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Preface

Microbial life on building façades is a worldwide known phenomenon and has often led to controversial discussions between scientists, architects and industry. On the one hand growth of microorganisms may be regarded as a decorative element on historical buildings, on the other hand it may represent stages of ruination and corrosion of building surfaces and is therefore recognized as a nuisance that should be eliminated. To understand microbial growth on façades comprehensive knowledge of its diversity, dynamics and ecology is required. This can help us to develop effective management and mitigation strategies. The diversity of organisms on buildings is of much broader variety than reflected in most discussions in which it is reduced to algal components only. Besides algae also fungi, lichens, mosses and other groups even of higher plant and animal life forms are members of the associations of pioneer organisms on such extreme habitats like façades. Numerous authors have discussed countermeasures against growth on buildings despite the vague knowledge of the biological background. Still an uncertainty remains regarding the composition of different types of growth and especially the very beginning of microbial colonization on man-made structures. Since 2001 when W. Hofbauer was appointed as scientific collaborator at the Fraunhofer Institute for Building Physics (IBP) in Holzkirchen, he started studying aeroterrestric algae and fungi on different external building components. During various projects into the beginning of microbial growth – initial colonization – specimens were exposed at the institute's outdoor area. Isolation and cultivation of microorganisms for taxonomic analysis as well as ecological experimental work resulted in W. Hofbauer's doctoral thesis (supervised by G. Gärtner) and are the main component of this book. G. Gärtner, who is an expert specialized in aeroterrestrial algae and their cultivation, participated in the taxonomical identification and provided support with cultures for comparison and literature.

Together with the descriptions of morphological and ecological data of the analysed organisms, the book provides information on chemical and physical processes of façades as well as notes on different methods for preventing growth on external building components. A dichotomous key for identifying micobial layers on building surfaces, a glossary of technical and biological terms and a broad list of references are also included.

Thanks are due to Werner Kofler for preparing scanning electron microscope (SEM) pictures, to the Fraunhofer IBP for support and to the Deutsche Bundesstiftung Umwelt (DBU) for funding a part of the investigations. Special thanks go to Maya Stoyneva for valuable discussions and to the editor Stephanie Wolf for her great patience.

The authors hope that the book will be of interest not only to specialists in aeroterrestrial organisms, but also provide technicians and building engineers with an essential reference to manage a biological phenomenon, which has been and is a part of our life since centuries.

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Introduction

Façades Colonized by Aerophytic Microorganisms

Since man-made buildings exist on earth, specialized microorganisms have found habitats on them, developing in different ways from positively accepted appearances as natural beauty to negatively conceived effects of deteriorating surfaces up to the destruction of artists' and architects' craft. In recent times, a lot of scientific research has been done, dealing with various aspects of microbial growth on man-made structures. Only a small minority of the enormous amount of publications dealt comprehensively with the whole spectrum of occurring organisms on modern building surfaces and almost none focused on the very beginning of the microbial colonization, the *primary colonization*, as we call it.

Façades as Functional Part of a Building

The façade of a building as the outermost layer of the construction has important protective functions, e.g., against driving rain or freezing conditions. But these are not the only functions of a façade, and it usually is also an element of decoration and design. Regarding the design and style of façades, there are many regional differences which are typical constituents of the flair of villages in different landscapes and economic zones. Usually, there are not only differences between geographical regions but also between rural and suburban or urban areas. The state of the art of house construction and the regional design are under continuous development, and the used materials and technologies are permanently optimized.

In our time, legal regulations have interfered in the design process substantially via, e.g., the German thermal insulation ordinance ("Wärmeschutzverordnung"). Producers

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as well as manufacturers have reacted accordingly in an adaptation of their products and techniques. In order to protect buildings as much as possible against loss of warmth and energy, different highly performing systems have been developed. Examples are among others: external thermal insulation compound systems (ETICS), facings with air space, insulated bricks and insulating mortars—all these may additionally be composed of different materials.

The Environment Façade

Physical structures, substrate chemistry and environmental conditions define a façade as an extreme environment. It can be colonized by organisms which endure the often harsh conditions. In view of the fact that approximately 99% of the whole biomass of all ecosystems on land and in water is produced by photosynthetic organisms (Larcher 2001; Raven et al. 2005), it seems feasible to have a close look on plant forms which are a main part of the initial growth on buildings as a base for further biological succession.

To what extent components of building coatings may support growth of organisms and how the pioneer phase proceeds has scarcely been investigated and is still discussed controversially. Not only algae are capable of colonizing the outer surface of buildings, as a rule complex biocoenoses consisting of various organisms (algae incl. Cyanoprokaryota, fungi, animals, etc.) are established.

Development of lichens and mosses on monuments or ancient buildings is a well-known and accepted phenomenon. The composition of microbial growth interfering with materials/substrates (mainly degradative but also protective) has been analyzed and documented mostly for historic buildings, monuments and in nature (e.g., growth on stone) (e.g., Richardson 1975; Krumbein and Jens 1981; Del Monte et al. 1987; Galun 1988; Jones 1988; Sabbioni and Zappia 1991; Nimis et al. 1992; Caneva 1993; Piervittori and Laccisaglia 1993; Crispim and Gaylarde 2005; Khobragade et al. 2006, Darienko et al. 2013).

Potential destructive influences of microbial growth on building surfaces are of vital importance for the assessment of the situation and for the design of countermeasures. In general, a visible microbiological colonization of a façade within the first few years after construction is seen as problematic and discussed under different points of view (e.g., Richardson 1975; Caneva 1993; Bagda et al. 1999; Saiz-Jimenez 1997; Künzel 2000; Künzel and Sedlbauer 2001; Sedlbauer and Krus 2001; Sedlbauer 2002; Hladik 2003; Rindi and Guiry 2004; Hofbauer et al. 2005a, b, c, 2006; Crispim et al. 2006). Recently, the growth of Cyanoprokaryota, algae, fungi and lichens on external walls has increased dramatically. This is also due to increased insulation and the thereby reduced drying potential of wet external walls. Surfaces of insulated walls, e.g., of ETICS which are loaded by dew or driving rain, may remain wet for a longer period of time which favors biological growth (Künzel and Sedlbauer 2001; Sedlbauer 2002). Different studies indicated that a change in the quality of outside air (e.g., less SO_2 content) enhances

the growth of certain aerophytic microorganisms (Hawksworth et al. 1973; Bates et al. 1990, 1996, 2001; Farmer et al. 1991, 1992; Gilbert 1992; Künzel 2000; Hauck et al. 2001, 2002; Hauck 2003, 2005; Schnug et al. 2004). An increasing eutrophication of the atmosphere (e.g., through increase of nitrogen compounds and hydrocarbons) and also climatic processes ("global change") are additionally recognized as factors which may favor the colonization of external building surfaces (Leathy and Colwell 1990; Cerniglia 1993; Pitcairn and Fowler 1995; Pitcairn et al. 2006; Ortega-Calvo and Saiz-Jimenez 1996; Saiz-Jimenez 1995, 1997; Leith et al. 1999, 2001; Mitchell et al. 2004; Raven et al. 2005). Frahm (2008) stated that the eutrophication of the atmosphere is also connected to the catalysts used in car engines. Microbial growth on building surfaces, which is relevant for damage cases, usually consists of different organisms. Not one single form (one alga or one fungus) alone is responsible for the perceived damage, but microbial growth is almost always caused by different organisms. Avoidance or reduction of unwanted growth is demanded, not only in a commercial view but also in a sustainable use of materials. Substantial growth on façades demands early and expensive renovation measures. In the long term, material damage or optic defacement and therefore greater use of materials cannot be ruled out. Added biocidal substances or chemical cures according to evident experiences have a certain time of action but do not last in the long term. Furthermore, if washed out, they may harm the environment. Common regulations within the EC result in a remarkable limitation in the choice of available biocides.

Within the scope of this book, general results regarding the diversity and ecophysiological parameters of biological growth on external building parts are presented.

Because of the rapidly and permanently changing conditions of temperature and moisture, building surfaces must be regarded as extreme environments. The constituents of biological crusts occurring in such environments are well equipped to face harsh conditions. They can withstand, e.g., extreme temperatures and other adverse influences. A filamentous soil crust alga (*Zygnema* sp.) was demonstrated to be insensitive to experimental UV (from 280 nm upwards) exposure (Holzinger et al. 2009).

History of Aerobiology in Respect of Research on Man-Made Surfaces

The history of the aerobiology is connected both to the development of microscopic instruments and techniques as well as to the establishment of laboratory cultures, as shown by Sitte et al. (2002). In the nineteenth century, Ferdinand Cohn, the founder of bacteriology, was able to keep *Haematococcus* (Chlorophyceae-Volvocales) in his laboratory in Breslau for a certain time. He named this process "cultivation" (Cohn 1850). The Russian plant physiologist Famintzin used for the first time Knop's solution for the cultivation of algae (Famintzin 1871). This culture medium with some inorganic compounds was developed by Knop for research on vascular plants in 1865 and is still in use today (Preisig and Andersen 2004). Aerobiology, as the study of aerophytic

microorganisms, was mainly influenced by the classical research of Louis Pasteur and Robert Koch (Deichfelder 1985) at the end of the nineteenth century, confirming the distribution of microorganisms by air. Further important steps in the research on aerophytic microorganisms were the foundation of culture collections, as was done by Chodat (e.g., 1913, 1928) and Pringsheim (1924), and the technology of maintaining isolated microorganisms, especially algae (Pringsheim 1954, Preisig and Andersen 2004). A specialized collection of aerophytic (soil, airborne and lichen) algae was established in Innsbruck based partially on the collections made by W. Vischer in Basel, Switzerland (Gärtner 2004).

Aerophytic cryptogams, with emphasis on aerophytic algae, were investigated by Puymaly (1924) in France. Aerophytic green algal layers and their components were studied by Brand (Brand and Stockmayer 1925) and aerophytic biocoenoses on rock and cryptogamic epiphytic coenoses including algal associations by Barkman (1969). For taxonomic studies of eukaryotic aeroterrestrial algae and phycobionts, see Ettl and Gärtner (1995, 2014).

The Fraunhofer Institute for Building Physics (IBP, founded 1929 as an institute for technical physics) in Holzkirchen/Bavaria is specialized in the investigations of building materials and constructions, and for many years the colonization of materials and constructions with microorganisms has been intensively studied.

Our Study on Aerophytic Organisms on Building Surfaces

In an interdisciplinary study (see also Hofbauer et al. 2006), we undertook many different investigations in connection with the initial biological succession on modern building surfaces. The applied research work was mostly carried out in the years 2002–2007. Apart from newly constructed specimens, exposed at three different study sites in Germany (Holzkirchen, Heggen/Finnentrop, Ernsthofen/Oberramstadt), also old specimens exposed for 10 years and many additional growth situations (Sect. 2.1) as well as background concentrations were measured. Qualitative and quantitative microbiological analyses as well as continuous observations of newly built specimens at different outdoor weathering stations were the main emphasis of the investigations (Sect. 2.2–2.4). Furthermore in cooperation with colleagues, also data of the structural–physical characteristics of the different building materials were gained (Sect. 4.2). Our work presents the first comprehensive investigation of the initial succession on modern building surfaces. Occurring organisms were differentiated as far as possible (especially algae, Cyanoprokaryota, fungi, bryophytes and lichens) and compiled with chemical–physical measurements and data from the literature (Chap. 3). The most important species of the initial succession were documented. In the course of our investigations, more than 220 different taxa were identified as part of the initial succession (see Sect. 4.1). The biggest part of the diversity was provided by algae and Cyanoprokaryota (ca. 85 species), followed by fungi (ca. 80 species). The remaining taxa were allocated to further groups of organisms. Remarkably also bryopsida (ca. 12 species) and lichens (ca. 15 species) contributed to the initial growth. Fungi were discovered to be prominent in the

initial phase of surface colonization on modern building surfaces, especially intensely pigmented forms like melanogenous fungi and Coelomycetes. Algae and Cyanoprokaryota were present after a certain lag phase. According to the given microclimatic conditions on the surfaces, especially the water availability mainly through dew or by high relative humidity, eukaryotic algae were favored and only few Cyanoprokaryota occurred. Surface growth was mostly dominated by aerophytic green algae (especially Trebouxiophyceae) and strongly pigmented fungi (dematiaceous fungi and Coelomycetes) on new specimens after 2 and 3 years' exposure, in additional growth situations and on old specimens. Overall, a correspondence existed between the different measurements regarding microclimate, local climate, material characteristics and the identified organisms. Growth was more intense on materials which stayed moist for a longer time or which contained more nutrients. Growth diminished on materials which were characterized by strong chalking. It became obvious that the contribution of nutrients to the surrounding environment plays an important function in initial succession. A further important result was that microbial growth develops in cycles. During the dry seasons (summer, winter), surface growth stagnated or decreased; whereas in the cool and damp seasons especially in autumn/early winter, a distinct increase of growth development happened. Furthermore, we demonstrated that driving rain is an important factor in the propagation and settlement of microorganisms involved in the initial succession on outer building surfaces. For all identified organisms, detailed data regarding taxonomy and physiology are provided (Chap. 3). The gained data are an important base for future work on initial succession on façades and can also be used to design effective countermeasures against "unwanted microbial growth". Target organisms for potential chemical measures were better defined. An ample culture collection of microorganisms relevant to building parts has been established. In the course of our study, more than 400 isolates were acquired and integrated into the collection. Our study clearly demonstrates that the control of moisture on building surfaces is of crucial importance. This can be reached by different measures and strategies. As the important organisms were determined also their needs regarding moisture, temperature and further influence factors were defined. The observed algae are known to be active in a range of relative air humidity of 68–100%; the fungi start at ca. 73.3% relative humidity. The upper temperature limits lay at 57 °C (active) or even at 100 °C (dormant stage). The lowest temperature for physiological activity of the identified organisms is ca. −15 °C. In Chap. 3 also the pH limits, tolerated salt concentrations, substrate specifications, etc., of the different observed forms are given. As examples, *Trentepohlia iolithus* (Ulvophyceae, Chlorophyta) and *Sarcogyne regularis* (Lecanoromycetidae, Ascolichenes) showed a preference for mineral surfaces (both species showed up in the second year of outdoor exposure or on older surfaces only). The gained data also offer an option for extended and enhanced accuracy of mathematical models in the assessment of damage tolerance/resilience of new materials and constructions regarding microbial growth.

Tools and Methods

For the experimental investigation and the analysis of *primary growth* (=initial growth) on building surfaces as conducted in the study by the authors, different methods were applied. In this chapter, we describe our investigation methods as well as some further or different approaches to assess microorganisms on modern building structures. Furthermore, testing methods for susceptibility of building products against microbial growth are discussed.

Specimens–Weathering Exposure–Locations

Specimens: To investigate the settlement and development (succession) of growth on surfaces of different ETICS, a special kind of specimen was created and exposed at different outdoor locations in Germany (Hofbauer et al. 2006). The structure of the specimens comprised the complete layer sequence of a real ETICS (from top to bottom: paint—top plaster—reinforcement and ground plaster—insulation layer). In all used materials, film conservation was excluded. Additionally, specimens were sealed on the side by a double layer of an epoxy coating. To reduce side effects by run-off water or stagnant water, the top of the specimens was slanted and at the lower end a drip edge was attached. The dimension of the specimens was ca. 35 × 30 cm. We did not provide the wall building material (e.g., concrete or brick) but glued the insulation layer onto a tile which functioned as a strengthening part and attached the specimens onto a supporting construction. Thus, the specimens were subjected to the influence of the outdoor climate from all directions and did not get any protection of a back wall. Real insulated ETICS walls may get tiny proportions of warmth from the room behind but rough calculations revealed that this effect could be neglected. The general composition of the specimens is illustrated in Fig. 2.1.

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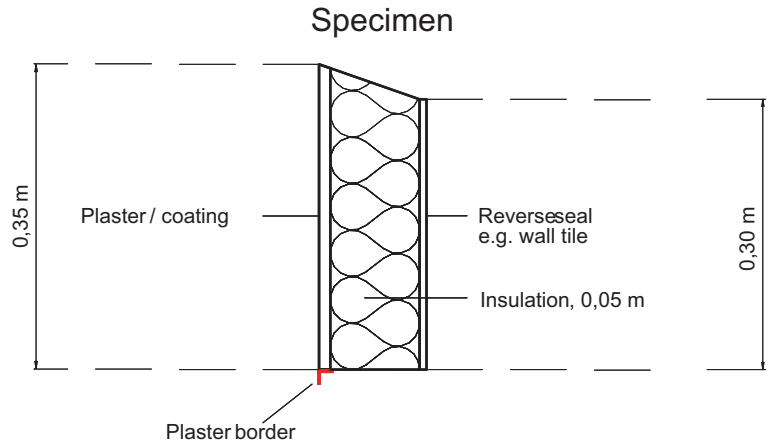

Fig. 2.1 Schematic structure of a specimen (Hofbauer et al. 2006; Hofbauer 2007)

Overall, the conditions on the surface of the specimens were slightly more challenging for the material itself and slightly more favorable for microbial growth than real wall constructions. Therefore, the results are reliable in respect of the long-term resistance of surface materials against growth. Altogether, 15 different variants were investigated comprising three different material groups (Table 2.1 for variants exposed in Valley/Holzkirchen).

For each variant, 18–21 replicates were produced, altogether about 300 specimens were involved in the investigations. In Valley/Germany, 10 specimens for each variant were exposed supplemented by extra specimens of some variants with "model character" which had attached PT100 thermoelements on the top layer to measure surface temperature and gain additional information on dew point shortfall. Since the attachment of thermoelements meant some disturbance of the surface, these specimens were excluded from the biological assessment. At additional locations (see below), four specimens of each variant again supplemented by "model specimens" with thermoelements were exposed. Specimens were attached to the supporting structure in approximately breast height (for better assessment of the surface) facing exactly west, and their angle was absolutely vertical. The outdoor exposure started in June 2002 (Fig. 2.2). In order to protect specimens against excess bird influence, a thread was attached above the specimens, as birds tend to sit on projecting structures in the field and leave droppings. Materials were varied in order to gain information about different factors which potentially influence the establishment of microbial growth. Generally, the surface was created in the typical way of a structure plaster with a structure kernel size of 2 mm, in one variant this was only 1 mm and one variant had a so-called scratched surface. Some variants had additional paint as finish, and some were without. For paints, also color and hydrophobicity were varied. Further, we had variants with super hydrophobicity and with infrared (IR) effect. Specimens in Holzkirchen were assessed (biological surface development) in a monthly or two monthly cycle, respectively.

Table 2.1 Overview of "new" variants exposed in Valley/Holzkirchen (main location) and in Heggen/Finnentrop and Ernsthofen/Oberrramstadt (exception: variants NV7–NV9 were only exposed in Holzkirchen)

Term	Plaster system	Top coat/color
NV1	Mineral thin	Dispersion silicate paint 1, purple, reduced hydrophoby
NV2	Mineral thin	Dispersion silicate paint 2, white, reduced hydrophoby
NV3	Mineral thin	Silicone resin paint 1, white
NV4	Mineral thin	Silicate paint 2
NV5	Mineral thick	Silicate paint 1, white
NV6	Mineral thin	Dispersion silicate paint 1, white
NV7	Mineral thin	Colored paint 1, gray
NV8	Mineral thin	Colored paint 1, gray, additional IR effect
NV9	Mineral thin	Silicone resin paint 3, white, super hydrophoby
NV10	Mineral thin	–
NV11	Mineral thin, structure kernels only 1 mm	–
NV12	Mineral thick, scratched surface	–
NV13	Silicone resin	–
NV14	Silicone resin	Silicone resin paint 1, white
NV15	Silicone resin	Silicone resin paint 2, white, super hydrophoby

Fig. 2.2 Exposition of the specimens at the main location in Valley/Holzkirchen shortly after the start. Above the specimens, bird protecting threads can be recognized (Hofbauer et al. 2006; Hofbauer 2007)

"Old" specimens: In addition to the newly built specimens, a double set of older specimens (already exposed to the local climate in Valley at an angle of 60° for 10 years [since 1992]) which remained of an earlier project were used. The structure of the "old" specimens was similar to the newly produced specimens apart from the following two differences: They were not slanted on top and did not have a drip edge. Some of the "old" specimens originally had film conservation, but it was only known which ones were equipped with it not the composition of the biocides. These older specimens were very useful in giving information on successional stages of growth development, and they also allowed us to evaluate and optimize methods of assessment since they were available from the beginning of the study. Eleven different variants of "old" specimens were chosen and biologically assessed (Table 2.2).

Whole wall constructions: For comparison and practical issues, a choice of different system compositions was installed on a whole building wall at the institute, again facing west (Fig. 2.3). The chosen system compositions (Table 2.3) mostly comprised the "model systems," and they were equipped with temperature sensors. Biological assessment was performed in areas not disturbed by the installed temperature sensors.

Additional locations: In order to have some variation in weather condition, additional locations for weathering exposure were chosen: Sauerland–Heggen at Finnentrop (outdoor area of company Weber and Broutin, Fig. 2.4) and Odenwald–Bergstraße–Ernsthofen at Oberramstadt (outdoor area of company Deutsche Amphibolin Werke, Fig. 2.5). Both

Table 2.2 Overview of the composition of "old" specimens

Term	Plaster system	Binders	Biocidal equipment
OV1	Synthetic resin plaster	Styrolacrylate Vinyl ester-terpolymer	+
OV2	Synthetic resin plaster	Styrolacrylate Vinylacetat/ethylene/vinyl chloride-copolymer	+
OV3	Mineral thin	Calcium silicate	–
OV4	Mineral thin	Lime–cement plaster	–
OV5	Mineral thin	Lime–cement plaster	–
OV6	Mineral thin	Lime-cement plaster	–
OV7	Mineral thin	Lime-cement plaster	–
OV8	Mineral thick	White lime hydrate white cement	–
OV9	Silicone resin plaster	Silicon resin emulsion KH dispersion	+
OV10	Silicate plaster	Potassium water glass Styrolacrylate	–
OV11	Silicate plaster	Potassium water glass Styrolacrylate	–

Fig. 2.3 View of the whole wall construction with attached ETICS facing west (Hofbauer et al. 2006; Hofbauer 2007)

Table 2.3 Variant specimens mounted as whole wall constructions with the same material types as used for new specimens

Term	Plaster system	Paint/color
NV1	Mineral thin	Dispersion silicate paint 1, purple, reduced hydrophoby
NV2	Mineral thin	Dispersion silicate paint 2, white, reduced hydophoby
NV3	Mineral thin	Silicone resin paint 1, white
NV4	Mineral thin	Silicate paint 2
NV5	Mineral thick	Silicate paint 1, white
NV6	Mineral thin	Dispersion silicate paint 1, white
NV7	Mineral thin	Colored paint 1, gray
NV8	Mineral thin	Colored paint 1, gray, additionally IR effect
NV9	Mineral thin	Silicone resin paint 3, white, super hydrophoby
NV10	Mineral thin	–

additional locations have a different climate compared to Valley/Holzkirchen with a tendency to be warmer and with more relative humidity. In contrast to the site in Holzkirchen, the additional locations are situated in depressions not far from running water. Therefore, some differences in the surface growth development were expected. Simultaneous to the installation of the specimens at the main exposure site also the exposure at the additional locations was started. The frequency of the surface assessment at the additional locations was for logistical reasons set at quarterly intervals.

Fig. 2.4 Exposition of the specimens in Heggen/Finnentrop (Hofbauer 2007)

Fig. 2.5 Exposition of the specimens in Ernsthofen/Oberramstadt (Hofbauer 2007)

Analysis of Microbial Growth

A major goal of our investigations was to systematically assess the composition and time course of the biological processes at the newly produced surfaces. Apart from taxonomical/systematical microbial analyses, different microscopic techniques (e.g., light and electron microscopy) were conducted.

A quantitative germ count was performed at defined intervals (½, 1 and 2 years). Special emphasis was laid on the qualitative composition and taxonomic background of the developing initial succession. This was also documented in defined intervals (½, 1,

2 and 3 years) and done for all three locations. Additionally, the qualitative assessment was compared to further analyses of established growth on building structures and on the older specimens. Visual growth development and pattern of initial growth were documented by a newly developed assessment method including a rating scale (Hofbauer et al. 2003) in monthly intervals.

Quantitative Biological Analysis

Visual Assessment

A special method was created on the base of synsystematic vegetation assessment to record the precise start of visible growth and developmental patterns (Hofbauer et al. 2003). Therefore, the specimens or part of wall areas were scanned, and the developed growth was rated by a special scale (Table 2.4). To avoid side effects, the upper

Table 2.4 Visual rating scale for assessing growth intensity on building surfaces. (Hofbauer et al. 2006; Hofbauer 2007; Künzel et al. 2011)

Rating figure	Rating symbol	Microbiological growth covering examined area on an average of
10	**+++++**	**Very intense**, almost the whole area is overgrown, >87.5–100% of the total area covered by clear surface growth or soiling
9	**++++(+)**	Surface growth/soiling **between intense and very intense**, >75–87.5% of the total area covered by surface growth or soiling
8	**++++**	**Intense** surface growth/soiling, >62.5–75% of the total area overgrown or soiled
7	**+++(+)**	Surface growth/soiling **between clear and intense,** >50–62.5% of the total area overgrown or soiled
6	**+++**	Surface growth/soiling **clear,** >37.5–50 % of the total area overgrown or soiled
5	**++(+)**	Surface growth/soiling **between moderate and clear,** numerous dots or marks, overgrown area >25–37.5%
4	**++**	**Moderate** surface growth, numerous dots or sparse spots, >5–25% of the total area affected by surface growth and soiling
3	**+(+)**	**Low** surface growth, some dots or marks, in all less than 5%
2	**+**	**Very low** surface growth, two to few single spots (clearly visible but less than 5%)
1	**(+)**	**Minimal** surface growth, 1–3 small single spots
0	**-**	No visually recognisable surface growth (0%)

4 cm of the specimens (slant) and the lower most 2 cm (plaster rim) were excluded. Differentiation of the scale is finer in the lower ranges in order to assess small differences in starting growth patterns, whereas higher ranges of the scale are oriented at the covered surface. This is in contrast to ASTM D3719-00 (2000) which only assesses growth on a linear scale based on the covered area. Emerging growth thus was regularly scanned on a monthly and bi-monthly (Valley/Holzkirchen) and half-year (Oberramstadt/Ernsthofen; Finnentrop/Heggen) basis, respectively.

In Figs. 2.6, 2.7 and 2.8, examples for the rating are given. In addition, growth patterns were assessed and documented by photography and by the aid of a template foil, which was used to transcribe the pattern on corresponding sketches (Fig. 2.9), in order to observe the development of the growth pattern in detail. Complementary to the regular assessment of the specimens, the analogous assessment was also done for the trial walls.

Fig. 2.6 Rating scale level + with detail of observed growth (Hofbauer et al. 2006; Hofbauer 2007)

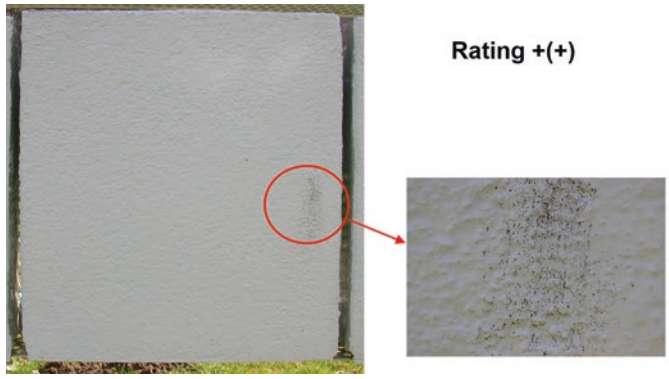

Fig. 2.7 Rating scale level +(+) with detail of observed growth (Hofbauer et al. 2006; Hofbauer 2007)

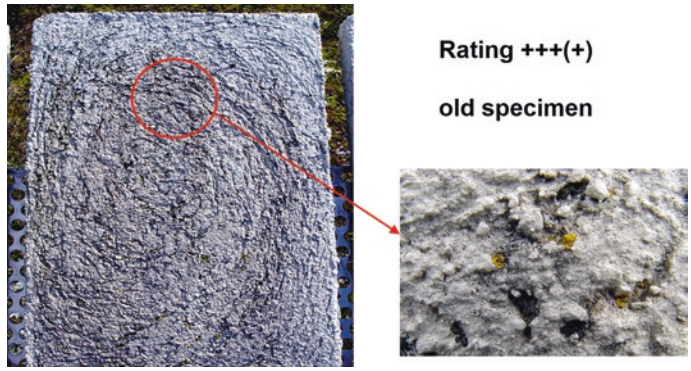

Fig. 2.8 Rating scale level +++(+) with detail of observed growth (Hofbauer et al. 2006; Hofbauer 2007)

Sample Collection and Preparation

At first, a set of "old" specimens (exposed for 10 years) was collected. Regarding the newly exposed specimens, a time scheme for collecting samples was established: Samples were taken after ½, 1 and 2 years of exposure. Specimens were taken into the laboratory in a dry state (not wetted by precipitation) for further processing. Of a representative area of the surface of a subsample, the size of 5×5 cm^2 was cut out in the thickness of the whole plaster and paint layers using a stone cutting saw. This was performed under conditions to avoid additional contamination and cross-contamination. The blade of the stone cutting saw was cleaned and disinfected with 70% ethanol or isopropanol before each specimen. For transport and/or temporary storage, the cutout samples were immediately transferred into fresh and sterile ziplock bags. Remaining parts of the old specimens were transferred back to the outdoor site as backup.

Further processing of the samples was done in a microbial laboratory under sterile conditions. Storage of samples, if necessary, took place under dark, dry and cool (room temperature) conditions.

Isolation of Microorganisms

Isolation of microorganisms was performed analogous to Berner et al. (1997) or Sterflinger and Prillinger (2001), respectively. Samples were cautiously manually grinded in an enamel mortar under laminar flow. This yielded a homogenous sample powder with grain size $\leq$0.1–0.5 mm and ensured the greatest possible recuperation of viable colony-forming units (CFU) under the aspect of more or less difference of hardness and elasticity of the sample material.

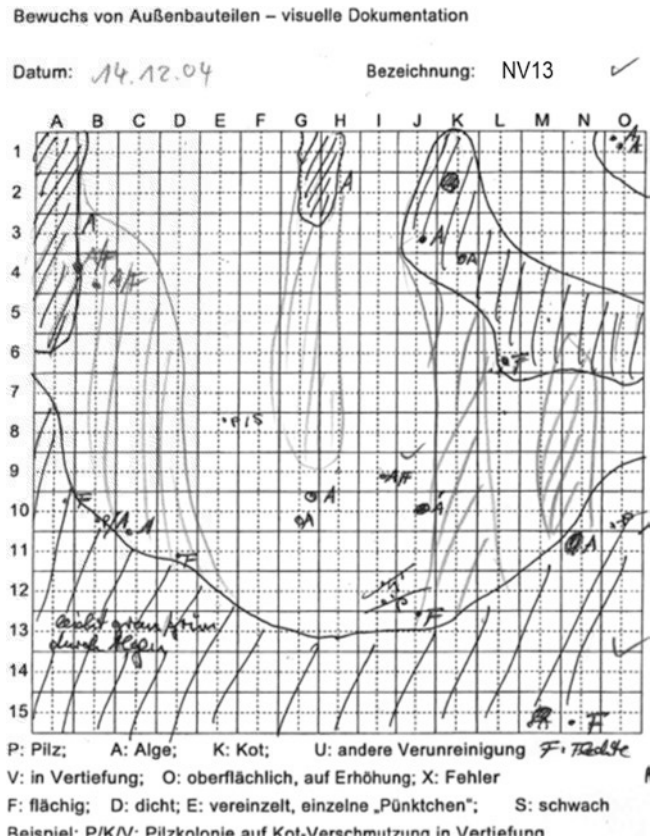

Fig. 2.9 Raster sketch of developing algal (A) and fungal (F) growth (Hofbauer et al. 2006; Hofbauer 2007)

Part of the gained homogenized powder (approx. 1 g) was given into 10 ml dilution solution (0.9% NaCl, 0.01% TWEEN 80) and shaken for 1 h at 300 rpm. A combination of physiological salt solution with the mild detergence TWEEN 80 has proven to be good in different microbial investigations and was applied, so that propagation units of microorganisms adhering to the substrate were removed in a mechanically and physiologically gentle way (e.g., Wollum 1982; Craig et al. 1987; Perrissol et al. 1993; Ehrlich 2002; Labuda et al. 2003; Ranilla and Carro 2003; Çelen and Kiliç 2004; Kłyszejko et al. 2005). After shaking, the powder suspension was allowed to settle for a few minutes. Working in a laminar flow, 100 µl of each diaspore-containing supernatant was plated onto Petri dishes containing different culture media by using Trigalsky spatula. In addition, a dilution series was performed according to the "most-probable-number-method" (MPN-method; Alexander 1982), employing three dilution steps (1:10 each,

always diluting 1 ml of the former suspension with 9 ml of dilution medium). After that the different dilutions were plated, resulting in a "diluted plating" (Skinner et al. 1952; Alexander 1982; Hoekstra et al. 2002; Samson et al. 2002). Further to the dilution series, a weighted amount of the sample powder (ca. 0.5 g up to ca. 1 g) was applied in "direct plating" (Skinner et al. 1952; Hoekstra et al. 2002; Samson et al. 2002). Direct plating was employed in order to cultivate organisms that appeared in low numbers on the investigated surfaces. In the direct plating trials, primarily algae-medium and a low nutrient medium were used because it was assumed that in the initial phase especially algae and other oligophilous microorganisms would be present at the investigated surfaces in low germ numbers. Each batch (dilution and medium) was done in triplicate. The inoculated Petri dishes were placed into culture cabinets (Binder Company), under a diurnal regime of 12/12 h light/dark and 25 °C (light) and 16 °C (dark), respectively. For irradiation, special plant culture light tubes (Osram Company) with an illuminance of ca. 1200 W/m^2 were chosen, according to previous experience in the cultivation of aerophytic algae (Ettl and Gärtner 1995; Gärtner 1996). In order to provide an additional selective effect for organisms that may survive on façade surfaces prone to light, not only the culture batches for algae but also the batches for fungi and bacteria were kept under daily variation of irradiation.

In the culture experiments, the following media were used: malt extract peptone agar (MEA) for mesophilic fungi (Booth 1971a; Samson et al. 2002; Domsch et al. 2007), Czapek-Dox agar (CD) undiluted for mesophilic fungi and diluted 1:50 for oligophilic/oligotrophic fungi and bacteria (Domsch et al. 2007), dichlorane-glycerin agar (DG18) for xerotolerant fungi (King et al. 1979, 1986; Hocking and Pitt 1980; Samson et al. 1992, 2002; Frändberg and Olsen 1999), plate count agar (PCA) for aerobic bacteria (Domsch et al. 2007; Samson et al. 2002) and Bold's basal medium (BBM) for algae (modified according to Bischoff and Bold 1963; Ettl and Gärtner 1995).

The Petri dishes were regularly controlled for several weeks because part of the investigated organisms was frugal or slow growing. For quantitative analyses, the emerging colonies (colony-forming units, CFU) were observed and counted after 10 and 90 days, respectively. In the total germ counts according to the MPN method (Alexander 1982), we distinguished between bacteria, fungi and algae (in single cases also for further organism groups like e.g. ferns). Finally, the total germ numbers were extrapolated to 1 g sample powder.

Assessment of Starter Germ Load of Materials

Of each material/composition, reserve samples were generated. The system composition (plaster and paint) was identical to specimens used in weathering experiments. Part of the reserve samples was used for assessment of the starter germ load immediately after completion of the hardening of the material while stored at room temperature. This was

undertaken to clarify the following questions: (1) If the specimens were loaded with germs of microorganisms right from the start, (2) what kind of microorganisms were present and (3) if microorganisms were present which caused damage later on. Reserve samples were called 0-probes, because they resembled the condition at time zero of the weathering exposure. Sample collection and sample preparation as well as the culture experiments were done as described previously. Cultures were assessed quantitatively and qualitatively. Aerobic bacteria were assessed as a whole group only. In principle, it was expected that starter germ loads of fresh finished coatings would be very low, because present germs usually are killed during the production of the material (e.g., rather high alkalinity of plaster in preparation, temperature evolvement, etc.). For data, see Hofbauer et al. (2006).

Culture-Based Taxonomic Analysis

Cultures on selective media with subsequent isolation of pure cultures allow an identification of the occurring microorganisms. Imperative for a taxonomic treatment are comparison and reference cultures, because most microorganisms cannot be compared with herbarium material like, e.g., vascular plants. Drying usually destroys important diagnostic characteristics of microorganisms. Furthermore, knowledge and investigation of different developmental stages, which can only be achieved in parallel with cultures, are often necessary for a successful differentiation (Gärtner 1986; Ettl and Gärtner 1995). At the IBP, a culture collection for the most important species known to occur on building surfaces has been built up, mainly based on proprietary investigations. This collection is still maintained and expanded. It comprises the only comprehensive culture collection which is specialized on building relevant microorganisms so far (Fig. 2.10; see also Hofbauer et al. 2003).

Taxonomy

Colonizers of building surfaces stem from almost all today accepted major groups of organisms and comprise representatives of prokaryota and eukaryota. In a more narrow sense, organisms of the regnums of bacterial life forms (incl. Cyanoprokaryota), fungi, plants, protists, etc., can be found.

In Chap. 3, basal groups are arranged mainly according to Ettl and Gärtner (1995, 2014), Hoek van den et al. (1995), Esser (2000), Komárek and Anagnostidis (1998), Graham et al. (2009), Komárek and Anagnostidis (2005) and Raven et al. (2005). For detailed questions regarding certain forms and species, specialized current literature was used, which is mentioned in the discussion of the respective forms. As much as possible, we tried to follow the natural system. The basis for the identification of the different taxa is a comprehensive taxonomic concept with emphasis on morphological

Fig. 2.10 Part of the culture collection of building relevant microorganisms at the IBP (Hofbauer 2007; Hofbauer et al. 2003, 2006; www.ibp.fraunhofer.de: Forschung im Fokus: Mikroorganismen)

characteristics (Bubrick et al.1984; Komárek and Fott 1983; Gärtner 1984, 1985; Komárek and Anagnostidis 1998; Graham et al. 2009). Additionally, synoptic works were used for the identification of diverse form groups such as Domsch et al. (2007), Ettl and Gärtner (1995, 2014), Bergey's Manual of Systematic Bacteriology (Boone and Castenholz 2001), Komárek and Anagnostidis (1998), Komárek and Anagnostidis (2005) and AlgaeBase (Guiry and Guiry 2019) as well as specialized literature which is specified at the respective groups. Technical equipment at hand was powerful reflected light and transmission microscopes employed in video, polarization and fluorescence microscopy. Magnifications up to 1000- to 1250-fold (immersions objectives) facilitated light microscopic analyses of cell morphology. For highlighting cellular structures different staining methods were used for microscopic preparation (see Ettl and Gärtner 1995), e.g., Lugol's solution, carmine acetic acid, methylene blue, Gram staining, sudan red and others. Microscopic investigations were accompanied by extensive photographic documentation. In some cases, also scanning electron microscope (SEM) techniques were used. Emphasis on determination of taxonomy was laid on photosynthetic active organisms (including algae, Cyanoprokaryota, bryophytes, ferns and lichens) and fungi. Bacteria were mainly assessed as a whole group (aerobic). Results of recent genetic–taxonomic research were also considered (e.g., Buchheim et al. 1990, 1996, 2001; Huss and Sogin 1990; Kantz et al. 1990; Buchheim and Chapman 1991; Lewis et al. 1992; Wilcox et al. 1992; Surek et al. 1994; Friedl 1995; Melkonian and Surek 1995; Bhattacharya et al. 1996; Nakayama et al. 1996; Booton et al. 1998; Chapman et al. 1998; An et al. 1999; Nedelcu et al. 2000; Turmel et al. 2002; Krienitz et al. 2003; Lewis and McCourt

2004; Pombert et al. 2004, etc.). Naming of the detailed taxonomic units was aligned to the International Code of Nomenclature for algae, fungi and plants (Shenzhen Code; Turland et al. 2018).

Pre-culture Investigation

In a pre-culture investigation, the surface of the specimens was scanned for traces of growth first by naked eye. The overall impression of the surface condition was noted (weathering, macroscopic discernible growth, etc.).

In the second step, probes of supposed growth or dirt/graying were investigated using scanning and transmission light microscopes. A detailed investigation of already visible established growth leads to subsequent cultures on selective media and helped to interpret the results. It cannot be ruled out that by chance forms developed in culture whose diaspores only accidentally had fallen on the surface of the specimens. Pre-culture analysis helped to assess the proportions that were measured in culture. Therefore, we tried to identify as much forms as possible in pre-culture investigation. This was only achieved in a limited way because many organisms showed reduced or altered features due to the changing and partly extreme outdoor conditions at the surfaces. It is well known that not all organisms that colonize building surfaces can be cultivated with standard media, as, e.g., lichens and some bryophytes. These organisms were identified mainly on original material from the surfaces; if enough differentiating characteristics were found, otherwise they were grouped within the next higher rank.

Sample Collection and Preparation

Collection of samples and preparation was the same as for the quantitative analysis which is described in Sect. 2.3.1. Raw cultures of the quantitative analysis after ½, 1 and 2 years of exposure in Holzkirchen were the base not only for the germ counts but also for the differentiation of the occurring microorganisms. Therefore, subsequent pure cultures of the isolates were established.

After 3 years of exposure in Valley/Holzkirchen, an additional survey was performed in a simplified procedure: With a lancet needle, the surface of the specimens was scratched at randomly selected points and the gained material was investigated microscopically, and the rest was spread evenly on Petri dishes with selective media for algae and fungi. In this way, raw cultures for further differentiation were gained. These cultures allowed to assess coarse abundance classes (dominant, subdominant, accessory/scattered).

Determination of the occurring organisms was conducted in the same way for the additional locations Ernsthofen/Oberramstadt and Heggen/Finnentrop. Furthermore, another set of samples of “additional variants/surfaces” (Table 2.5) was also investigated based on scratch samples and subsequent cultures.

Table 2.5 Overview of additional variants and surfaces

Symbol	Location	Substrate/building material/construction	Age (in years)
AV1	Ernsthofen	Specimen, ETICS,	ca. 10
AV2	Oberramstadt	Specimen, ETICS,	ca. 2
AV3	Oberramstadt	Specimen, ETICS,	ca. 3
AV4	Oberramstadt	Specimen, ETICS,	ca. 3
AV5	Holzkirchen, IBP	Wall base, ETICS, shady	Unknown (>10)
AV6	Holzkirchen, IBP	Concrete slab, horizontal	Unknown (>10)
AV7	Holzkirchen, IBP	Specimen: sandstone, cut (“Asterix”)	ca. 20
AV8	Innsbruck	Concrete base of lantern pole	Unknown (>10)
AV9	Innsbruck	Algal crust on pavement	Several years
AV10	Kufstein fortress	Base of travertine built wall	Unknown (>10)
AV11	Innsbruck	Plaster surface, massive construction, green stain	Unknown (>10)
AV12	Pfaffenhofen	Dark spots at ETICS	Unknown (>10)
AV13	Berlin	Flat roof	Several years
AV14	Innsbruck	Algal crust at fair-faced concrete	Unknown (>10)
AV15	Kufstein/Zell	Concrete surface	Unknown (>10)
AV16	Stuttgart	ETICS, green stain	Unknown (>10)
AV17	Gladbeck	Specimen, coated façade facing slab	Unknown (>10)
AV18	Nürnberg	ETICS, green stain	Unknown (>10)
AV19	Wesel	Special type of concrete, pre-fabricated construction part	Several years
AV20	Alzenau	Special type of concrete, pre-fabricated construction part	Several years
AV21	Grevesmühle	ETICS, green stain	Unknown (>10)
AV22	Wismar	Timber surface, green stain	Unknown (>10)
AV23	Holzkirchen, IBP	Algal crust, window sill	Several years
AV24	München	Travertine, zoological garden	Unknown (>10)
AV25	Schleswig	ETICS, green stain	Unknown (>10)

Establishment of Pure Cultures

In general, isolation of microorganisms was performed as described above, analogous to Berner et al. (1997) or Sterflinger and Prillinger (2001), respectively. To establish pure cultures, one or two cleaning steps were needed during which the respective forms were transferred to further culture or selective media. If necessary, also micromanipulator techniques were performed by use of specially produced thin glass tips or threads