

RESEARCH

Open Access



The potential of fibre optic UV-Vis-NIR spectroscopy to distinguish vital and devitalised forms of microbial contamination of paper substrates

Patrícia Paračková^{1*}, Michal Čepčan¹, Barbora Kaliňáková², Milena Reháková¹ and Lukáš Gál¹

Abstract

Microorganisms, especially microscopic filamentous fungi, represent one of the most significant factors influencing the degradation of cultural heritage cellulosic objects. Early detection, identification and characterisation of microbial communities can help select a suitable conservation intervention and, in addition, allow possible control of its effectiveness. A combination of spectral methods and statistical data processing appears to be a suitable alternative to conventional methods for monitoring microbial contamination. The main goal of the presented research was the investigation of the spectral properties of vital and devitalised filamentous fungi *Alternaria alternata*, *Aspergillus niger*, *Cladosporium herbarum*, *Penicillium chrysogenum*, and *Trichoderma atroviride*, inoculated on a paper substrate Whatman in two phases—dormant (conidia) and active (mycelium). The combination of the UV-Vis-NIR and NIR Fibre Optics Reflection Spectroscopy (FORS) with the Principal Component Analysis (PCA) was set to determine whether the spectra of vital and devitalised forms of studied samples differ. The obtained results prove differences between the spectra of vital and devitalised forms of filamentous fungi, at least in one studied spectral region. Therefore, UV-Vis-NIR and NIR FORS combined with PCA seems to be a promising tool for monitoring paper-based objects' microbial contamination.

Keywords Filamentous fungi, Biodeterioration, Fibre optics reflection spectroscopy, Cultural heritage, Paper, Principal component analysis

Introduction

The protection of cultural heritage is a crucial activity that ensures the preservation and transmission of information from various areas of human history to future generations. In addition to natural ageing, all objects of

cultural heritage, including those found on paper carriers, are exposed to several factors, which can significantly accelerate degradation processes leading to a change in chemical composition, a decrease in mechanical strength, and even the destruction of the object itself. Furthermore, as a result of improper storage conditions and insufficient preventive measures, these objects are often located in an environment that provides suitable conditions for potential microbial contamination. An important source of contamination in archives, libraries, and other memory institutions is a group of microorganisms called microscopic filamentous fungi (Fig. 1). Besides the degradation of works of art, fungal spores may affect the health of memory institution employees and visitors [1].

*Correspondence:

Patrícia Paračková
patricia.parackova@stuba.sk

¹ Faculty of Chemical and Food Technology, Department of Graphic Arts Technology and Applied Photochemistry, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovak Republic

² Faculty of Chemical and Food Technology, Institute of Biochemistry and Microbiology, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovak Republic



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.



Fig. 1 A set of contaminated paper-based cultural heritage objects from the collection of the Slovak National Archive

The reasons for microbial contamination are various. It is generally known that filamentous fungi are ubiquitous and cultural heritage objects are therefore constantly exposed to the risk of microbial deterioration [2]. Fungi are able to get into archives and libraries through dust particles, which contain fungal spores [3]. Another way how micromycetes can get into the indoor environment of memory institutions is through newly admitted contaminated objects or human interactions [4]. Low-quality ventilation systems combined with different surface temperature lead to the formation of local micro-climates with higher water availability, consequently creating favourable conditions for fungal growth [5].

A wide variety of fungi can be found in archives and libraries, most often from the genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Trichoderma* and *Chaetomium* [6, 7]. Paper, as a hygroscopic substrate made of cellulose fibres containing various additives (glues, binders, etc.), represents an excellent source of nutrients for filamentous fungi as they are chemoheterotrophs, requiring organic compounds as energy and carbon source [3, 8].

Microscopic filamentous fungi are able to deteriorate paper-based objects by the action of extracellular cellulolytic enzyme complex (cellulase) that is capable of hydrolysing β -1,4-glycosidic bonds in cellulose. Enzymatic degradation results in a transformation of cellulose

into glucose molecules—the primary carbon and energy source for fungi [9]. At the same time, paper heritage objects are exposed to mechanical damage associated with the penetration of fungi hyphae in the substrate [9]. The enzymatic activity of filamentous fungi can affect the chemical, physical and aesthetic properties of contaminated artefacts [10]. This phenomenon is known as biodeterioration, defined as “any undesirable change in the properties of a material caused by the vital activities of organisms” [11]. As a result of the irreversible degradation of cellulose fibres, paper-based heritage objects become brittle and cracked [12].

Moreover, certain types of filamentous fungi are characterised by the production of pigments and weak organic acids, making the documents unreadable and diminishing its aesthetic value. The stains on the substrate caused by the activity of fungi may have different colouration (e.g. yellow, brown, black) [9, 13]. A special case of fungal deterioration, appearing as local changes in a paper’s colour, is called foxing [14]. Foxing spots are described as “random circular and irregular yellowish to brownish-red stains on the surface of old paper” [15].

To inhibit biodeterioration processes, memory institutions use various methods. The most available method is applying UVC irradiation, but due to its poor penetration, it is mainly used to sterilise directly exposed surfaces

and air. Its germicidal effect is based on damaging microbial RNA and DNA, making replication of genetic information impossible and thus preventing reproduction [16, 17]. Despite the fact that microwave radiation is not traditionally used for decontaminating heritage objects, some authors [18, 19] demonstrated the potential of the method for the devitalisation of microorganisms. However, the mechanism is not yet fully understood. It is not entirely clear whether the devitalisation is caused only by thermal effects, i.e. the heat generated as a result of the absorption of microwave radiation by water, or by complex organic systems with permanent or induced polarization, or it occurs due to a non-thermal microwave effect, i.e. by the direct transfer of energy from the electromagnetic field to the vibrational states, which leads to a change in their conformation [20]. Another widely used method for a long time is the application of ethylene oxide as an effective biocidal agent [10, 21, 22]. Although it belongs to one of the most effective decontamination methods, it has a huge disadvantage—it is carcinogenic and mutagenic [4]. Its biocidal effects on microorganisms completely stop their biological processes through the alkylation reaction of cellular constituents (nucleic acids, proteins). The application of electron beam irradiation is a promising and effective alternative to ethylene oxide [23]. Interaction between accelerated electrons and microorganisms leads to the cleavage of their DNA chains. The method's advantages include a short exposure time and no residues after the sterilization process [24].

Cultivation techniques have been conventionally used to detect and identify microorganisms inhabiting cultural heritage objects and materials [25]. However, the main drawback of culture-dependent methods is that they provide limited information, as the majority of microorganisms is not cultivable in laboratory conditions [26]. In recent years, interest in new methods has been growing, and other methods of detecting and identifying microorganisms have been created. Among the most used are DNA amplification by polymerase chain reaction (PCR) or DNA sequencing. However, these methods are often expensive, time-consuming and performed *ex-situ* [27, 28].

In order to choose the most adequate and effective ways of protection, it is necessary to know all the essential information about microbial communities inhabiting objects of cultural value, i.e. type of microorganisms, their characteristics, relationship to the contaminated objects and activity level (viable or devitalised form). Early detection and characterisation of fungal contamination make it possible to apply relatively non-invasive methods for its removal before visible and permanent damage of attacked artefacts becomes apparent [29].

Information about viability enables the selection of an appropriate conservation method, and in addition, it can help to check the effectiveness of the conservation intervention.

With a focus on determining the viability of filamentous fungi, a few techniques are currently used. In addition to the cultivation techniques, the method based on the measurement of ATP by bioluminescence showed its potential [30]. Other techniques, such as solid-phase cytometry [31], fluorometric assays or dye exclusion assays [32] are used in various fields (medicine, microbiology, etc.), but they have not yet been applied in the field of heritage protection. Ongoing and intense research aims to develop new, time-saving, easier, non-destructive, and non-invasive methods that could be used directly where microbial contamination is present. In this regard, UV-Vis-NIR and NIR Fibre Optics Reflection Spectroscopy (FORS) seems to be a promising method to fulfil these requirements. The devices are quite small, portable, and the measurements take only a few seconds. They can be used directly in collections-keeping institutions. Furthermore, measurements with optical fibre devices are not limited by the size and shape of the analysed objects. The method has already been successfully used in the cultural heritage field to identify dyes and pigments [33, 34] or iron-gall inks [35]. On the other hand, the method has not yet been sufficiently investigated to detect, characterise or identify microbial contamination of heritage objects. However, several studies from different fields (e.g. microbiology of food, environment, agriculture or medicine) demonstrate the method's applicability and other spectroscopic techniques for this intent [36–39].

The aim of this paper is to apply UV-Vis-NIR and NIR FORS to study the fungal contamination of paper substrates and evaluate the possibility of using the method to monitor the state of contaminated objects of cultural value.

Materials and methods

Model systems

Model systems mimicking microbial contamination of paper consisted of two main components—a paper substrate and filamentous fungi, which were inoculated, either in the form of conidia or mycelia. The filamentous fungi used in the experiment were selected based on a literature review as representatives of the fungi genera most often found in the environment of memory institutions. Three groups of model systems were prepared—viable, devitalised and reference. Reference model systems were exposed to the same conditions as viable and devitalised ones, but they were not inoculated with filamentous fungi. Four paper carriers inoculated with conidia or mycelia of filamentous fungi were prepared for each

investigated model system. Two samples were intended for spectra measurement, and two were used for viability control.

Model substrate

Chromatographic paper Whatman 1 CHR with smooth surface, 0.18 mm thick, basis weight 87 g/m², surface pH 7, manufactured without additives. A sheet of paper was cut into squares 2×2 cm and sterilized by autoclaving (120 °C, 120 kPa, 20 min).

Model microorganisms

Alternaria alternata CCM F-128 (AA), *Aspergillus niger* CCM 8189 (AN), *Cladosporium herbarum* CCM F-159 (CH), *Penicillium chrysogenum* ATCC 9480, CCM F-362 (PCH) and *Trichoderma atroviride* CCM F-534 (TV) (Czech Collection of Microorganisms, Masaryk University in Brno, Czech Republic) were cultivated on malt extract agar (MEA; Biolife, Italy).

Inoculation of paper substrates

A few millilitres of sterilized distilled water were added to the 10 day-old cultures of filamentous fungi, and the conidia were released using a sterile L-shaped spreader. The fungal spore suspensions were transferred into a Falcon-type test tubes (50 mL) and filtered through a sterile polyamide/nylon sieve (opening size 0.43 µm). Fungal spores were separated by centrifugation (10 min at 2500 rpm) and washed with water three times. A diluted solutions of the spore suspensions were prepared in a sterile Eppendorf tubes. The Bürker chamber was used for counting spores. The concentration of spores was adjusted to 10⁶ conidia/50 µl, except for *Alternaria alternata* – 10⁵ conidia/50 µl. Model systems consisting of paper carriers and conidia of filamentous fungi were prepared by inoculating the paper substrates with a 50 µl of spore suspension using a pipette.

A model systems consisting of a paper carrier and mycelia of filamentous fungi were prepared as follows: 1.5 mL of Czapek-Dox broth (CZDB; Biolife, Italy) in sterile Eppendorf microtubes were inoculated by 50 µL of spore suspension and cultivated for 48 to 72 h on a reciprocal shaker. Mycelia were separated from the medium by centrifugation (15 min at 13.400 rpm) and washed three times with water. Subsequently, the solid mycelium was quantitatively transferred to a paper carrier using a pipette.

Devitalisation of model systems

Five available methods for the devitalisation of samples were selected, whose goal was to capture the properties of the widest possible range of devitalised sample products. A model system consisting of a paper substrate and

conidia or mycelia of individual filamentous fungi was prepared for each devitalisation method. Namely, UVC irradiation (UVC), microwave radiation (MW), ethylene oxide (EO), accelerated electrons (AE), and anaerobic conditions (-O₂) were used as devitalisation methods.

UVC irradiation (UVC)

The devitalisation process based on applying UVC irradiation was carried out using the germicidal lamp BakMed LB-301.1 (BakMed; Poland), emitting ultraviolet rays with a wavelength of 253.7 nm. The distance between the samples and the light source was 8 cm, and the irradiation of samples lasted 15 h.

Microwave radiation (MW)

The devitalisation method based on the biocidal effects of microwave radiation was carried out using the Candy CMW 2070 M microwave oven, which has a power of 700 W. The samples were exposed to microwave radiation with a high power level (100% power output) for 15 min.

Ethylene oxide (EO)

Ethylene oxide sterilization took place in cooperation with the Slovak National Gallery (Zvolen, Slovakia). The ethylene oxide fumigation chamber (DE LAMA; Italy) has several programs, which differ in the humidity, temperature and pressure used, depending on the material and the degree of damage. The selected sterilization program P4 lasts 22 h—from when the object is inserted into the chamber until the end of sterilization and ventilation. The parameters of the P4 program are summarized in Table 1.

Accelerated electrons (AE)

Devitalisation of model systems using a linear electron accelerator UELR-5-1S (FGUP NIIIEFA, Russia) took place in cooperation with the Progres Final SK in the Centre of electron accelerator (Trenčín, Slovakia). The process parameters are in Table 2.

Anaerobic conditions (-O₂)

The anaerobic box with AnaeroGen™ sachet (Oxoid, UK) was used to create anaerobic conditions. After inserting

Table 1 P4 program used for ethylene oxide devitalisation

Parameter	
Temperature	35 °C
Relative Humidity	55%
Pressure	1.5 barr
Sterilant	ETO:CO ₂ (10:90)

Table 2 Parameters of accelerated electrons devitalisation process

Parameter	
Electron energy	5 MeV
Scan frequency	1 Hz
Repetition rate	120 Hz
Scanning width	45 cm
Geometry	One-sided irradiation
Required dose	25 kGy
Real dose	27,5 ± 5% kGy

the sachet into the anaerobic box, oxygen is absorbed, and carbon dioxide CO₂ is produced. When used correctly, the system is able to reduce the amount of oxygen below 1% in 30 min. The resulting amount of CO₂ ranges from 9 to 13%. The active ingredient is ascorbic acid. A moisture absorber—a sachet with silica gel—was used to reduce the humidity. It can reduce humidity to 40% in a closed system. The samples were subjected to anaerobic conditions for four weeks.

Viability control

The effectiveness of the selected devitalisation processes was verified by the cultivation of samples. Samples intended for viability control were aseptically transferred to a sterile CZDB medium (Biolife, Italy), put on a rotary shaker, and left to culture for 72 h. After this time interval, a visual check of the viability of filamentous fungi followed. Insufficient devitalisation of conidia was observed as germination and mycelium formation. In case of unsuccessful devitalisation of the mycelia, we could observe their growth on the paper substrate.

Spectroscopy methods

Before the actual measurement of UV-Vis-NIR and NIR spectra, all prepared model systems were conditioned in a climatic chamber for 24 h (23 °C, 50% RH). Spectral data of samples in the UV-Vis-NIR and NIR spectral regions have been obtained using Ocean Optics fibre optics spectrophotometric devices (Ocean Optics, Dunedin, FL, U.S.):

UV-Vis-NIR region—spectrometer HR4000CG, light source DH-200-BAL and standard reflection accessory with 45°/45° geometry; NIR region—spectrometer NIR256-2.5, light source HL-2000-FHSA and standard reflection accessory with 45°/45° geometry. The measurements were performed with the OceanView 1.6.7 (Ocean Optics, Dunedin, FL, U.S.) software package with the following settings: integration time, automatic; the number of averaged spectra, 5; boxcar, 10.

Two parallel samples were measured at five different spots to limit the impact of uneven inoculation. The result of the measurements of each sample was a set of 10 spectra, which were statistically processed.

Processing of data

1. The original spectral data were interpolated in the wavelength range of 300–1050 nm (UV-Vis-NIR) and 1000–2200 nm (NIR), 150 points.
2. Subtraction of the average spectrum of reference samples from each spectrum of viable or devitalised samples.
3. Normalization of each spectrum in the interval 0–1.
4. Calculation of the average normalized spectrum for each sample, calculation of the standard deviation SDO of the set of spectra.

In the case of a high standard deviation value (above 0.10), possible outlier spectra of the set were identified using the Principal Component Analysis (PCA) and excluded from the data set. Spectra were displayed graphically and analysed by the PCA method.

Principal component analysis (PCA)

Modern spectrometric systems generate enormous amounts of data. Therefore, a simple evaluation is not always possible without losing valuable information. In such a situation, it is helpful to use chemometric-numerical data processing. The methods based on factor analysis are used most often.

Among the factor analysis methods, the multivariate statistical method Principal Component Analysis (PCA) is an especially useful tool for processing spectral data sets. The PCA method uses a matrix decomposition of spectral data and a corresponding reduction in the number of variables to describe the spectra of the studied samples in a reduced space. The PCA allows to detect the tendency of samples with comparable properties to cluster into groups and reveal the latent internal structure of the data. The Unscrambler X 10.5 programme (CAMO Softwares AS., Oslo, Norway) was used for the analysis.

Results and discussion

The aim of this work is to evaluate the possible application of UV-Vis-NIR and NIR FORS combined with the multivariate statistical method PCA as an effective approach to monitor microbial contamination. Several spectra were measured and analysed to evaluate the potential of Principal Component Analysis of UV-Vis-NIR and NIR spectra as a tool for the differentiation of viable and devitalised forms of filamentous fungi.

Despite several repeated attempts, we did not achieve the devitalisation of *Alternaria alternata* (mycelium) using UVC irradiation and *Aspergillus niger* (conidia and mycelium) using anaerobic conditions. *Alternaria alternata* has multi-celled spores with thick, melanised cell walls that can create a way of protection against UV irradiation [40]. Moreover, UVC radiation is characterised by low penetration, and it is possible that not all cells were exposed to its germicidal effects. *Aspergillus* species are obligate aerobic microorganisms. However, decreasing oxygen level increase sporulation. Therefore, it is possible to survive adverse conditions in this dormant form without losing viability.

The results of the PCA method—two-dimensional score scatter diagrams—allow observing the distribution of samples into clusters. It is important to note that

Table 3 The principal component analysis (PCA) results—a noticeable and clear difference of the spectra of viable and devitalised forms—Yes, no/poor distinction of the spectra of viable and devitalised forms—No, the distinction is only partial—Partially

Filamentous fungi		UV-Vis-NIR	NIR
<i>Alternaria alternata</i>	Conidia	Yes	Yes
	Mycelium	Yes	No
<i>Aspergillus niger</i>	Conidia	Yes	Partially
	Mycelium	No	No
<i>Cladosporium herbarum</i>	Conidia	Yes	Yes
	Mycelium	No	Yes
<i>Penicillium chrysogenum</i>	Conidia	Yes	Yes
	Mycelium	Yes	Partially
<i>Trichoderma atroviride</i>	Conidia	Yes	Yes
	Mycelium	No	Yes

samples located in one cluster are similar to each other, and at the same time, they are different from samples located in other clusters. Table 3 shows in which scatter score diagrams a noticeable and clear difference of the spectra of viable and devitalised forms was found (Yes), in which no/poor distinction of the spectra of viable and devitalised forms was observed (No) and in which the distinction is only partial (Partially). It turns out that the difference between viable and devitalised forms was found in almost all studied systems, at least in one studied spectral range, except for *Aspergillus niger* mycelia.

Figures 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 show UV-Vis-NIR and NIR spectra of viable and devitalised forms (conidia, mycelium) of examined fungi and corresponding two-dimensional score scatter diagrams. Different groups of samples have different colours: viable samples (VIT)—black; samples devitalised using UVC irradiation (UVC)—red; microwave radiation (MW)—blue; ethylene oxide (EO)—green; accelerated electrons (AE)—cyan and anaerobic conditions (-O₂)—magenta.

UV-Vis-NIR spectra of viable conidia and mycelium of *Alternaria alternata* (Figs. 2, 4) form separated clusters clearly distinguishable from the spectra of samples devitalised by selected methods. In the case of *Alternaria alternata* conidia (Fig. 2), PCA analysis in the UV-Vis-NIR spectral region led to the separation of several groups corresponding to viable and devitalised samples. Viable samples are clearly differentiated from devitalised ones and are characterised by a negative score value of both components (PC-1, PC-2). Most devitalised samples have a positive score value of component PC-1, except for samples devitalised by anaerobic conditions. UV-Vis-NIR spectra of *Alternaria alternata* mycelium (Fig. 4) show similar behaviour, but in this case, viable samples are characterised by a positive score value of the

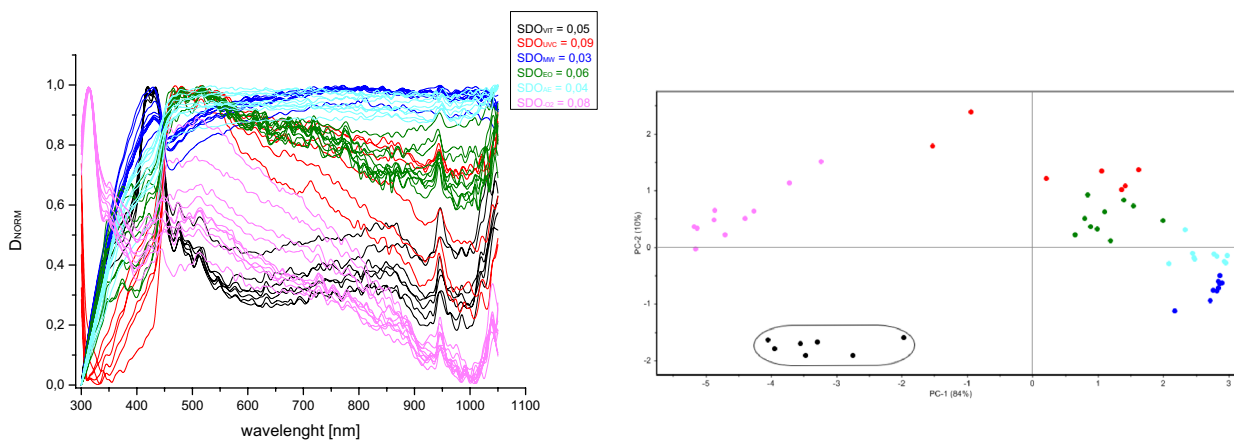


Fig. 2 UV-Vis-NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Alternaria alternata* conidia samples

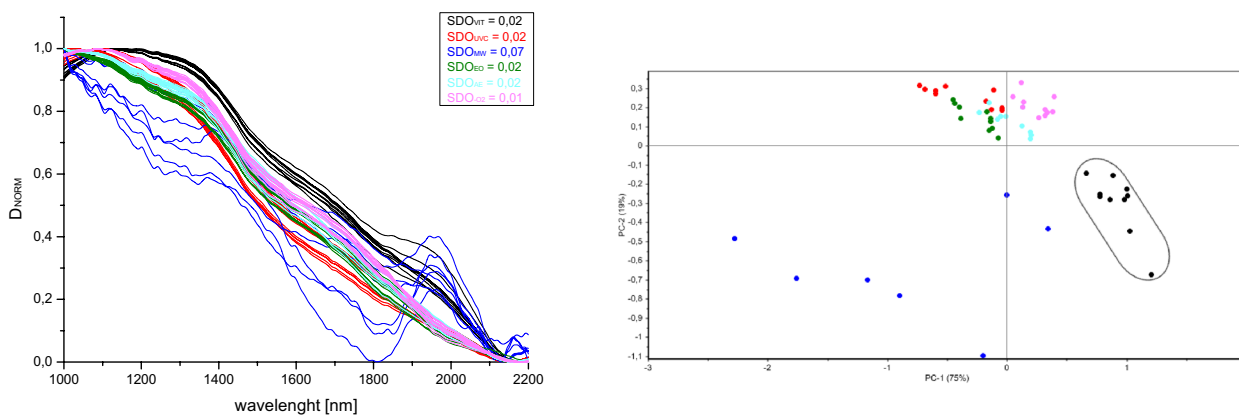


Fig. 3 NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Alternaria alternata* conidia samples

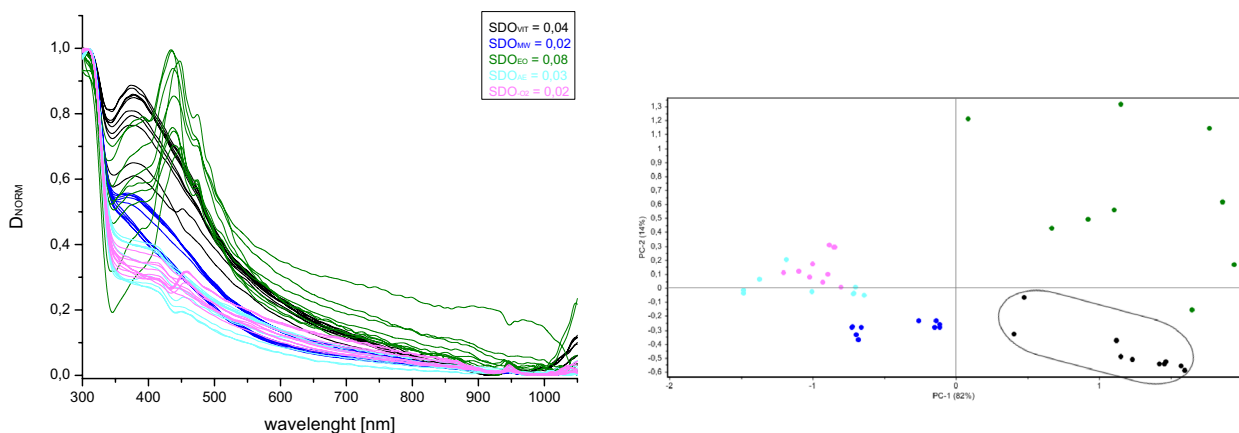


Fig. 4 UV-Vis-NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Alternaria alternata* mycelium samples

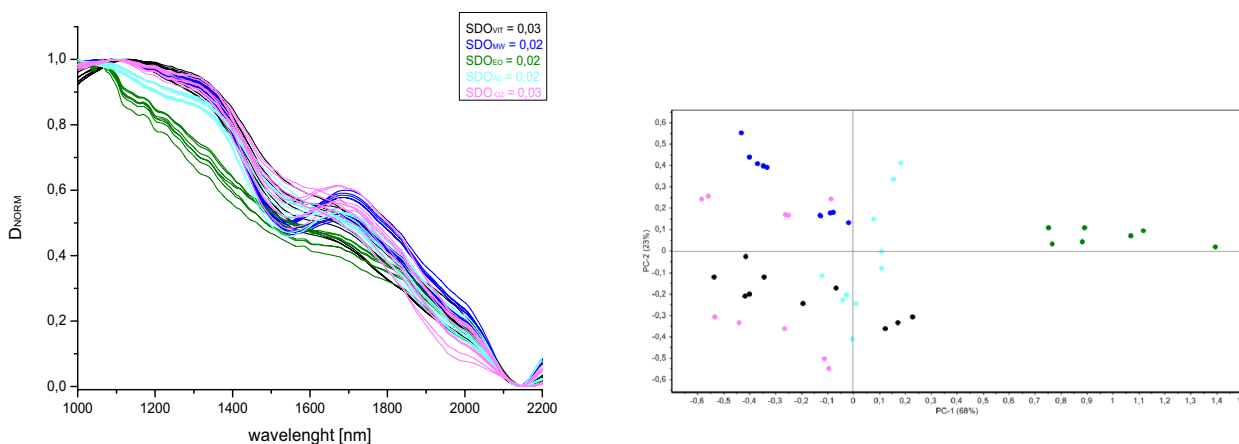


Fig. 5 NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Alternaria alternata* mycelium samples

first component, while most devitalised samples have a negative score value, except for samples devitalised with ethylene oxide. NIR spectra and score scatter diagrams

revealed differences between viable and devitalised *Alternaria alternata* conidia (Fig. 3) as well. The cluster corresponding to NIR spectra of viable conidia is separated by

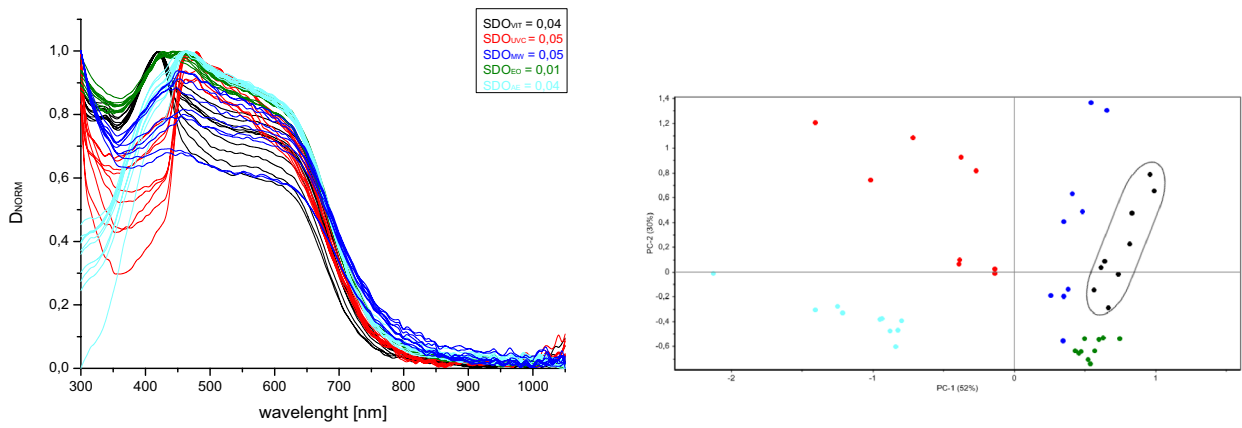


Fig. 6 UV-Vis-NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Aspergillus niger* conidia samples

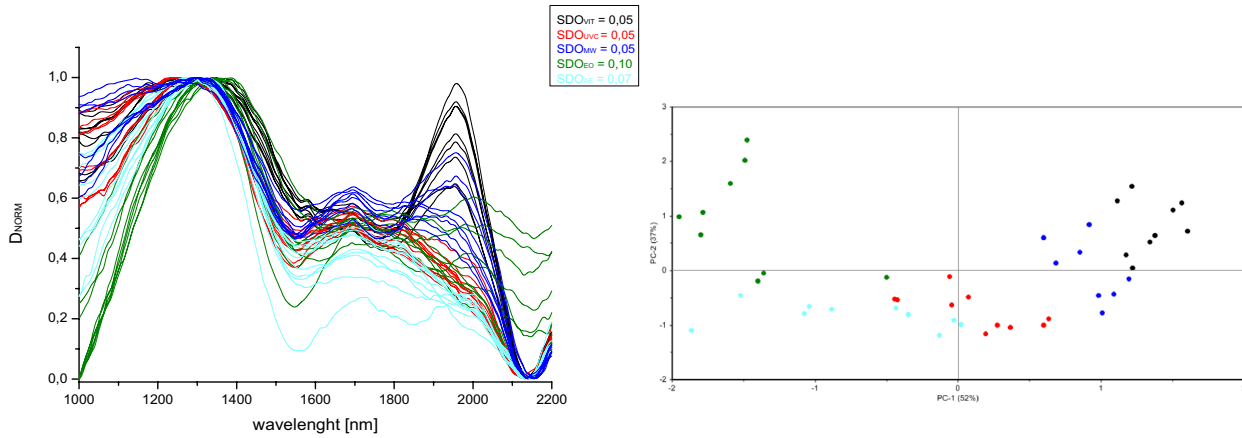


Fig. 7 NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Aspergillus niger* conidia samples

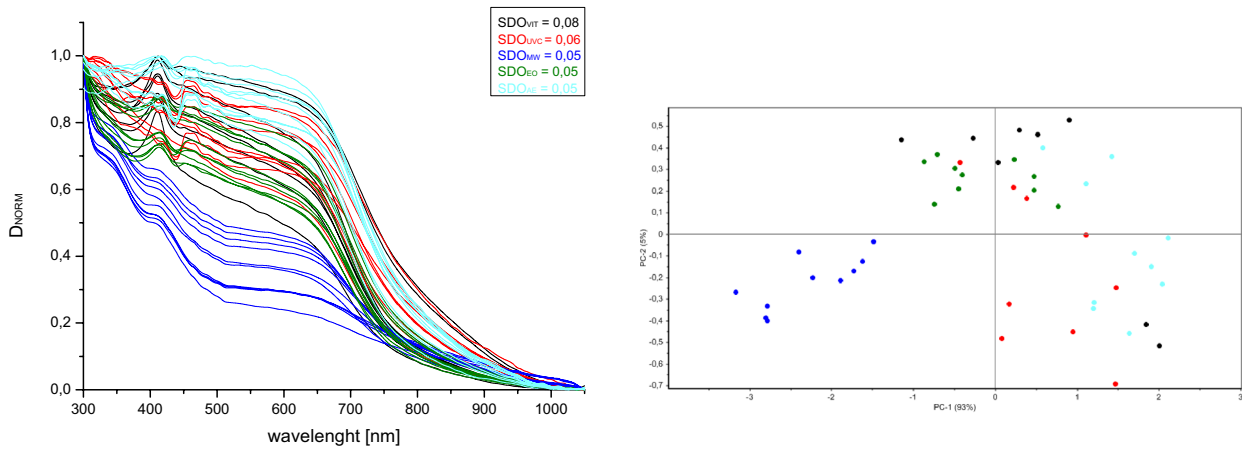


Fig. 8 UV-Vis-NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Aspergillus niger* mycelium samples

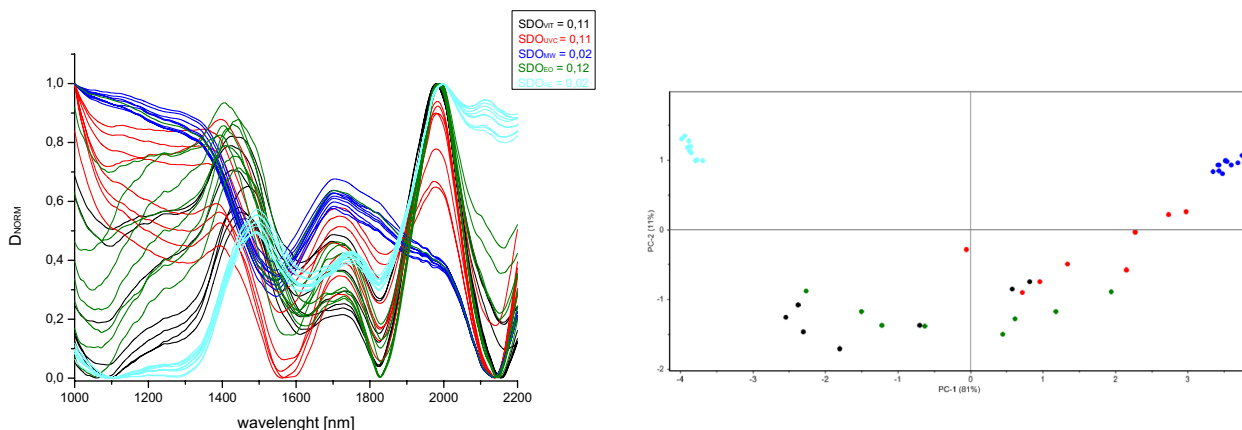


Fig. 9 NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Aspergillus niger* mycelium samples

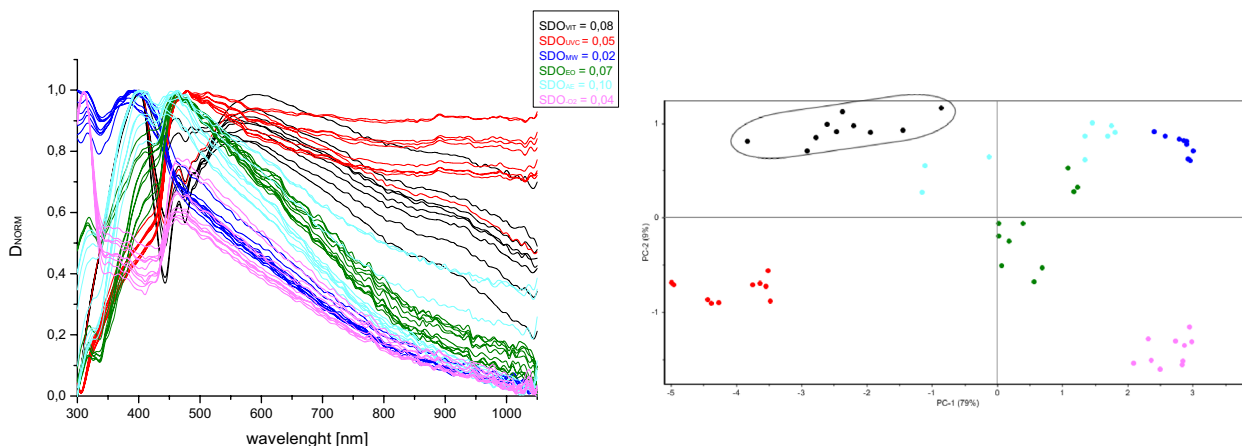


Fig. 10 UV-Vis-NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Cladosporium herbarum* conidia samples

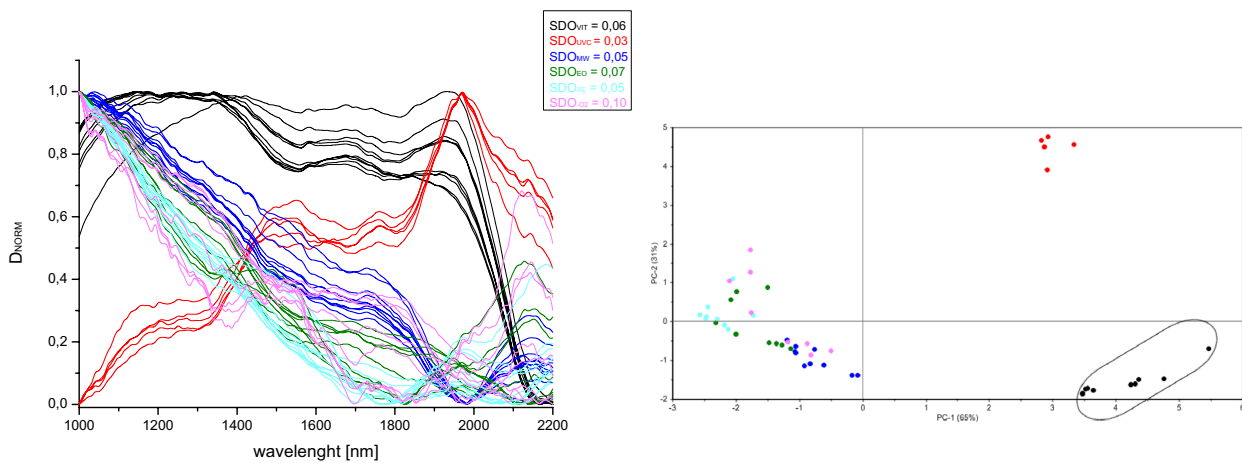


Fig. 11 NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Cladosporium herbarum* conidia samples

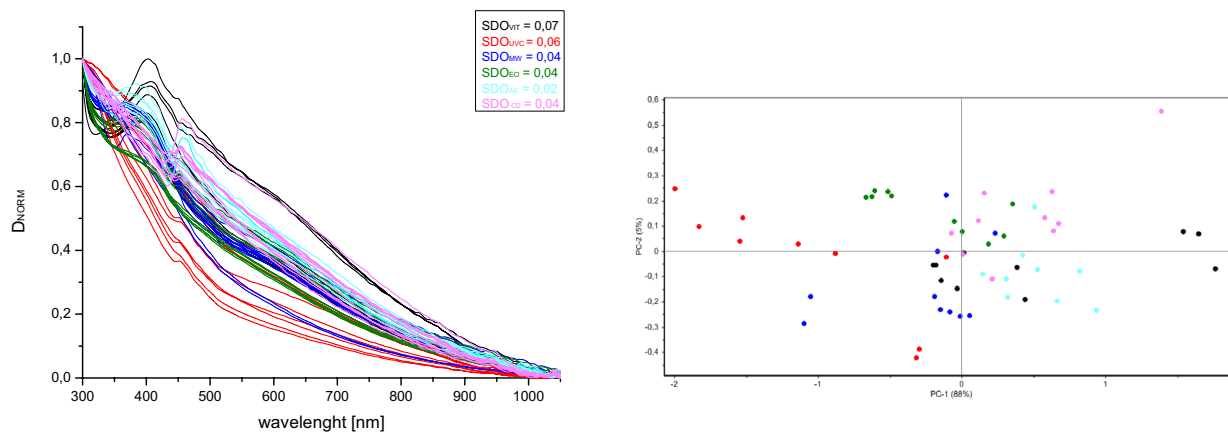


Fig. 12 UV-Vis-NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Cladosporium herbarum* mycelium samples

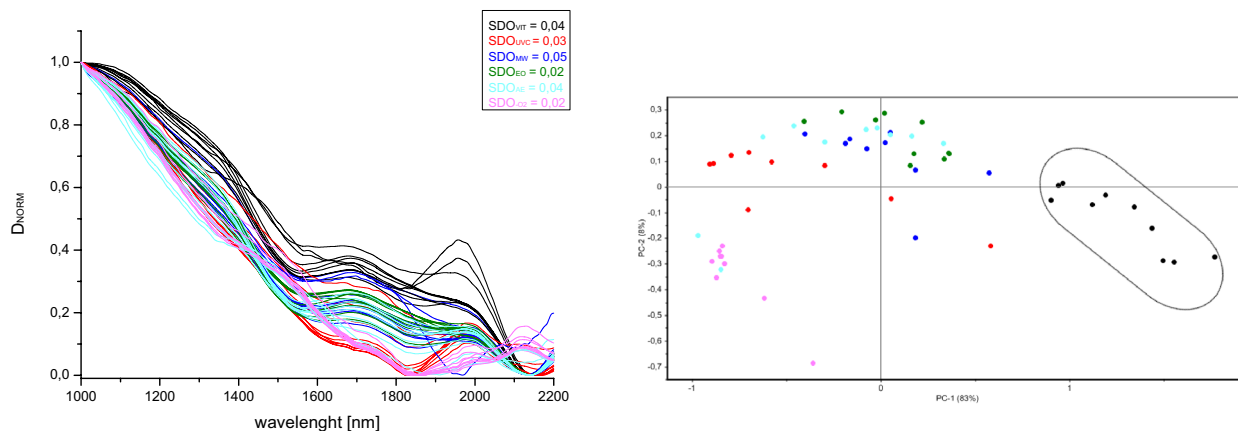


Fig. 13 NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Cladosporium herbarum* mycelium samples

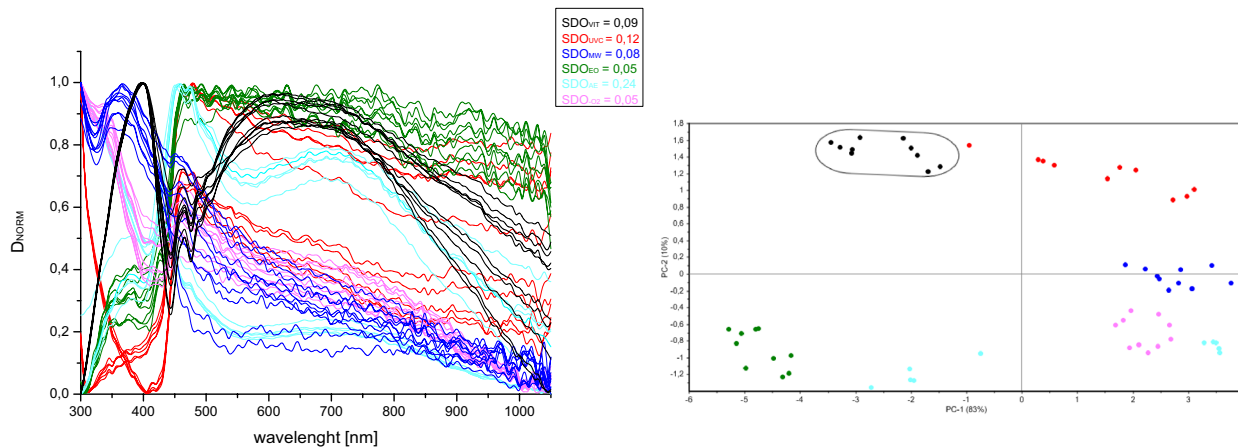


Fig. 14 UV-Vis-NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Penicillium chrysogenum* conidia samples

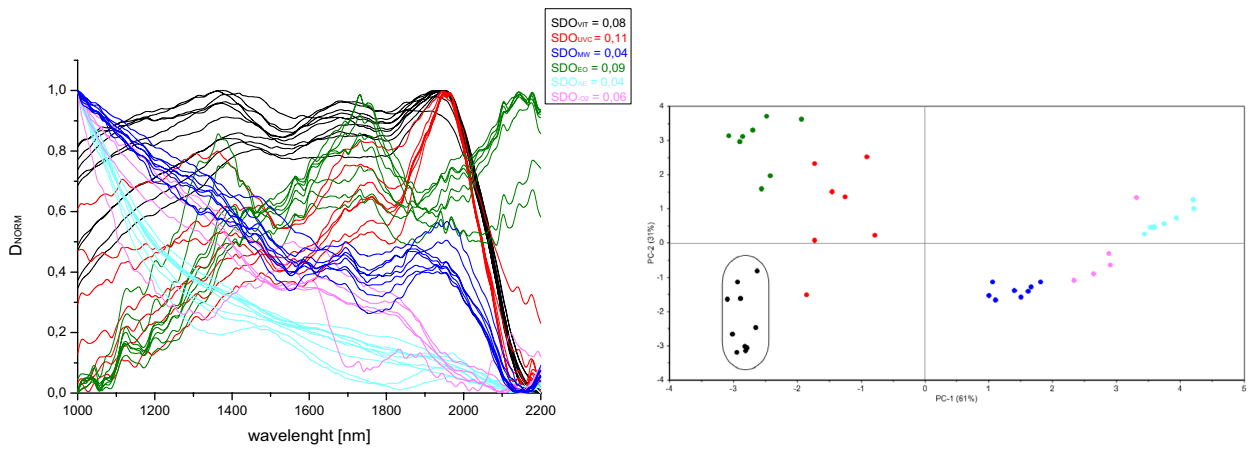


Fig. 15 NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Penicillium chrysogenum* conidia samples

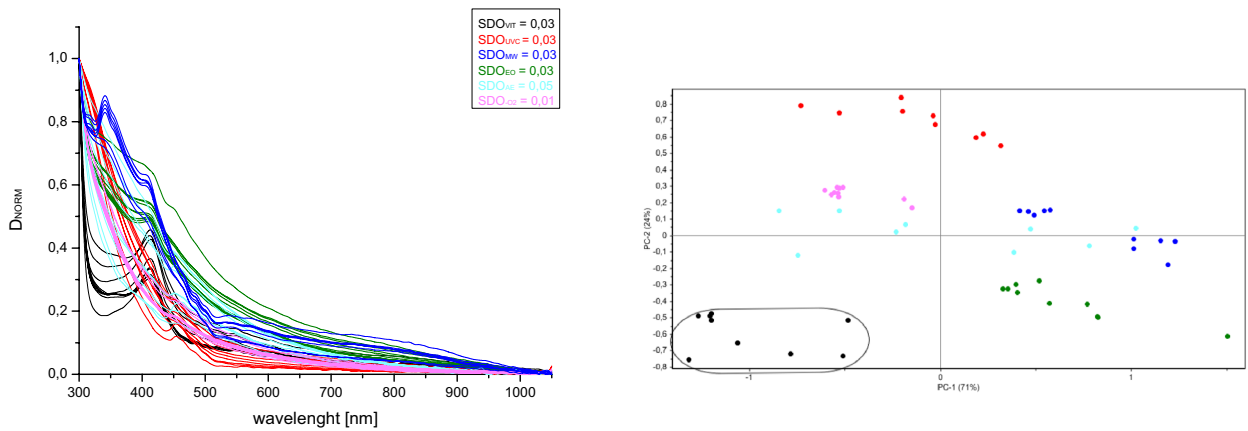


Fig. 16 UV-Vis-NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Penicillium chrysogenum* mycelium samples

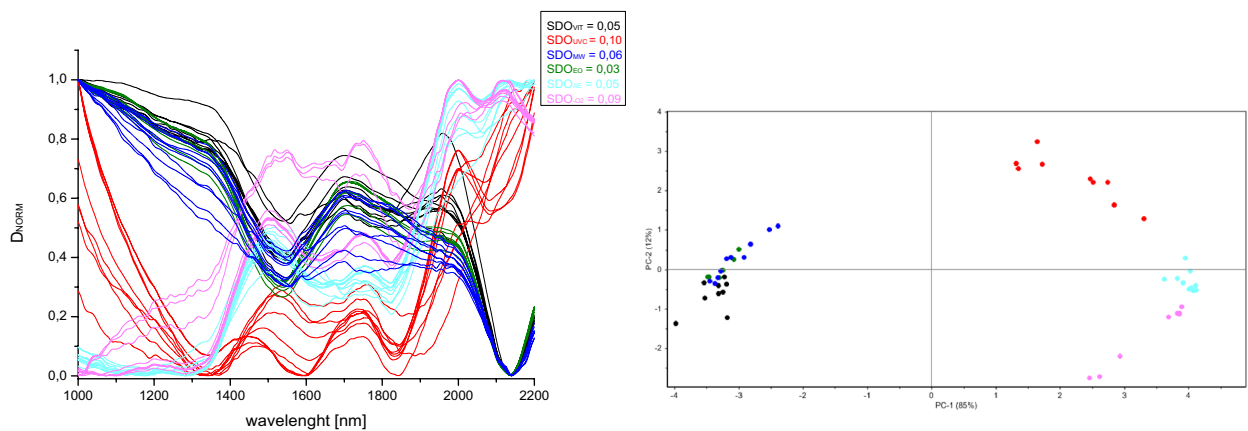


Fig. 17 NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Penicillium chrysogenum* mycelium samples

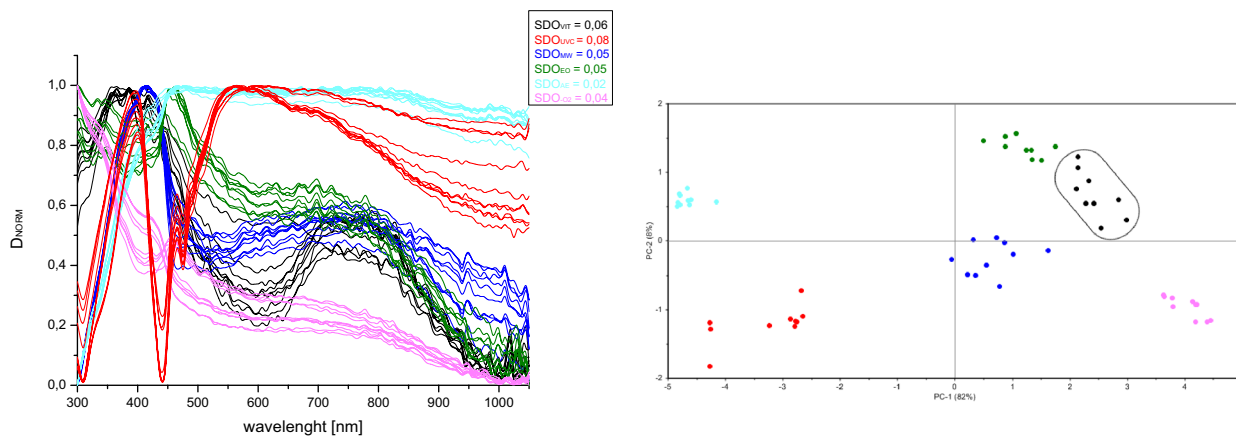


Fig. 18 UV-Vis-NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Trichoderma atroviride* conidia samples

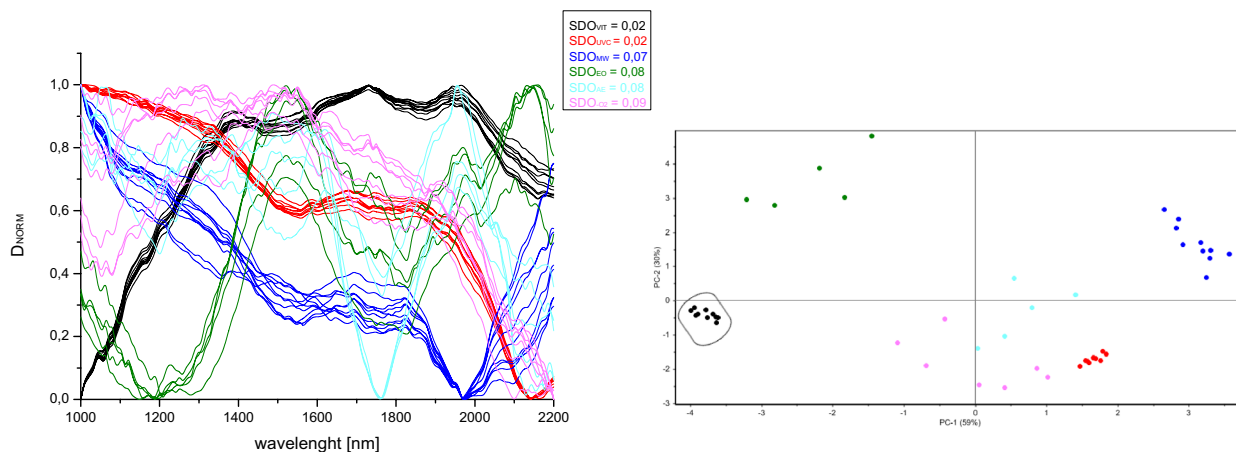


Fig. 19 NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Trichoderma atroviride* conidia samples

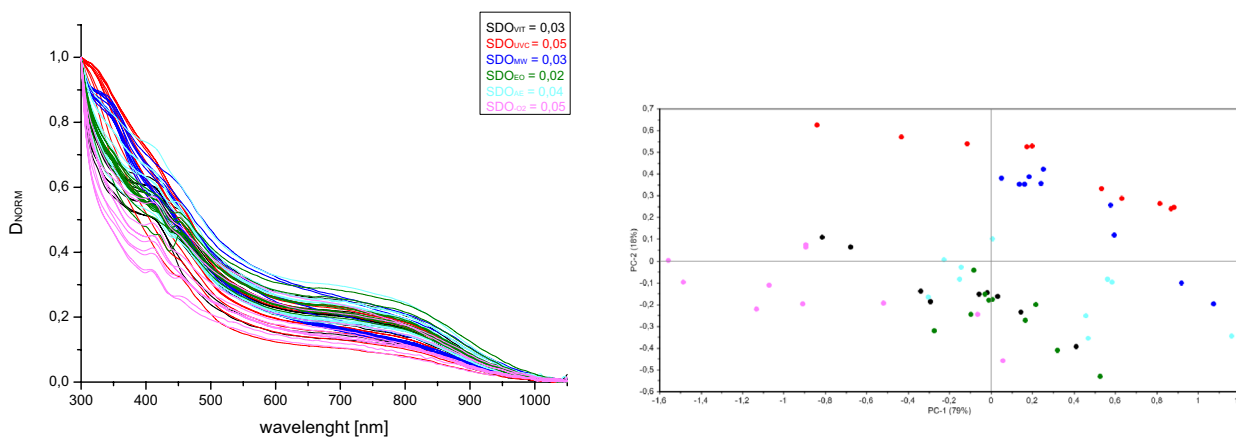


Fig. 20 UV-Vis-NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Trichoderma atroviride* mycelium samples

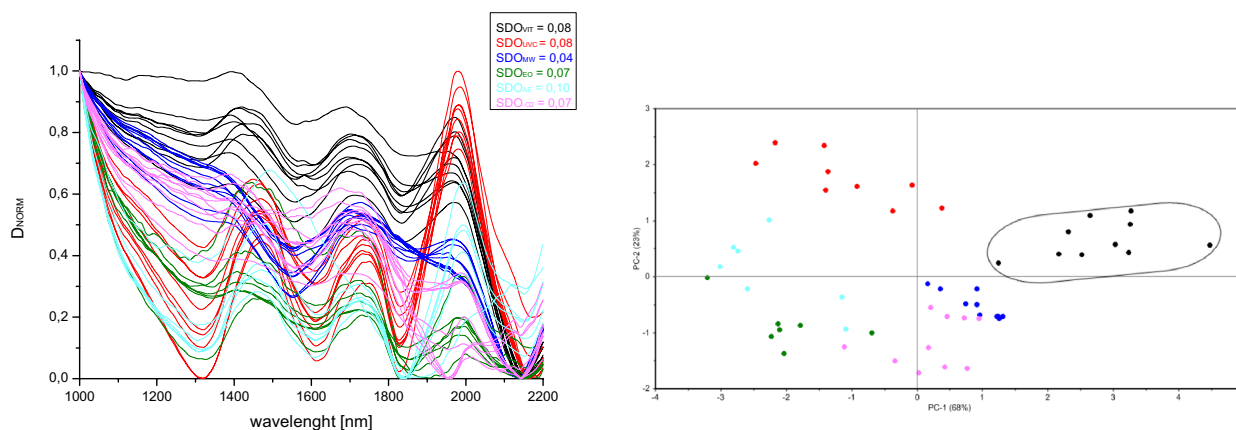


Fig. 21 NIR spectra and two-dimensional score scatter diagram—viable and devitalised *Trichoderma atroviride* mycelium samples

the positive score value of PC-1 and negative score value of PC-2 from the spectra of devitalised samples. However, in the case of mycelium (Fig. 5), the differentiation of viable and devitalised samples was not shown.

In the case of *Aspergillus niger* conidia, PCA analysis in the UV-Vis-NIR spectral region led to the separation of viable samples (Fig. 6). In the NIR spectral region (Fig. 7), the spectra of viable conidia are relatively close to the spectra of samples devitalised by microwave radiation and the distinction of the viable samples is only partial. The PCA analysis of UV-Vis-NIR and NIR spectral data of *Aspergillus niger* mycelium (Figs. 8, 9) could not differentiate the spectra of viable and devitalised samples, and samples remained unresolved from each other.

The analysis of spectral data of *Cladosporium herbarum* conidia and mycelium results in the complete separation of viable conidia samples in both spectral regions (Figs. 10, 11) and of viable mycelium in the NIR spectral region (Fig. 13). The UV-Vis-NIR spectra of viable conidia samples (Fig. 10) have a positive PC-2 score value and negative PC-1 score value what makes them distinguished from devitalised samples. PCA of NIR spectra corresponding to *Cladosporium herbarum* conidia samples (Fig. 11) shows that viable and devitalised samples are clearly distinguished along the PC-1. In this case, most devitalised conidia samples are characterised by negative PC-1 score values. As can be seen in the score scatter diagram (Fig. 12), points corresponding to viable mycelium spectra in the UV-Vis-NIR region overlap with the points of devitalised mycelium spectra. PCA did not reveal differences between the spectra of viable and devitalised samples.

The situation is very similar for *Penicillium chrysogenum* samples. The spectra corresponding to viable conidia samples form clusters clearly separated from the spectra of devitalised samples in both studied spectral

regions (Figs. 14, 15). In the score scatter diagram of conidia samples in the UV-Vis-NIR region (Fig. 14), spectra of viable samples created a separated group with positive score values, both PC-1 and PC-2. In the case of the NIR spectral region (Fig. 15), viable samples are characterised by negative score values and well distinguished from devitalised samples. Separation of viable samples can be observed as well for *Penicillium chrysogenum* mycelium in the UV-Vis-NIR spectral region (Fig. 16). Differentiation between the spectra of viable and devitalised mycelium samples in the NIR spectral region is only partial (Fig. 17), and the points corresponding to viable samples' spectra overlap with the points of samples devitalised using microwave and ethylene oxide devitalisation.

The UV-Vis-NIR and NIR spectra of viable *Trichoderma atroviride* conidia were distinguished from the spectra of devitalised conidia samples (Figs. 18, 19). UV-Vis-NIR spectra of viable conidia samples (Fig. 18) and samples devitalised using ethylene oxide are both characterised by positive score values, but they are still quite well distinguishable from each other. NIR spectra of viable conidia (Fig. 19) form a separate group that is very compact and clearly differentiated from the devitalised samples. The viable and devitalised mycelium samples in the UV-Vis-NIR spectral region remained undifferentiated from each other (Fig. 20). However, in the NIR spectral region, as seen in Fig. 21, the viable mycelium samples clearly distinguish and form a separate cluster.

In cases where it is impossible to clearly confirm the differences between the spectra of viable and devitalised fungi, based on a simple visual assessment of scatter diagrams, it will be helpful to extend the data processing with more sophisticated statistical analysis, for example Linear discriminant analysis (LDA), Partial least squares discriminant analysis (PLS-DA), Cluster analysis or

Multivariate analysis of variance (MANOVA), that are used in other fields (e.g. forensic science) [41–43].

Conclusions

The results of this study suggest that UV-Vis-NIR and NIR Fibre Optics Reflection Spectroscopy (FORS) coupled with Principal Component Analysis (PCA) can be used as the basis of the method for monitoring the microbial contamination of paper substrates. The PCA analysis has been applied to the UV-Vis-NIR and NIR spectral data of viable and devitalised filamentous fungi in two different forms—conidia and mycelium. The obtained results prove differences between the spectra of viable and devitalised forms at least in one studied spectral region, except for one sample—*Aspergillus niger* mycelium. A combination of spectroscopic techniques and statistical data processing seems to be a promising, alternative, relatively quick and non-destructive tool, which may help memory institutions characterise microbial contamination and evaluate the effectiveness of sterilization processes.

Abbreviations

CZDB	Czapek-Dox broth
FORS	Fibre optics reflection spectroscopy
MEA	Malt extract agar
PCA	Principal component analysis
PCR	Polymerase chain reaction

Acknowledgements

The authors wish to thank the company Progres Final SK for irradiating the samples in the electron beam accelerator and the Slovak National Gallery for the ethylene oxide sterilization of samples.

Author contributions

All authors contributed to the study conception and design and approved the final manuscript.

Funding

This research received a financial contribution from the STU Grant scheme for Support of Young Researchers, the Slovak Research and Development Agency under the contract APVV, Grant No. 20–0410, and the Scientific Grant Agency VEGA, Grant No. 1/0602/19.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests

The authors declare that they have no competing interests.

Received: 31 March 2023 Accepted: 17 June 2023
Published online: 27 July 2023

References

- Boniek D, de Abreu CS, dos Santos AFB, de Resende Stoianoff MA. Filamentous fungi in Brazilian indoor cultural heritage as potential risk to human health and biodeterioration of artworks. *Air Qual Atmos Health*. 2022;15(2):339–46.
- Sterflinger K, Pinzari F. The revenge of time: fungal deterioration of cultural heritage with particular reference to books, paper and parchment. *Environ Microbiol*. 2012;14(3):559–66.
- Borrego S, Lavin P, Perdomo I, Gómez de Saravia S, Guaiamet P. Determination of indoor air quality in archives and biodeterioration of the documentary heritage. *ISRN Microbiol*. 2012;2012:1–10.
- Pietrzak K, Otlewska A, Dybka K, Danielewicz D, Pangallo D, Demnerová K, et al. A modern approach to biodeterioration assessment and disinfection of historical book collections. Poland: Arden Prepress Studio Sp; 2016.
- Sterflinger K, Piňar G. Microbial deterioration of cultural heritage and works of art—tilting at windmills? *Appl Microbiol Biotechnol*. 2013;97(22):9637–46. <https://doi.org/10.1007/s00253-013-5283-1>.
- Pinheiro AC, Sequeira SO, Macedo MF. Fungi in archives, libraries, and museums: a review on paper conservation and human health. *Crit Rev Microbiol*. 2019;45(5–6):686–700.
- Skóra J, Gutarowska B, Pielech-Przybylska K, Stępień Ł, Pietrzak K, Piotrowska M, Pietrowski P. Assessment of microbiological contamination in the work environments of museums, archives and libraries. *Aerobiologia*. 2015;31(3):389–401.
- Singara Charya MA. Fungi an overview. In: Bahadur B, Venkat Rajam M, Sahijram L, Krishnamurthy K, editors. *Plant biology and biotechnology*. New Delhi: Springer; 2015. p. 197–215.
- Savković Ž, Stupar M, Unković N, Knežević A, Vukojević J, Ljaljević Grbić M. Fungal deterioration of cultural heritage objects. In: Mendes KF, de Sousa RO, Mielke KC, editors. *Biodegrad Technol Org Inorg Pollut*. London: Academic; 2021.
- Cappitelli F, Cattò C, Villa F. The control of cultural heritage microbial deterioration. *Microorganisms*. 2020;8(10):1–20.
- Hueck HJ. The biodeterioration of materials—an appraisal. *Int Biodeterior Biodegrad*. 2001;48(1–4):5–11.
- Pinzari F, Gutarowska B. Extreme colonizers and rapid profiteers. In: Joseph Edith, editor. *The challenging world of microorganisms that attack paper and parchment*. Chem: Springer; 2021. p. 2021.
- Dunca SI, Tanase C, Padurariu C, Balaes T, Ardelean E, Puica NM. Study of the contaminating microbiota of old paper supports. *Eur Sci J*. 2014;3:237–51.
- Volkov VV, Perry CC. Fungal pigments on paper: Raman and quantum chemistry studies of *Alternaria* Sp. *Dye Pigment*. 2021;195: 109719.
- Rakotonirainy MS, Bénaud O, Vilmont LB. Contribution to the characterization of foxing stains on printed books using infrared spectroscopy and scanning electron microscopy energy dispersive spectrometry. *Int Biodeterior Biodegrad*. 2015;101:1–7.
- Ramos CCR, Roque JLA, Sarmiento DB, Suarez LEG, Sunio JTP, Tabungar KIB, Tengco GSC, Rio PC, Hilario AL. Use of ultraviolet-C in environmental sterilization in hospitals: a systematic review on efficacy and safety. *Int J Health Sci (Qassim)*. 2020;14(6):52–65.
- Tiano P. Biodegradation of cultural heritage: decay mechanisms and control methods. *CNR-Centro di Studio Sulle Cause Deperimento e Metodi Conservazione Opere d'Arte*. 2001;9(October):1–37.
- Riminesi C, Olmi R. Localized microwave heating for controlling biodeteriogens on cultural heritage assets. *Int J Conserv Sci*. 2016;7(SpecialIssue1):281–94.
- Cuzman OA, Olmi R, Riminesi C, Tiano P. Preliminary study on controlling black fungi dwelling on stone monuments by using a microwave heating system. *Int J Conserv Sci*. 2013;4(2):133–44.
- Górny R, Mainelis G, Wlazlo A, Niesler A, Lis O, Marzec DS, et al. Viability of fungal and actinomycetal spores after microwave radiation of building materials. *Ann Agric Environ Med*. 2007;14(2):313–24.
- Zajac I, Szulc J, Gutarowska B. The effect of ethylene oxide and silver nanoparticles on photographic models in the context of disinfection of photo albums. *J Cult Herit*. 2021;51:59–70.
- Engel P, Sterflinger K, Eckhart R. Ethyleneoxide fumigation for mouldy archival material. *Restauratorenblätter/Papers Conserv*. 2014;32(October):241–55.

23. Chmielewska-Śmietanko D, Gryczka U, Migdał W, Kopeć K. Electron beam for preservation of biodeteriorated cultural heritage paper-based objects. *Radiat Phys Chem.* 2017;2018(143):89–93. <https://doi.org/10.1016/j.radphyschem.2017.07.008>.
24. Gunay-Silindir M, Özer AY. Sterilization methods and the comparison of E-beam sterilization with gamma radiation sterilization. *Fab J Pharm Sci.* 2009;34(1):43–53.
25. Adamiak J, Otlewska A, Tafer H, Lopandic K, Gutarowska B, Sterflinger K, Piñar G. First evaluation of the microbiome of built cultural heritage by using the Ion torrent next generation sequencing platform. *Int Biodeterior Biodegrad.* 2018;131:11–8.
26. Pyzik A, Ciuchcinski K, Dziurzynski M, Dziewit L. The bad and the good—microorganisms in cultural heritage environments—an update on biodeterioration and biotreatment approaches. *Materials.* 2021;14(1):1–15.
27. Honsig C, Selitsch B, Hollenstein M, Vossen MG, Spettel K, Willinger B. Identification of *Filamentous* fungi by MALDI-TOF mass spectrometry: evaluation of three different sample preparation methods and validation of an in-house species cutoff. *J Fungi.* 2022;8(4):383.
28. Suchorab Z, Frać M, Guz Ł, Oszust K, Łagód G, Gryta A, Bilińska-Wielgus N, Czerwiński J. A method for early detection and identification of fungal contamination of building materials using e-nose. *PLoS ONE.* 2019;14(4):1–17.
29. Konkol NR, McNamara CJ, Hellman E, Mitchell R. Early detection of fungal biomass on library materials. *J Cult Herit.* 2012;13(2):115–9.
30. Rakotonirainy MS, Dubar P. Application of bioluminescence ATP measurement for evaluation of fungal viability of foxing spots on old documents. *Luminescence.* 2013;28(3):308–12.
31. Méheust D, Le Cann P, Gangneux JP. Rapid quantification of viable fungi in hospital environments: analysis of air and surface samples using solid-phase cytometry. *J Hosp Infect.* 2013;83(2):122–6.
32. Kamiloglu S, Sari G, Ozdal T, Capanoglu E. Guidelines for cell viability assays. *Food Front.* 2020;1(3):332–49.
33. Dal Fovo A, Mazzinghi A, Omarini S, Pampaloni E, Ruberto C, Striova J, Fontana R. Non-invasive mapping methods for pigments analysis of Roman mural paintings. *J Cult Herit.* 2020;43(311):318.
34. Liu W, Li M, Wu N, Liu S, Chen J. A new application of fiber optics reflection spectroscopy (FORS): Identification of “bronze disease” induced corrosion products on ancient bronzes. *J Cult Herit.* 2021;49:19–27.
35. Reháková M, Gál L, Belovičová M, Oravec M, Dvonka V, Stojkovičová D, Čeppan M. Identification of iron-gall inks in historical drawings by fibre optics reflection spectroscopy—extension to the NIR spectral range. *J Cult Herit.* 2017;27:137–42.
36. Saif FA, Yaseen SA, Alameen AS, Mane SB, Undre PB. Identification and characterization of *Aspergillus* species of fruit rot fungi using microscopy, FT-IR, Raman and UV-Vis spectroscopy. *Spectrochim Acta - Part A Mol Biomol Spectrosc.* 2021;246: 119010.
37. Cebrián E, Núñez F, Rodríguez M, Grassi S, González-Mohino A. Potential of near infrared spectroscopy as a rapid method to discriminate ota and non-ota-producing mould species in a dry-cured ham model system. *Toxins.* 2021;13(9):620.
38. Fazio AT, López MM, Temperini MLA, de Faria DLA. Surface enhanced Raman spectroscopy and cultural heritage biodeterioration: Fungi identification in earthen architecture from Paraíba Valley (São Paulo, Brazil). *Vib Spectrosc.* 2018;97:129–34.
39. Shapaval V, Schmitt J, Møretø T, Suso HP, Skaar I, Asli AW, Lillehaug D, Kohler A. Characterization of food spoilage fungi by FTIR spectroscopy. *J Appl Microbiol.* 2013;114(3):788–96.
40. Wong HJ, Mohamad-Fauzi N, Rizman-Idid M, Convey P, Alias SA. Protective mechanisms and responses of micro-fungi towards ultraviolet-induced cellular damage. *Polar Sci.* 2019;20:19–34.
41. Oravec M, Beganović A, Gál L, Čeppan M, Huck CW. Forensic classification of black inkjet prints using fourier transform near-infrared spectroscopy and linear discriminant analysis. *Forensic Sci Int.* 2019;299:128–34.
42. Gál L, Oravec M, Kiššová M, Gemeiner P, Čeppan M. Forensic discrimination of black laser prints by a combination of chemometric methods and μ -ATR-FTIR spectroscopy. *Chem Pap.* 2020;74(10):3269–77.
43. Kumar R, Sharma V. Chemometrics in forensic science. *TrAC - Trends Anal Chem.* 2018;105:191–201.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen® journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)