to this the conditions for growth are rather unfavorable and it takes much more time for the microbes to establish on this type of material.

The isolation and enrichment of fungi and bac-

Figure 2: Carrara marble in Parco di Boboli (Florence, IT). Left uncleaned sculpture with dense lichen growth, right cleaned figure: in the close-up it is clearly visible that lichens are already growing out of the rock structure and form new colonies.

Figure 3: Sandstone decoration on the roof of the Hofburg (Vienna, AT): the slurry is being pushed away from below by fungi and lichen growing out of the pore space.

teria initially indicates a high diversity of fungi (Table 3). However, it must be said in restriction that the predominant proportion of fungi isolated from the rocks consists of transients (Table 3). It can be assumed that fungi of the genera *Phialophora, Cryptococcus, Rhodotorula* and *Ochrocladosporium* grow on the rock and – together with algae and cyanobacteria – are responsible for the dark encrustations on the surface of the rock (Fig. 7). The genera *Cyptococcus* and *Phialophora* are typical rock colonizers (Caretta et al, 2002) and, like the genus *Ochrocladosporium*, the fungi have dark pigmentation. The diversity of bacteria appears to be low compared to that of fungi. However, it must be noted that only bacteria that are capable of growing on common culture media have been detected. In the future, molecular biological methods (metagenomics) will be used to determine the diversity in greater detail (see for example Dyda et al, 2019; Piñar et al, 2019).

From the ecological point of view it is interesting that the fungal and bacterial microflora, as far as it was detectable by the classical enrichment technique, is dominated by organisms that are cryophilic or cryotolerant (cold loving or able to grow at very low temperatures). For example, the genus *Cryptococcus* contains fungi that occur on rocks of Antarctica, the fungus *Ochrocladsporium* sp. was originally isolated from a cold store (Crous, 2007). The bacterium *P. glaciei* originates from permafrost regions, E. undae was isolated in Antarctica and bacteria of the genus Pseudomonas are also known for their growth in cold environments such as cold storage cells. This occurrence of cryotolerant organisms – although sampling took place in summer – is certainly related to the relatively cool climate in Mauerbach.

The results of this study give us a first impression of the time it takes for biofilms to form, especially

Figure 4: Stone figures after 13 years of exposition.

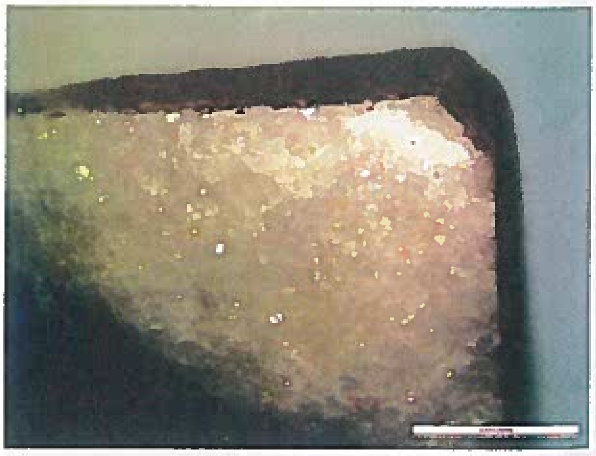

Figure 5: PAS stain of the biofilm on Carrara marble with practically no penetration of the stone (scalebar = 5 mm).

Figure 6: PAS stain of St. Margarethen sandstone (scale-bar = 5 mm).

Table 1: Colonization depth [mm] according to the PAS stain.
*due to natural fissures in the material

Lindabrunn	< 18*
Aflenz	16
Margarethen	12
Laas	< 1
Carrara	< 1

Table 2: Fungal and bacterial cell counts in the 5 rock types [cfu/g].

rock type	bacteria	fungi
Lindabrunn	5,2 × 106	1500
Aflenz	5,5 × 106	8300
St. Margarethem	8 × 107	8000
Laas	5,7 × 104	50
Carrara	2,7 × 103	0

Table 3: Fungal and bacterial species isolated from the 5 stone types. Species possibly established on an in the rock are printed in bold.

Fungal species	Bacterial species
Alternaria burnsii	Arthrobacter sp.
Ascochyta medicaginicola	Bacillus safensis
Boeremia exiqua	Exiguobacterium undae
Chalara sp.	Paenisporosarcina sp.
Cladosporium aspertulatum	Planomicrobium glacei
Cladosporum aspertulatum	Pseudomonas spp.
Cryptococcus sp.	
Cryptococcus tephrensis	
Cystobasidiomycetes sp.	
Didymella fabae	
Fomes fomentarius (tinder fungus)	
Fusarium oxysorum	
Hormonema sp.	
Lecanicillium sp.	
Mortierella sp.	
Mucor sp.	
Mycosphaerella sp.	
Ochrocladosporium sp.	
Penicillium sp.	
Phialophora sessilis	
Rhodotorula sp.	
Sarocladium strictum	
Sordariomycetes sp.	
Sporidiobolales sp.	
Ustilago spp.	
Valsa cypri	

in natural stone. It is clearly visible that the depth of settlement is determined individually by the respective type of rock and its physical conditions – in the first instance this will be the water balance. The surrounding climate, on the other hand, is responsible for the type of colonisation and the biodiversity.

Conclusions and outlook

In ancient Greece, there was a special class of professionals for sanctuaries and temples, the kosmetoi, who were responsible for the regular cleaning of deity statues (Casiello, 2008). This special care named "kosmesis", was considered the most delicate moment of conservation and could be defined practically as a kind of 'therapy' for sculptures and was carried out in a kind of ceremony, the therapeèia (Holleaux, 1890). We should take this historical fact as an opportunity to be aware of the importance of every intervention on the historical object and treat the objects with appropriate restraint and respect. In order to be able to take appropriate cleaning measures, it is absolutely necessary to consider each type of rock individually, taking into account the respective climatic conditions. To deepen the initial insights gained in this study, we plan the following: (1) Continuation of the current long-term experiment, (2) Metagenomic analysis for complete coverage of biodiversity. (3) Preparation of petrographic thin sections for

Figure 7: Black biogenic crust on Aflenz sandstone (scale-bar = 5 mm).

analysis of the rock structure and possible structural changes.

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