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Article

Microwave-Assisted Extraction Combined with HPLC-MS/MS for Diagnosis of Fungal Contamination in Building Materials

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Ergosterol foi usado como indicador de contaminação fúngica para avaliar a presença de mofo em materiais de construção. Saponificação assistida por radiação micro-ondas seguida por extração com pentano foi utilizada na etapa de preparo de amostra. Analisaram-se amostras de tijolo, gesso, cimento e granito por cromatografia líquida de alta eficiência acoplada à espectrometria de massas sequencial (HPLC-MS/MS). As concentrações de ergosterol em amostras infectadas em laboratório variaram de 6 a 16 ng g^{-1}, enquanto que em amostras de materiais de ocorrência natural variou de 66 a 3020 ng g^{-1}. Os limites de detecção e de quantificação foram 1,7 e 5,5 ng g^{-1}, respectivamente. As recuperações e o efeito de matriz foram estimados a partir de diferentes experimentos de adição e recuperação (100 ng g^{-1}). A recuperação média e o efeito de matriz médio foram 65,9 e 111,6%, respectivamente. Efetuaram-se observações microscópicas e medidas de bioluminescência para as amostras infectadas para confirmação adicional da presença de fungos. A concentração de ergosterol determinada por HPLC-MS/MS em materiais de construção mostrou ser um indicador adequado para estimar a contaminação fúngica. O método desenvolvido foi consideravelmente mais rápido para este objetivo do que medidas de bioluminescência e métodos de cultura tradicionalmente usados.

Ergosterol was used as indicator of fungal contamination to assess mould presence on the building materials. Microwave-assisted saponification followed by pentane extraction was used in the sample preparation step. Samples of brick, plaster, concrete and granite were analyzed by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Ergosterol concentrations in laboratory infected samples ranged from 6 to 16 ng g^{-1}, while in samples of naturally occurring materials ranged from 66 to 3020 ng g^{-1}. Limits of detection and of quantitation were 1.7 and 5.5 ng g^{-1}, respectively. Recovery and matrix effect were tested using samples spiked at 100 ng g^{-1}. Mean recovery and mean matrix effect were 65.9 and 111.6%, respectively. Microscopic observations and bioluminescence measurements were carried out for the infected samples to make additional confirmation of fungi presence. The ergosterol concentration determined by HPLC-MS/MS in building materials proved to be a suitable indicator to estimate fungal contamination. The developed method was considerably faster for this aim than bioluminescence measurements and traditionally used culture methods.

Keywords: ergosterol, building material, microwave extraction, bioluminescence imaging

Introduction

Fungi can be found in almost every place in the environment. Some of them adversely influence human beings and animals. There are fungal allergies diagnosed in up to 10% of human population.[1] Moreover, between 25 and 30% of asthma cases are connected with mould allergies.[2] Non-allergic respiratory symptoms and other adverse health effects can be connected with toxic metabolic by-products of fungi known as mycotoxins.[3,4] Therefore, many studies were undertaken to measure contamination of food and animal feed caused by fungi.[5-7] Official norms were also created to reduce consumption of such products.[8,9] However, allergic and toxic influence of fungi can be also connected with contaminated building materials. The surface of these materials can be destroyed in damp buildings with poor ventilation, tight sealing and overheating. This creates ideal conditions for growth of fungi in plaster, brick and

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other materials. Inhalation of indoor dust particles from such buildings leads to non-specific symptoms including irritative reactions, headache and fatigue. In connection with that, several diseases were diagnosed including asthma, respiratory infections, conjunctivitis, urticaria and hypersensitivity pneumonitis.[10] Therefore, studies were undertaken to measure fungi content in both building materials and indoor dust.[11-15] Typically, amount of fungi can be determined with the use of culture methods. However, these procedures are long lasting and laborious. Therefore, it is highly recommended to use other analytical methods for the determination of fungi presence. Several approaches can be used for this purpose including microscopic observations, measurement of bioluminescence and chromatographic analysis of selected chemical markers of fungal contamination.

Microscopic observations are relatively simple to make but require use of not easily available and expensive electron microscopes. Also, measurements are time consuming. Nevertheless, the obtained results give insight into the level of contamination of the studied materials and thickness of fungi infected zone.

The bioluminescence is another technique used for study of the surface of tested materials.[16] Living fungi show both bioluminescence and light-induced luminescence. Biochemical processes in the living fungi provide energy needed to excite electrons which is then partially lost leading to luminescence. This can be used to monitor the presence of living organisms. However, external light-induced luminescence can be also observed. This type of emission can be found in living organisms as well as in many different materials. Therefore, examined objects must be placed in darkness to prevent formation of light-induced luminescence.

Fungal contamination can also be assessed with the use of appropriate chemical markers. Both mycotoxins and ergosterol can be analyzed for this purpose, although not all fungi produce mycotoxins and the presence of these compounds should rather be used to asses risk for humans. The markers can be analyzed using chromatographic techniques. Seits *et al.*[17] suggested usage of ergosterol as this compound is present in fungal cell membranes while it is absent or is a minor constituent of cell walls in higher plants.[18] Ergosterol in fungal cells is present in both free and bounded form. In the bounded form, various esters can be found which have to be saponified to give ergosterol in its free form. Therefore, three different types of sample preparation can be performed. Free ergosterol can be extracted without saponification. Total neutral ergosterol is yielded using saponification of alcoholic extracts containing esters. Finally, total alkali ergosterol is obtained by simultaneous extraction and saponification which results in samples containing also ergosterol from cell membrane bounded esters.[19,20] For this purpose, the conventional liquid-liquid extraction (LLE) has been widely used. But the conventional extraction procedure is time-consuming and requires large amounts of reagents and sample.[21,22] To overcome the problems associated with LLE, the microwave-assisted extraction (MAE) was proposed by Young.[22]

Ergosterol can be determined with the use of different chromatographic techniques including high-performance liquid chromatography with ultraviolet detection (HPLC-UV)[5,22-24] or mass spectrometric detection (HPLC-MS)[24,25-27] and gas chromatography with mass spectrometric detection (GC-MS).[13,22,28,29] HPLC analysis with UV detection can be performed in almost any laboratory as it requires relatively low cost equipment. However, the limit of detection obtained with this technique is relatively high. The use of gas chromatography leads to lower limit of detection but the derivatization of ergosterol must be performed before sample injection. HPLC-MS offers both low limit of detection and analysis without derivatization.

This work presents the development of the analytical method for determination of low ergosterol concentrations in building materials. Its application to laboratory infected and real contaminated building materials is presented.

Experimental

Reagents and chemicals

Ergosterol analytical standard was purchased from Sigma-Aldrich (St. Louis, MO, USA). The stock solution containing this analyte was prepared in ethanol at 400 μg mL^{-1}. Stock solution was diluted with methanol to 10 μg mL^{-1}. Stigmasterol used as internal standard was from POCh (Gliwice, Poland). Stock solution containing stigmasterol was prepared in ethanol at 400 μg mL^{-1}. Solution was diluted with methanol to 10 μg mL^{-1}. Combined solutions of ergosterol and stigmasterol were prepared at 0.5 μg mL^{-1} each. The 10 μg mL^{-1} solution of stigmasterol was also used for spiking samples before extraction.

MS-grade methanol was from POCh. Water was prepared by reverse osmosis in a Demiwa system from Watek (Ledec nad Sazavou, The Czech Republic), followed by double distillation from a quartz apparatus (Heraeus Bi18, Hanau, Germany). Pentane for HPLC and analytical grade NaOH were both from POCh.

Fungi propagation on building materials

Four different building materials were selected for this study: brick, plaster, concrete and granite. These

materials were selected because of their wide usage in both old and modern buildings. The fungi for propagation were chosen on the basis of previous, unpublished study on old buildings with visible biological corrosion. *Penicillium chrysogenum* belongs to the most widely occurring fungi, *Cladosporium cladosporioides* was dominant in the tested materials and *Aspergillus niger* are one of the most dangerous species. These fungi were cultivated on Difco™ potato-dextrose agar (PDA) from Becton, Dickinson and Company (Franklin Lakes, NJ, USA) acidified with tartaric acid from Sigma-Aldrich (St. Louis, MO, USA) to pH 5.7. After incubation for 14 days, the spores were rinsed to a flask with 2% PDA solution. The suspension was diluted to 10^5 spores *per* mL. A volume of 1 mL of this propagation structures was transferred with a sterile pipette onto wet 5.0 × 5.0 × 0.8 cm plates. The plates containing fungi propagation structures were kept at 21-23 °C and 96-99% relative humidity.

Sample preparation procedures

About 100 to 200 mg of the sample were placed in 12 mL glass culture tube, and 2 mL of methanol followed by 0.5 mL of 2 mol L^{-1} NaOH were added. The tube was sealed with a rubber lined screw cap, and ergosterol was extracted with the use of three different extraction procedures.

Focused microwave system

The culture tube was placed in a high-pressure polytetrafluoroethylene vessel and subjected to irradiation in a UniClever focused microwave system (Plazmatronika, Wrocław, Poland) operating at 2450 MHz and 300 W maximum output. A computer-controlled system was used for continuous monitoring of microwave power, temperature and pressure. Maximum temperature was set at 300 °C and pressure at 100 bar. The sample was microwave irradiated at 300 W for selected time period and cooled inside microwave system for 5 min.

Microwave oven

The culture tube was placed in a 200 mL screw capped high-density polyethylene bottle (Bel-Art Products, Wayne, USA). A Microchef 460 microwave oven (Moulinex, Caen, France) operating at 2450 MHz and 1300 W maximum output was used for irradiation of the samples. The irradiation was performed at 300 W for selected time period, and then the sample was removed from the oven and allowed to cool to room temperature.

Laboratory dryer

The culture tube was heated at 80 °C for 60 or 90 min in a gravity convection oven SML 32/250 Zelmed (Łomianki, Poland). Then, it was cooled outside the oven to room temperature.

Extraction with pentane

After irradiation in the microwave systems or heating in the dryer, the cooled samples were subjected to pentane extraction. Before extraction, 25 µL of internal standard (stigmasterol) were added to the tube. Then, the sample was extracted four times with 1 mL portions of pentane. Pentane extracts were separated by 1 min centrifugation at 4000 rounds *per* min. The extracts were combined and evaporated with a gentle stream of nitrogen that could be easily achieved because of low boiling temperature of pentane. Then, the samples were reconstituted in 0.5 mL of methanol and filtered through the 0.2 µm polytetrafluoroethylene syringe filter.

Liquid chromatography with mass spectrometry

A chromatographic system UltiMate 3000 RSLC from Dionex (Sunnyvale, CA, USA) was used. 10 µL samples were injected into a Gemini-NX C18 column (100 mm × 2.0 mm i.d.; 3 µm) from Phenomenex (Torrance, CA, USA) maintained at 35 °C. The isocratic mobile phase employed in the analysis consisted of methanol and water (95:5, v v^{-1}) at a flow rate of 0.4 mL min^{-1}. The LC column effluent was directed to the atmospheric pressure chemical ionization (APCI) interface of the API 4000 QTRAP triple quadrupole mass spectrometer from AB Sciex (Foster City, CA, USA). The APCI source operated in positive ion mode. The following settings for the ion source and mass spectrometer were used: curtain gas 10 psi, nebulizer gas 20 psi, temperature 400 °C, nebulizing current 3 µA and collision gas 10 psi. Declustering potential was 65 V and the dwell time for each mass transition detected in the selected reaction monitoring mode was set to 200 ms. Multiple reaction monitoring (MRM) was employed for data acquisition. The quantitative transition was from 379.3 to 69.1 *m/z* at collision energy set to 45 V and the confirmatory transition was from 379.3 to 145.1 *m/z* at collision energy set to 22 V for ergosterol. The transition for internal standard was from 395.3 to 147.1 *m/z* at collision energy set to 38 V. Abundance for the confirmatory transition for ergosterol standard was equal to 30% of the quantitative transition. Therefore, according to the EU guidelines,[30] the maximum

permitted tolerance for relative ion intensity was set to ± 25%. Simultaneously mass spectra were taken for the ergosterol peak in the enhanced product ion (EPI) mode. These were used for additional confirmation of ergosterol presence in the samples. The EPI mode was turned on in the information dependent acquisition, i.e., after intensity in the MRM mode exceeded 1000 counts *per* s. Collision energy in the EPI mode was set to 30 V to obtain less fragments than at 45 V used for quantitation. Scan speed in the EPI mode was set to 1000 amu s^{-1} and the dynamic fill time was turned on. The mass spectrum of ergosterol standard was added to the library and used for confirmation of ergosterol presence in the samples. The fit value for confirmation was set to at least 70%. Typical chromatogram and mass spectra are presented in Figure 1.

Method performance

The calibration graph was obtained by implementing the proposed method with a series of standard solutions in the range of 0.001 to 0.5 μg mL^{-1}. The limits of detection (LOD) and of quantitation (LOQ) were calculated on the basis of signal to noise (S/N) ratio. The S/N ratio of 3 was used for calculation of LOD and the S/N ratio of 10 for calculation of LOQ. The matrix effect was determined according to the procedure proposed by Matuszewski *et al.*[31] at 100 ng g^{-1}. Three sets of data were prepared. Set A included the results gained for standards. Set B contained data gathered for the samples spiked after extraction and set C for the samples spiked before extraction. The matrix effect (ME), recovery of the extraction procedure (RE) and overall process efficiency (PE) were calculated according to Matuszewski *et al.*[31] as follows:

$$ME\ (\%) = (B/A)\ 100 \quad (1)$$

$$RE\ (\%) = (C/B)\ 100 \quad (2)$$

$$PE\ (\%) = (C/A)\ 100 = (ME \times RE)/100 \quad (3)$$

The A, B and C in equations 1, 2 and 3 are peak areas obtained for sets A, B and C, respectively. Precision (as the relative standard deviation) of the method was calculated based on the results for the spiked samples.

Data analysis

One-way analysis of variance (ANOVA) was used to compare the means in both method development and analysis of samples of laboratory contaminated building materials. Two-way ANOVA with repetitions was used to test existence of interactions between type of building material and different fungal species. All statistical tests were carried out with a level of significance equal to 0.05. Calculations were performed using Microsoft Excel Analysis toolpak.

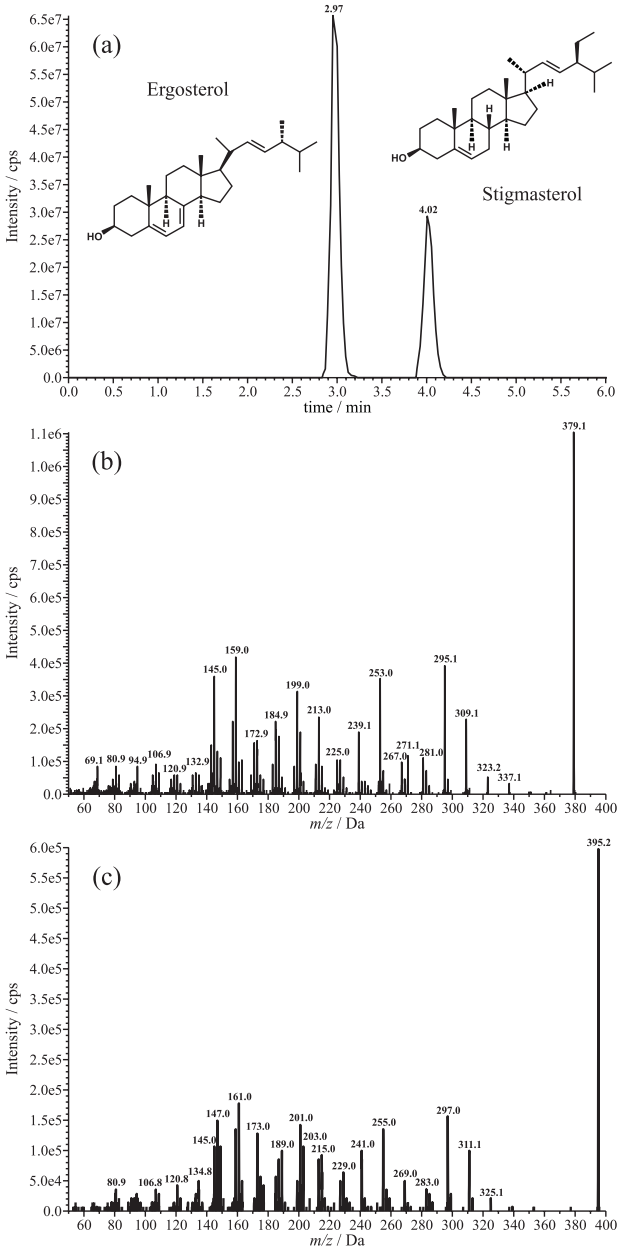

Figure 1. Typical chromatogram (a) with spectra of ergosterol (b) and stigmasterol (c).

Microscopic studies

A scanning electron microscope type VEGA TS 5135 MM from Tescan (Cranberry Twp., PA, USA) was used. The samples were coated with conductive material and scanned in vacuum with the use of the microscope.

Equipment for imaging luminescence

A charge coupled device (CCD) Night OWL LB 981 Imaging System from EG & G Berthold (Bad Wildbad, Germany) was used for imaging of the enhanced luminescence without any external excitation by chemicals or light. The device consisted of a light-sensitive dark box, inside which a CCD slow scan camera was mounted. The size of the observed area containing a sample with ^{63}Ni-porcelain-radioluminescence light standard was regulated by shifting the camera (up-down) by a camera lift. The applied camera consisted of an air cooler Peltier of a converter imaging CCD chip. The converter temperature was 200 K. Optical system wetting was eliminated by the separation of the cold part from the part at room temperature by a vacuum-quartz gate. The image of the observed object was projected onto CCD using a quartz lens. The camera was equipped with an automatic focus system. The image from the converter was directed by an imaging wire and an imaging interface to a computer. The entire device was controlled by a camera stacking wire (RS 232 J). All required procedures of regulation, control, measurement and analysis of the obtained images were carried out by the WinLight software supplied by the producer. The Night Owl camera was equipped with a slow scan light-sensitive CCD matrix, lighted from the back, with the resolving power of 512 × 512 points, sensitive to luminous radiation within the range of 200-1100 nm. The obtained image was generated through the accumulation of a charge resulting from the action of photons with the matrix material. The method of image generation adopted here is called single photon counting imaging and makes possible to record emissions of very low intensity, for methodology details see Górski *et al.*[32]

The first stage of the applied procedure was to record photography of samples (1 month after infection) under illumination in the growth chamber. The second stage was to record a series of luminescent images after turning off the lights of the measuring chamber. The time of recording a single image was 1 h. After reaching homeostasis of fungi, the Boramon fungicide from Altax (Poznań, Poland) was sprayed in darkness onto the samples from 0.2 m to achieve a uniformly covered surface. A series of luminescent images was taken again with 1 h acquisition time for each image.

Results and Discussion

Contamination of building materials with fungi poses a serious problem for human health as many of fungal species produce toxins. These toxins can be found on walls, ceilings and in the interior air of the human dwellings. Analysis of fungi presence with culture methods is long and difficult. Therefore, chemical analysis of appropriate marker of fungi can be made to overcome these problems. The analysis of ergosterol as fungi marker was described by Seitz *et al.*[17] who extracted it with methanol. It was modified by Griffiths *et al.*[33] who used ethanol-KOH solution enabling saponification of ergosterol bounded in fungi cell walls. The extraction process can be performed by heating the samples to about 80 °C under reflux or in closed tubes using a dryer or microwave system. However, the optimization of the sample preparation step has to be done to obtain reliable analytical method for selected type of sample matrices. This work presents the development of a procedure for ergosterol extraction from building materials and its use in analysis of laboratory infected samples and real materials from different buildings with visible fungi presence. The extracted ergosterol was determined with the use of liquid chromatography tandem mass spectrometry.

The optimization of the sample preparation started from the two-step microwave-assisted extraction similar to procedures described in the literature.[23,34] A system with focused microwave energy was used for this purpose and a number of procedures was tested. There were four different procedures applied with two heating time periods ranged from 20 to 60 s and a 2 min cooling time between them. As presented in Figure 2a, the procedure with two-times 20 s extraction led to scattered results. Thus, it was rejected. The three tests with longer heating times led to similar results. Therefore, there were compared using ANOVA. The ANOVA calculation confirmed that there was no statistical difference between mean values obtained as the calculated *p*-value was 0.933. Then, the neutralization of the sample extract before its transfer to pentane was also tested (N marked sample in Figure 2a). The results for this test, together with results for two-step process with 40, 50 and 60 s, of extraction were subjected to the ANOVA calculation. Again, the hypothesis of equality between means could not be excluded as the obtained *p*-value was equal to 0.823. Therefore, no neutralization was used in further experiments. As the two-step process was labor-intensive, a one-step process was checked. The test included 60 and 70 s long focused microwave-assisted extraction. The 70 s maximum time was selected because longer time caused leaking of the tubes and loses of the samples. Probably it was caused by too high pressure and temperature inside the tubes. The results from these tests were subjected to the ANOVA calculation to compare the mean values with those obtained during two-step procedure. The *p*-value obtained for the six tests was 0.064. Although the hypothesis of equality between means could not be

excluded, the *p*-value was close to the 0.05 limit. Therefore, the equality of means for one-step 60 and 70 s procedures was tested. The obtained *p*-value was 0.027, i.e., the two means were not the same. As a result, the simple one-step procedure with 70 s extraction was taken for further studies.

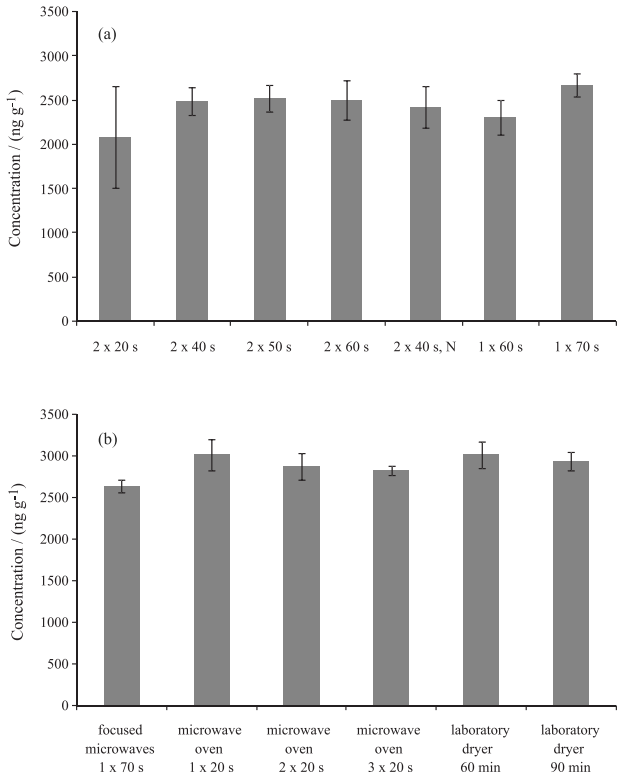

Figure 2. Optimization of the extraction conditions in the focused microwave system (a) and comparison of the three studied systems: focused microwave system, microwave oven and laboratory dryer (b). The error bar is the standard deviation (SD, n = 3).

The results obtained for focused microwave system were compared with those obtained with microwave oven and laboratory dryer. A time of 20 s of irradiation was used for the microwave oven as a maximum safe time and use of repeated microwave heating steps was also studied. The heating in the dryer was performed for 60 or 90 min. The results presented in Figure 2b were statistically compared using the ANOVA calculation. The obtained *p*-value was 0.027, i.e., the mean values presented in Figure 2b were not equal. Indeed, it could be found that the extraction with the use of both microwave oven and laboratory dryer led to higher efficiency than extraction using the focused microwave system. The removal of results gained for the 70 s long focused microwave-assisted extraction led to much higher *p*-value, i.e., 0.268. Equality of results obtained with the use of the microwave oven and laboratory dryer extraction could not be excluded. As a result, the simplest tested extraction process was used in further studies that included 20 s long one-step extraction in the microwave oven.

The optimized method was validated. The linearity was tested from 0.001 to 0.5 μg L^{-1} and correlation coefficient (0.9998) was obtained. The limits of detection and of quantitation were 1.7 and 5.5 ng g^{-1}, respectively. The recovery and matrix effect were tested using samples spiked with 100 ng g^{-1} of ergosterol according to the procedure proposed by Matuszewski *et al.*[31]. The mean recovery and mean matrix effect were 65.9 ± 3.8 and 111.6 ± 5.9%, respectively. Thus, the overall process efficiency expressed as product of matrix effect and recovery divided by 100 was 73.5 ± 4.3%. The complete recovery of ergosterol could not be achieved even though multiple microwave extraction was tested during optimization. However, the obtained results were repeatable. The precision expressed as relative standard deviation for five measurements of sample solution was 5.8%.

The developed method was used for ergosterol determination in building materials intentionally infected with different species of fungi and in selected building materials sampled from several buildings. The infected samples were left for one month in controlled conditions (21-23 °C, 96-99% relative humidity). The results obtained for infected samples presented in Figure 3 show that for the particular building materials there was no significant difference between the samples infected with three fungi species. This was confirmed using the ANOVA calculation performed for granite, brick, plaster and concrete that provided 0.092, 0.105, 0.433 and 0.153 *p*-values, respectively. These results also enabled easier comparison between different building materials. Data for each material could be grouped and subjected to the ANOVA test. The obtained *p*-value below 0.001 confirmed that the mean results for granite, brick, plaster and concrete were substantially different. Multiple comparison test was also done to test whether there is a statistical difference between granite and brick, brick and plaster as well as plaster and concrete. The *p*-values for these pairs were 0.007, 0.012 and 0.002, respectively, i.e., results for all tested pairs differ. The highest amounts of ergosterol were found for granite samples. Lower results were obtained for brick and further deceleration was noted for plaster. The lowest amounts of ergosterol were found in concrete samples. The results gained in the experiment were also subjected to two-way ANOVA test. This allowed checking if there is an interaction between the two selected variables (building materials and fungi). The *p*-value obtained for the interaction of these variables was only 0.021, i.e., the existence of interaction was confirmed. Indeed, the amount of ergosterol found for materials propagated by

Aspergillus niger was much more differentiated than for two other fungi tested.

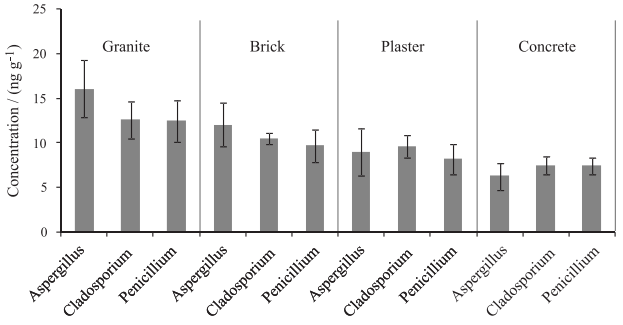

Figure 3. Concentration of ergosterol in different building materials measured one month after fungi propagation. The error bar is the standard deviation (SD, n = 3).

The microscopic observations and bioluminescence measurements were carried out for the infected samples in order to additionally confirm the fungi presence. The use of an electron microscope enables relatively fast assessment of fungi presence on the surfaces of the building materials. Photographs taken during microscopic investigation of granite infected with different fungi can be found as an example in Figure 4. Fungal colonies can be clearly observed on the surface of the samples. However, electron microscope observation cannot be the basis for a quantitative measurement as the fungal colonies grow with unequal coverage of the surface. This can be clearly observed with the use of bioluminescence which is measured on much larger surfaces than these observed in the microscopic studies.

Photographs taken for different stages of the luminescence measurement for the granite samples infected with *Cladosporium cladosporioides* can be found in Figure 5. These photographs document the unequal coverage of the surface with fungal colonies and allow for visual examination of the samples. Each photograph was taken with 1 h of exposure time. The mean intensity of light-induced luminescence from three infected samples and reference sample can be obtained together with light intensity of a standard. The curve obtained for light-induced infected samples lowers considerably as a result of the biochemical process being suppressed. Fungi aim to establishment of a new "dark" homeostasis. The samples were left in darkness over 40 h, until no changes in light intensity could be measured and then, three luminescence photographs were taken with acquisition time equal to 1 h each. The mean intensity of luminescence from infected samples established at constant level equal to this noted for the reference sample. The samples were taken from the measurement chamber and sprayed with a solution of the commercial antifungal agent Boramon. The use of the antifungal substance forced fungi to leave the "dark" homeostasis and activated both defensive and decay processes. These processes are connected with light emission.

The sprayed samples were again placed in the measurement chamber and a series of luminescence photographs was taken. As it can be observed, the intensity of light for the samples infected with fungi increased twice in comparison to the uninfected reference sample. The observed increase in the light emission from the infected samples treated with antifungal agent may be an evidence of the living fungi presence on the surface of the tested material. Nevertheless, this method cannot be used for quantitative study of fungal infections on the building materials at low levels. Moreover, it is time-consuming technique in comparison to both microscopic studies and ergosterol determination as a fungal marker with the use of HPLC-MS/MS.

The applicability of the developed chromatographic method was tested also for the determination of ergosterol in real building materials. All analyzed samples had signs of the fungal presence, visible to the "naked eye". Some of these samples contained considerable amount of stigmasterol that made impossible to use this compound

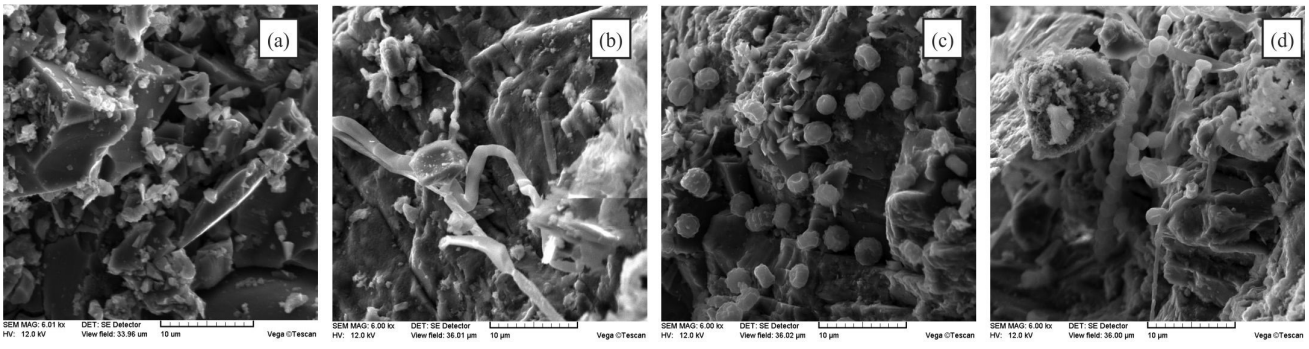

Figure 4. Electron microscope images of granite (magnification 6000 ×) taken 1 month after fungi propagation. Control, uncontaminated sample (a), sample infected with *Cladosporium cladosporioides* (b), sample infected with *Aspergillus niger* (c) and sample infected with *Penicillium chrysogenum* (d).

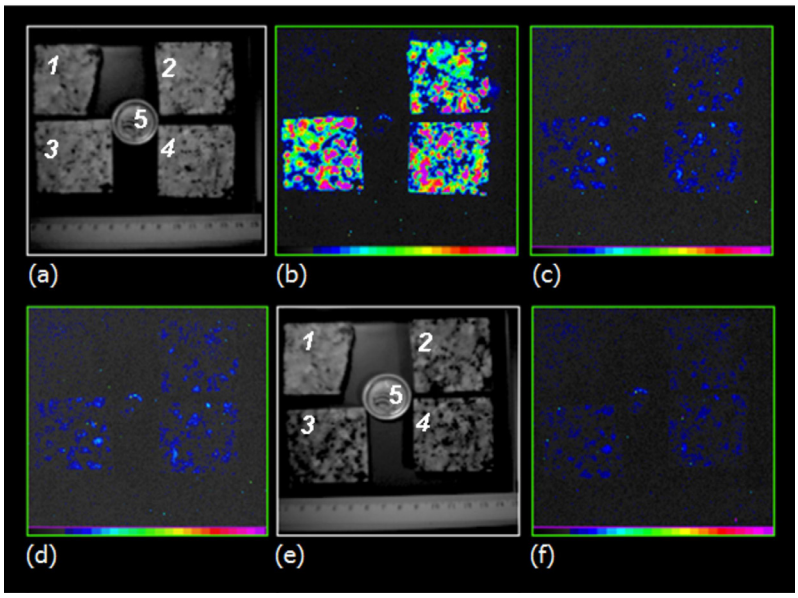

Figure 5. Photographs and luminescence images of granite samples 1 month after infection with *Cladosporium cladosporioides*. 1: noninfected sample, 2, 3 and 4: infected sample, 5: radioluminescence light standard. Photography (a), initial luminescence image (b), luminescence image after 18 h (c), luminescence image after establishment of "dark" homeostasis (d), photograph after spraying with fungicide (e) and luminescence image after spraying with fungicide (f).

as the internal standard. The use of isotopically-labelled internal standard was considered but a satisfactory precision of the obtained results was achieved without using internal standard. The results presented in Figure 6 show different levels of contamination in the materials. Very high concentrations of ergosterol were observed for the samples of cellular concrete and plaster having porous structure and no paint protection on the surface.

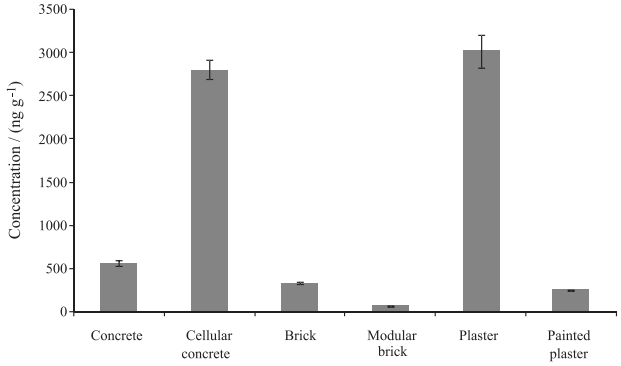

Figure 6. Concentration of ergosterol in different real building materials contaminated with fungi.

Conclusions

A rapid analytical methodology was developed for determination of ergosterol as a marker of fungi contamination in different building materials. Microwave-assisted extraction and HPLC-MS/MS determination were successfully used for analysis of ergosterol in intentionally infected samples of granite, brick, plaster and concrete. The developed method can be used for determination of ergosterol in different building materials at a wide concentration range.

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