mgr inż. Michał Komar[1)*)]
ORCID: 0000-0001-6354-6885
dr hab. inż. Justyna Szulc[2)]
ORCID: 0000-0002-6601-2376
prof. dr hab. Beata Gutarowska[2)]
ORCID: 0000-0002-9223-2001

New methods of determining fungi and algae in construction

Nowe metody oznaczeń grzybów i glonów w budownictwie

DOI: 10.15199/33.2024.05.06

Abstract. Microorganisms inhabit building materials from the first days of their use. They cause biodeterioration and contribute to economic losses, and in the case of building interiors, they may negatively affect the health of residents. Early detection of fungi and algae on building materials is important. The following article contains a literature review of research methods for detecting fungi and algae on building materials and presents a new method for assessing the resistance of coatings against fouling.
Keywords: fungi; algae; mycological analysis; algological analysis; biodeterioration of building materials.

Streszczenie. Mikroorganizmy zasiedlają materiały budowlane od pierwszych dni ich użytkowania. Powodują biodeteriorację i przyczyniają się do strat ekonomicznych, a w przypadku wnętrza budynków mogą negatywnie oddziaływać na zdrowie mieszkańców. Istotne jest wczesne wykrywanie grzybów i glonów na materiałach budowlanych. Poniższy artykuł zawiera przegląd literaturowy metod wykrywania grzybów i glonów na materiałach budowlanych oraz przedstawia nową metodę oceny trwałości powłok przed porastaniem.
Słowa kluczowe: grzyby; glony; analiza mykologiczna; analiza algologiczna; biodeterioracja materiałów budowlanych.

Algae appear on building materials from the first days of use, as their growth is only activated by light and air humidity. In the next stage, fungi begin to grow, requiring small amounts of organic matter and material moisture. Both microbial groups cause biodeterioration of building materials: aesthetic (e.g. formation of discoloration, slimy surfaces, patinas), chemical (e.g. excretion of metabolites, assimilative and dissimilatory processing of material) and geophysical (e.g. mechanical stress, water binding, change in the volume of biofilms and bound water) [1, 2] contributing to significant economic losses [3]. In the case of the building interiors, they may also have a negative impact on the health of residents and cause the so-called sick building syndrome [4]. Therefore, early detection of fungi and algae on building materials using modern and accurate research methods is particularly important. Their use should allow for quick threat elimination or enable determination of the coating resistance against the growth of harmful microorganisms. So far, studies on the growth of microorganisms have been performed in accordance with standards PN-EN 15457:2022-08 [5] and PN-EN 15458: 2022-08 [6] in order to evaluate the effect of active substances in protective coatings applied to building materials. However, these methods do not indicate the efficacy of the coating in protecting itself or the material beneath and do not take into account the influence of environmental conditions, especially washing out, as well as UV radiation, which may accelerate the wear of the coating and determine the effectiveness of photoactive compounds (e.g. TiO_2) [7, 8]. Due to the lack of consideration for coating wear occurring with atmospheric factors and lack of standardization methods, the development of a new procedure for assessing the durability of building materials is necessary. The following article contains a literature review of research methods for detecting fungi and algae on building materials and presents a new procedure for assessing the durability of coatings against fouling.

Methods for assessing fungal growth on building materials

Mycological analysis can assess the susceptibilityof new building materials to fungal growth, especially those with the addition of biocides [5]. It can also quantitatively and qualitatively evaluate the growth of fungi in used buildings to determine the degree of their contamination [9]. In quantitative assessment, the most frequently used are the classical cultivation methods along with their modifications, which use microbiological media to obtain fungal growth and count the so-called cfu/100 cm^2 of material surface. The use of different media is recommended, in order to increase the chances of detecting all species present on the tested materials. This especially applies to xerophilic fungi (growth under low water activity) [10]. In the next step, the obtained fungal colonies are identified to the species level, using the comparison of morphological features with taxonomic keys [11]. Currently, identification is carried out using molecular methods

[1)] Politechnika Łódzka, Interdyscyplinara Szkoła Doktorska
[2)] Politechnika Łódzka, Wydział Biotechnologii i Nauk o Żywności
[*)] Correspondence address: michal.komar@dokt.p.lodz.pl

with various genetic markers (ITS1, ITS2, rpb1, rpb2, mcm7, tef1α, benA, cmd, hogA, h3-h4 etc.) [12]. However, the most reliable identification method is full genome sequencing. For identification, determining the profile of volatile organic compounds (VOCs), characteristic for individual species, is also used [13]. Quantitative analysis also uses chemical methods, which have many advantages compared to cultivation methods, such as: short analysis time; high accuracy and assessment of both live and dead fungi that may have an allergenic effect. These techniques involve the extraction of mycelium components (ergosterol, glucans) from the building material with organic solvents and their determination using chromatographic techniques (HPLC, GC) [14]. So far, no standards have been developed for the interpretation of mycological tests on building materials, both quantitatively and qualitatively. However, the literature contains recommendations on how to interpret results obtained using the cultivation method (<104 cfu/100 cm^2 – no fungal growth; 104 – 106 cfu/100 cm^2 – warning levels, active fungal growth, >106 cfu/100 cm^2 – warning levels, very active fungal growth, hazardous for human health). The ergosterol content in a building material sample > 4 mg/m^2 means high fungal infection of the material [15]. In relation to quality, fungi identified in terms of species should be classified according to the so-called BSL classification (bio-safety level) and divided into hazard groups according to the Regulation of the Minister of Health on harmful biological agents [16]. These classifications indicate the harmful impact of fungal species on the health of residents or employees living and working in moldy buildings. If it is necessary to confirm diseases related to the presence of fungi and the mycotoxins they produce, mycotoxins in building materials can be determined by performing immunological analyzes using monoclonal antibodies (ELISA tests). Blood serum testing and skin tests for the presence of fungi isolated in rooms may confirm the occurrence of allergic reactions in residents [17].

Methods for assessing algal growth on building materials

In a temperate climate zone, aerophytic algae are represented primarily by green algae, which constitute an equally common threat to building facades as molds. Until recently, qualitative analysis of the so-called green biofilms was performed primarily using traditional microscopic methods by distinguishing morphological features using light microscopy (LM) [18] or more advanced scanning (SEM) and transmission electron microscopy (TEM) [19, 20]. Due to the fact that many species of aerophytic algae show high morphological similarity [21] and some characteristic features may depend on environmental conditions [22], current identification is based on molecular analysis and gene sequencing [23, 24]. Microscopic methods can also be used in quantitative analysis [25], allowing the determination of algal cell number present on the façade surface. Another biomarker used is chlorophyll-a, the basic assimilation pigment of green algae, which concentration is proportional to the number of photoautotrophic cells [26, 27]. The measurement technique involves a relatively simple extraction procedure, spectrophotometric measurement or chromatography. Unfortunately, the collection of biofilm, especially from very porous materials, may be difficult, reducing the accuracy of the analysis. Moreover, this method may be ineffective in detecting early signs of facade colonization [28, 29]. As an alternative, measurement of chlorophyll fluorescence is used, allowing for the quantitative analysis of green biofilms and the examination of their photosynthetic activity. Traditionally, it is based on microscopic methods, but modern devices use Pulse Amplitude Modulation (PAM) allowing for non-invasive analysis of facades, also in situ [30, 31]. The main undesirable effect of algae growth on building materials is most often green or yellow-green discoloration. The change in the color of the substrate related to the production of pigments, such as chlorophylls and carotenoids, measured using colorimeters or spectrophotometers can be an effective indicator of the degree of the fouling [32]. Such measurement does not require a complicated procedure, is non-invasive and can be used in field conditions, while modern measuring devices allow the recording of large quantities of data. On the other hand, the complexity of the method and interpretation of the results increases in the case of materials and facades with non-homogeneous surfaces. Appropriate adjustment of the spectrophotometric color change measurement may allow early detection of changes indicating undesirable algae growth, even before they are visible to the human eye. Considering the functionality of facades, the most practical is a visual assessment of the growth, most often expressed as a percentage of surface coverage. The effectiveness of the method decreases in the initial stages of biofilm development and may require a qualified employee. However, it can be successfully used in laboratory tests of the durability of building coatings. Currently, in order to reduce the subjectivity of the assessment, digital image analysis techniques are being additionally introduced [33], which in the future may be even supported by machine learning methods [34].

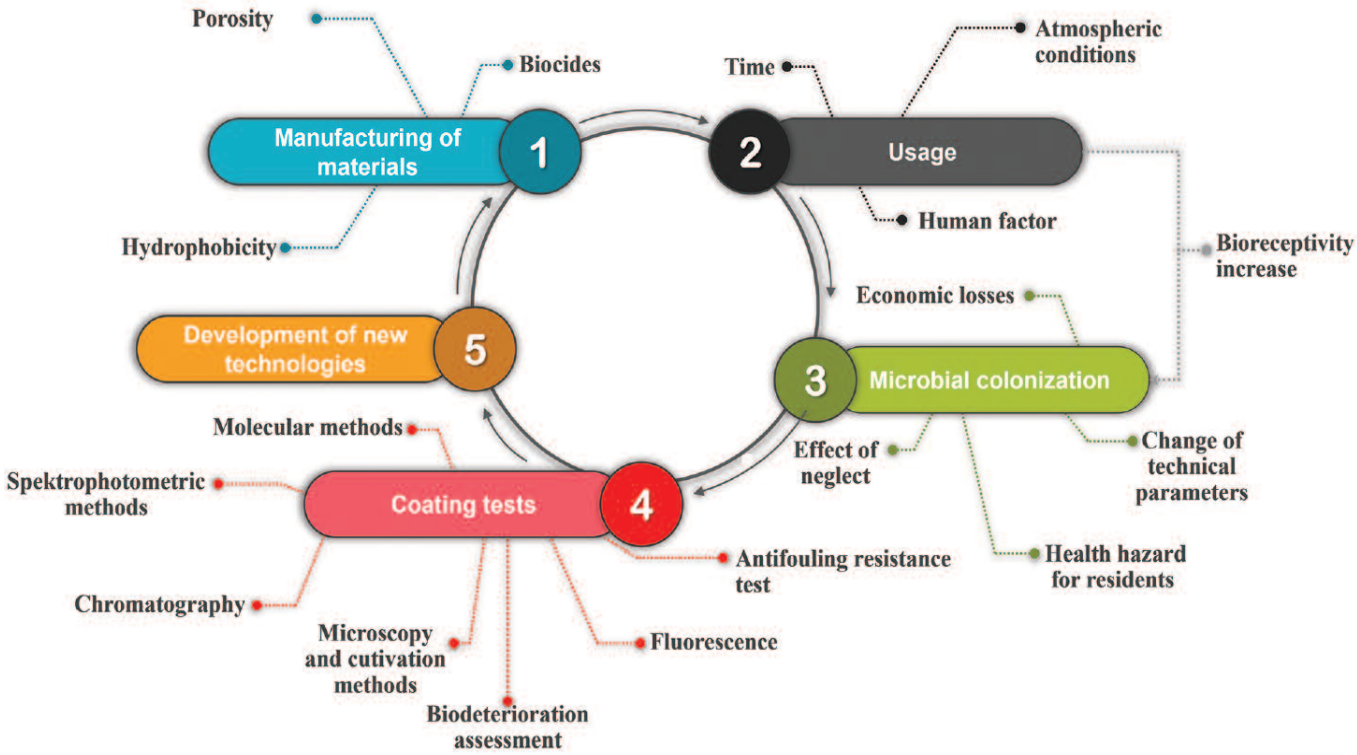
A novel method for testing the durability of building materials against algae and fungi growth

The effectiveness of the abovementioned methods in relation to building coatings was analyzed in laboratory tests presented in [29, 35]. Increasing the assessment efficacy often requires specialized equipment, reduces the widespread use and increases the analysis time. For producers of building materials and their users, standardization plays an important role in reliable warranty of durability. The key information is how long the coating will remain resistant to the growth of microorganisms. This will depend on many factors, including: type of materials and conditions of use (figure). Unlike methods for

assessing the degree of coating fouling, no method has been developed so far to determine its durability. Normative methods, e.g. [5, 6], make it possible to check whether the protection of the tested material is effective or not, but they do not enable determining the time during which the coating will not be colonized. This parameter is most often determined by manufacturers internal tests and is rarely subjected to model and environmental tests. Therefore, differences in individual methods make it impossible to compare them reliably. In recent years, the Department of Environmental Biotechnology of the Lodz University of Technology has been conducting research concerning methods allowing to assess the degree of building materials fouling and their antimicrobial resistance. The result of over three years of research conducted in laboratory and environmental conditions was the development of a new method for assessing the durability of building coatings against algae and fungal growth [29, 35, 36].

The basis of the method are model tests, repeated cyclically until the material subjected to the test is covered in at least 10% of the surface. One test cycle is divided into 4 distinct stages, including: (1) sample conditioning; (2) inoculation of materials; (3) incubation and (4) evaluation. Material samples prepared in accordance with EAD 040083-00-0404, 2020 [37] in the form of discs with a diameter of 50 mm are used as the test material. During the conditioning stage, samples are soaked and exposed to UV light. This is intended to reduce the resistance of the coating, which in natural conditions occurs as a result of atmospheric precipitation and sunlight. So far, the impact of environmental factors on the antimicrobial resistance of the coating has not been taken into account in the normative methods [5, 6], achieving higher protection than in reality. Conditioning parameters, e.g. water volume, were selected based on the analysis of climatic data provided by the Institute of Meteorology and Water Management, National Research Institute and the Chief Inspectorate of Environmental Protection. The amount of water should be selected so that it creates a layer above the sample that is at least 4 cm high. The exact volume can be calculated based on the average annual rainfall and the area of samples of a given material e.g. the average annual rainfall of 500 mm of water column corresponds to 500 l of water falling on an area of 1 m^2 and 50 ml of water for each cm^2 of material surface. Samples with an area of 20 cm^2 should therefore be washed in at least 1 liter of distilled water. According to the method, the washing process lasts for 24 hours, and then the samples are exposed to UV light for 3 hours on each side. This approach allows for easy adaptation of the model to demanding ecological niches or ongoing climate changes.

The species of molds and algae used in the inoculation process were selected on the basis of quantitative and qualitative analysis of the species most commonly colonizing building facades in Central Europe. The algae mixture is prepared in liquid Bold's Basal Medium (BBM) by mixing equal volumes of actively growing strain suspensions of *Coenochloris signiensis* (environmental

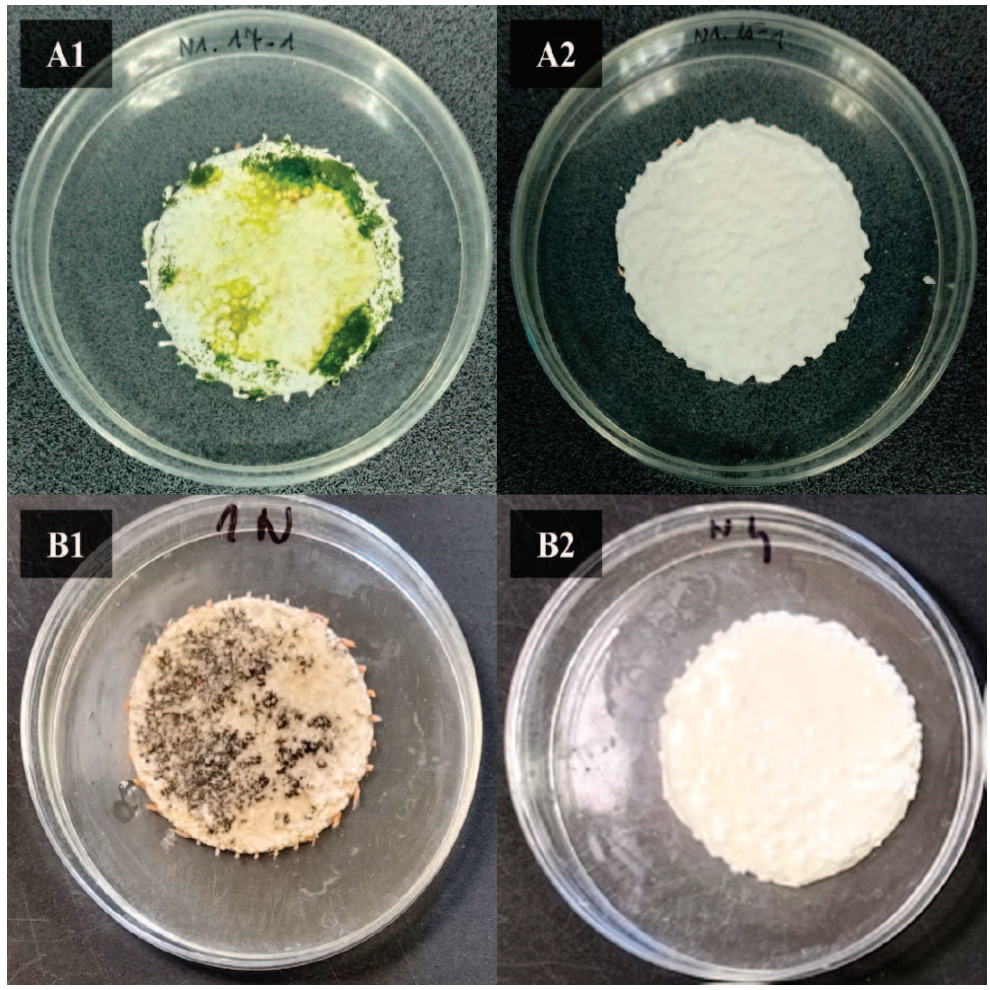


Life cycle of building coatings
Cykl życia powłok budowlanych

isolate), *Pseudochlorella signiensis* (environmental isolate), *Stichococcus Bacillaris* (collective strain) and *Nostoc commune* (collective strain). The cell density of the mixture is determined by counting cells using a hemocytometer (e.g. Thoma chamber) and adjusted to 10^6 cfu/ml. The fungi inoculum mixture includes mold species of *Penicillium citrinum* (environmental isolate), *Cladosporium cladosporoides* (environmental isolate), *Apergillus niger* (collective strain) and yeasts *Rhodotorula mucilaginosa* (collective strain). The mixture is made in 0.85% saline solution with the addition of Tween80 (0.01% v/v). The density of individual suspensions is determined by densitometric measurement, and the density of the fungi inoculation mixture should correspond to 10^4 cfu/ml. Agar medium is applied to the surface of each material sample and spread gently with a soft brush before it solidifies (BBM in the case of algae and maltose medium in the case of fungi). For samples with an area of 20 cm^2, approximately 1 ml of semi-liquid medium is used. The prepared surface is inoculated by applying 1 ml of a previously assembled, well-blended mixture. The samples are placed in Petri dishes and left for 24 hours to absorb the inoculum, then parafilmed and left to incubate. To determine antifungal durability, samples are incubated for 21 days at a temperature of 25 ± 2°C and air humidity of 50%. The algae incubation time was extended to 28 days, with the same humidity, air temperature of 22 ± 1°C and artificial lighting with an average intensity of 1200 Lux in a cycle of 16 hours of the day and 8 hours of the night. The parameters of the incubation process were developed based on the analysis of literature data, supported by environmental research conducted on experimental plots and long-term exposures [29, 35].

After the incubation process, each cycle ends with an assessment of the degree of the tested materials fouling. Due to the high correlation with actual conditions of use and the low complexity of the analysis, the assessment is performed on the basis of visual assay or visual assay supported by digital image analysis techniques. In this approach, a trained laboratory technician independently or using image analysis techniques (e.g. programs such as Image J [38]) determines the surface area of the sample that has been colonized. If the degree of fouling is less than 10% the tested sample passes the test. The samples are then carefully cleaned to remove any biofilm formed and sent for the next cycle of tests. The number of cycles in which the sample passed the visual inspection corresponds to the number of years the material will remain resistant to fouling (Figure 1). Unlike methods based on the extraction of characteristic compounds (e.g. determining the concentration of chlorophyll -a), which require more time-consuming procedures and interference in the tested substrate (related to, inter alia the method of biofilm collection, grinding of the material or the use of organic solvents) [29], the described evaluation method allows for reduction of analysis time and the number of samples necessary to carry out the tests.

The developed procedure is one of the first documented techniques enabling determination of the resistance of building coatings exposed to microbial colonization [36]. The method does not require complicated, expensive



Samples tested for antialgal (A) and antifungal (B) resistance after 8 research cycles: A1, B1 – samples non-resistant to biofouling; A2, B2 – samples resistant to biofouling for more than 8 years
Próbki poddane analizie odporności przeciwglonowej (A) i przeciwgrzybowej (B) po 8 cyklach badawczych: A1, B1 – próbki nieodporne na porastanie; A2, B2 – próbki wykazujące odporność dłuższą niż 8 lat

research equipment and takes into account the often overlooked simulation of environmental conditions, the parameters of which can be easily adjusted to different geographical areas. The analysis is not invasive and therefore does not require a larger number of samples. Additionally, the procedure is based on the species of microorganisms confirmed by biodiversity studies and includes both collective strains and environmental isolates. The development of traditional and modern methods for assessing the degree of colonization on materials and their long-term resistance to fouling creates a real potential for procedures standardization, beneficial from the perspective of consumers of construction solutions and fair trade competition.

Literature

[1] Cutler N, Viles H. Eukaryotic microorganisms and stone biodeterioration. Geomicrobiol. J. DOI: 10.1080/01490451003702933.

[2] Allsopp D, Seal KJ, Gaylarde CC. Introduction to Biodeterioration, 2nd ed. Cambridge: Cambridge University Press. 2004; DOI: DOI: 10.1017/CBO9780511617065.

[3] Dergunova A, Piksaykina A, Bogatov A, Salman ADSD, Erofeev V. The economic damage from biodeterioration in building sector. IOP Conf. Ser. Mater. Sci. Eng. 2019; DOI: 10.1088/1757-899X/698/7/077020.

[4] Zyska B. Zagrożenia biologiczne w budynku. Warszawa: Arkady, 1999.

[5] PN-EN 15457:2022-08. Farby i lakiery – Laboratoryjna metoda badania skuteczności w powłoce środków ochrony powłok przed grzybami. 2022.

[6] PN-EN 15458:2022-08. Farby i lakiery – Laboratoryjna metoda badania skuteczności w powłoce środków ochrony powłok przed glonami. 2022.

[7] Wiejak A. Ocena skuteczności działania środków ochrony powłok elewacyjnych przed grzybami pleśniowymi i glonami. Pr. Inst. Tech. Bud. 2011 40: 15 – 2.

[8] Kata I, Stasica Z, Szafran K. Czynniki sprzyjające korozji mikrobiologicznej ocieplonych elewacji i sposoby jej zapobiegania. Materiały Budowlane. 2021; DOI: 10.15199/33.2021.01.02.

[9] Gutarowska B, Piotrowska M, Koziróg A. Grzyby w budynkach, Zagrożenia, ochrona, usuwanie. Wydawnictwo Naukowe PWN, 2019.

[10] Gutarowska B, Piotrowska M. Methods of mycological analysis in buildings. Build. Environ. 2007; DOI: https://doi.org/10.1016/j.buildenv.2006.02.015.

[11] Lindemann V, Schleiner T, Maier U, Fels H, Cramer B, Humpf H-U. Analysis of mold and mycotoxins in naturally infested indoor building materials. Mycotoxin Res. 2022; DOI: 10.1007/s12550-022-00461-3.

[12] Lewinska A, Peuhkuri R, Rode C, Andersen B, Hoof J. Rapid detection and identification of Stachybotrys and Chaetomium species using tissue PCR analysis. J. Microbiol. Methods. 2016; doi: 10.1016/j.mimet.2016.09.005.

[13] Garbacz M, Malec A, Duda-Saternus S, Suchorab Z, Guz Ł, Łagód G. Methods for Early Detection of Microbiological Infestation of Buildings Based on Gas Sensor Technologies. Chemosensors. 2020; DOI: 10.3390/chemosensors8010007.

[14] Szponar Band, Larsson L. Determination of microbial colonisation in water-damaged buildings using chemical marker analysis by gas chromatography – Mass spectrometry. Indoor Air. 2000; 10: 13 – 18.

[15] Gutarowska B, Żakowska Z. Elaboration and application of mathematical model for estimation of mould contamination of some building materials based on ergosterol content determination. Int. Biodeterior. Biodegradation. 2002, DOI: https://doi.org/10.1016/S0964-8305 (02) 00063-X.

[16] Rozporządzenie Ministra Zdrowia z 11 grudnia 2020 r. zmieniające rozporządzenie w sprawie szkodliwych czynników biologicznych dla zdrowia w środowisku pracy oraz ochrony zdrowia pracowników zawodowo narażonych na te czynniki. Ministerstwo Zdrowia, 2020.

[17] Gutarowska B, Kosmowska M, Wiszniewska M, Palczynski C, Walusiak-Skorupa J. An Investigation of Allergenic Proteins Produced by Moulds on Building Materials. Indoor Built Environ. – INDOOR BUILT Env. 2012; DOI: 10.1177/1420326X11413911.

[18] Uher B. Spatial distribution of cyanobacteria and algae from the tombstone in a historic cemetery in Bratislava. Slovakia-Fottea. 2008; 9: 81 – 92.

[19] Piontek M, Lechów H, Paradowska E, Nycz M. Examination of deteriogenic biofilms on building facades with scanning electron microscopy. Civ. Environ. Eng. Reports. 2016; 20 (1): 79 –88.

[20] Czerwik-Marcinkowska J, Mrozińska T. Algae and cyanobacteria in caves of the Polish Jura. Polish Bot. J. 2011; 56(2): 203 – 243.

[21] Rindi F. Diversity, Distribution and Ecology of Green Algae and Cyanobacteria in Urban Habitats. 2006; DOI: 10.1007/978-1-4020-6112-7_34.

[22] Rindi F, Guiry MD. Composition and spatial variability of terrestrial algal assemblages occurring at the bases of urban walls in Europe. Phycologia. 2004; DOI: 10.2216/i0031-8884-43-3-225.1.

[23] Cheung SL, Allen DG, Short SM. Specific quantification of Scenedesmus obliquus and Chlorella vulgaris in mixed-species algal biofilms. Bioresour. Technol. 2020; DOI: https://doi.org/10.1016/j.biortech. 2019.122251.

[24] Hallmann C, Rüdrich J, Enseleit M, Friedl T, Hoppert M. Microbial diversity on a marble monument: A case study. Environ. Earth Sci. 2010; DOI: 10.1007/s12665-010-0772-3.

[25] Starmach K. Plankton roślinny wód słodkich. Warszawa: Państwowe Wydawnictwo Naukowe, 1989.

[26] Tang Y et al. Hybrid Xerogel Films as Novel Coatings for Antifouling and Fouling Release. Biofouling. 2005; DOI: 10.1080/08927010500070935.

[27] Chaudhury M, Finlay J, Chung J, Callow M, Callow J. The influence of elastic modulus and thickness on the release of the soft-fouling green alga Ulva linza (syn. Enteromorpha linza) from poly (dimethylsiloxane) (PDMS) model networks. Biofouling. 2005; DOI: 10.1080/08927010500044377.

[28] Prieto B, Silva B, Lantes O. Biofilm quantification on stone surfaces: Comparison of various methods. Sci. Total Environ. 2004; DOI: 10.1016/j. scitotenv. 2004.05.003.

[29] Komar M, Szulc J, Kata I, Szafran K, Gutarowska B. Development of a Method for Assessing the Resistance of Building Coatings to Phoatoautotrophic Biofouling. Applied Sciences. 2023. DOI: 10.3390/app13148009.

[30] Eggert A, Häubner N, Klausch S, Karsten U, Schumann R. Quantification of algal biofilms colonising building materials: Chlorophyll α measured by PAM-fluorometry as a biomass parameter. Biofouling. 2006, DOI: 10.1080/08927010600579090.

[31] Giakoumaki A, Philippidis A, Siozos P, Pyrri I, Anglos D, Pouli P. Development of a methodology for the characterisation and assessment of biodeteriogens on archaeological surfaces by use of a portable LED-induced fluorescence instrument. Herit. Sci. 2022; DOI: 10.1186/s40494--022-00827-x.

[32] Nakajima M, Hokoi S, Ogura D, Iba C. Field survey of the relationship between environmental conditions and algal growth on exterior walls. Build. Environ. 2020, DOI: https://doi.org/10.1016/j. buildenv. 2019.106575.

[33] Kukletová I, Chromková I. Testing of algae colonization growth risk on building materials. IOP Conf. Ser. Mater. Sci. Eng. 2018; DOI: 10.1088/1757-899X/379/1/012041.

[34] Bloomfield NJ, Wei S, Woodham BA, Wilkinson P, Robinson AP. Automating the assessment of biofouling in images using expert agreement as a gold standard. Sci. Rep. 2021; DOI: 10.1038/s41598-021-81011-2.

[35] Szulc J, Komar M, Kata I, Szafran K, Gutarowska B. Novel Method for Assessing the Protection Lifetime of Building Coatings against Fungi. Coatings, vol. 13, no. 12. 2023. DOI: 10.3390/coatings13122026.

[36] Gutarowska B, Szulc J, Komar M. Sposób laboratoryjny oceny czasu trwałości zabezpieczenia przeciwgrzybowego i przeciwglonowego tynków budow. P. 444942, 2023.

[37] European Organization for Technical Assessment (EOTA. External Thermal Insulation Composite Systems (Etics) With Renderings – EAD 040083-00-0404. 2020.

[38] Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods. 2012; DOI: 10.1038/nmeth.2089.

Accepted for publication: 02.05.2024 r.