

# Effects of microwave heating for the conservation of paper artworks contaminated with *Aspergillus versicolor*

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**Abstract** The conservation of the cultural heritage, such as old books, manuscripts, paintings etc. is particularly important, for both their artistic and historical values. These types of materials are often exposed to usage or storage conditions where efficient biodeterioration mechanisms take place. Deterioration of these materials occurs naturally as a result of aging, but it can be accelerated by poor storage conditions (humidity) that lead to fungi growth and negative chemically effects. Firstly, this work concerns with isolation and identification of a fungal species that infects an 18th century book. The identification was based on morphological analysis made by light and SEM microscopy and on ribosomal DNA loci amplification and sequencing. One fungal strain, *Aspergillus versicolor*, was identified as responsible of book biodeterioration. Then, *A. versicolor* was used as biodeteriogen to contaminate paper samples exposed

two degradation processes (exposure to wet atmosphere and to acidic attack) simulating storage conditions of 18th century book. Secondly, microwave heating at three different temperatures (30, 58 and 63 °C) was applied on paper samples affected by spots originating from *A. versicolor* in order to evaluate the effectiveness of microwave in cleaning of artworks from fungi. Scanning electron microscopy and cellulose degree of polymerization were used for visual inspection and characterization of the paper samples before and after the treatments respectively. The best results were obtained by exposure of paper samples for few minutes at 58 and 63 °C, while the lower temperature (30 °C) didn't inhibit *A. versicolor*'s growth.

**Keywords** Foxing · *Aspergillus* · Biodeterioration · Microwave heating · Degree of polymerization

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## Introduction

Library documents can be deteriorated by different organisms, including insects, rodents and microorganisms, generally called biodeteriogens (Canhoto et al. 2004; Pinzari et al. 2006). Because the paper is an organic material, it is a food source for heterotroph organisms. It is chiefly made of cellulose of various origin (cotton, linen, hardwood, softwood), but in addition paper usually contains traces of metallic

elements (such as iron, copper) and other substances (such as inks, pigments, etc.) which make it a complex and heterogeneous medium (Pinzari et al. 2006). In particular, fungal species causes significant disfigurement of library materials, especially books and paper. Apart from the undesirable aesthetic aspects, including discoloration by weak acids produced by fungi and/or the accumulation of pigments that may stain the paper in a phenomenon known as foxing (Mesquita et al. 2009), the presence of these microorganisms can lead to the irreversible degradation of the paper.

Environmental factors such as relative humidity (RH), temperature, light and gas composition (Magan 1997) favor the germination and growth of fungal species on paper. To prevent fungal growth, books and archives are commonly stored in controlled environments, with temperature and humidity maintained in a defined range, and periodically cleaned of dust (Pinzari et al. 2003).

In literature, are reported numerous works on the identification of fungal species causing foxing (Rakotonirainy et al. 2007; Szczepanowska and Cavaliere 2000; Montemartini Corte et al. 2002; Florian and Manning 2000) such as *Penicillium*, *Aspergillus* and *Chaetomium*.

Identification of fungal species was based, in the past, on morphological and cultural methods, using a variety of standardized media. Today this scenario is shifting in favor of modern molecular techniques supported by various studies demonstrating that morphology alone may not be a sufficiently objective method for species determination (Balajee et al. 2005; Hong et al. 2005).

Currently, comparative sequence-based identification strategies can be considered a new method for fungal species identification (Summerbell et al. 2005). This method is based on PCR amplification of a selected region of genomic DNA, followed by sequencing. The Internal Transcribed Spacers (ITS regions), which are nested in the nuclear rDNA repeat, have been selected to investigate the fungal diversity. This regions possess a high variation between taxonomically distinct fungal species and even within the species (Sterflinger 2010), and has been used for species complex-level identification of *Aspergillus* (Hinrikson et al. 2005) and most other species. The identification of fungal species is of great relevance since allows to choose the best conservative approach to the restoration. Among physical methods,

microwave (MW) heating is safe, pollution free (Riminesi and Olmi 2016) and has been successfully used on wooden cultural heritage objects for disinfection from woodworm (*Hylotrupes bajulus* L., *Oligomerus pitilinoides* Wollaston and *Drosophila melanogaster*) (Andreuccetti et al. 1994; Plaza et al. 2007; Cuzman et al. 2013). The effectiveness of MW is due to the ability of inactivating microbial contaminants not only on the exposed surfaces but inside them as well (Gorny et al. 2007).

In this work, we analyzed a book of 18th century characterized by a consistent foxing deterioration. The isolation of fungal species was based on culture, morphological and molecular identification. *A. versicolor* was used as biodeteriogen to contaminate paper samples exposed two degradation processes (exposure to wet atmosphere and to acidic attack) simulating storage conditions of 18th century book.

Finally, we investigated the use of MW as physical method to protect biological attack due to fungi in paper artworks. MW heating at three different temperatures (30, 58 and 63 °C) was applied on different types of paper samples affected by spots originating from *A. versicolor*. Cellulose degree of polymerization (DP) of paper samples and scanning electron microscopy (SEM) were evaluated after prefixed times of fungal attack, respectively.

## Materials and methods

### Materials

The private collector asked our laboratory to examine the book “Trattato delle acque minerali” printed 1783 on which foxing stains had been found, in order to investigate the cause of this damage. This book was left in a room with high relative humidity. The book did not appear to be in good condition as shown in Fig. 1a. In fact, all pages are characterized by irregular shaped, rusty red and uniformly discoloured spots. Figure 1b reports several observed foxing spots, as confirmed by Wood light (CTS ART lux 70, mod. 808).

Isolation and molecular identification of fungal species from 18th century book damaged by foxing



**Fig. 1** a “Trattato delle acque minerali” dating from 18th century b foxing spots observed by Wood light

### Sample isolation and culture

Fungi were isolated by sterile swab rubbed on the fluorescent area to allow a sufficient amount of material and then placed directly into liquid medium MEB (Malt Extract Broth). Incubation was carried out at 25 °C. Liquid fungal cultures were then inoculated in solid medium MEA (Malt Extract Agar) and incubated at room temperature.

According to Urzì and De Leo (2001), sampling of the fungal species from the book for microscopic observation has been done by adhesive strips (Fungi Tape DID, Calsystem, CS, Italy).

### Microscopy analysis

#### *Light microscopy*

Adhesive strips were gently applied to the surface of paper and were then immediately placed on sterile glass microscope slides. A drop of sterile water, Malachite Green solution (Calsystem, DID, CS, Italy) was poured between the glass slide and the tape. A cover glass was placed on the top in order to keep the tape as flat as possible (Urzì and Albertano 2001). Direct observations of samples were carried out using a light microscope Leitz Laborlux 12 POL (Germany). Petri dishes with MEA were also observed with a light microscope equipped with phase contrast (Leica, Germany) to see the typical fungal morphology.

#### *Scanning electron microscopy (SEM)*

For SEM observations, a piece of adhesive tape (Fungi Tape DID, Calsystem, CS, Italy) was mounted face up on a stub with a biadhesive tape (Assing, Monterotondo, Italy). Observations were carried out using a SEM Leo 420 (Leo electron microscope Ltd, Cambridge, England) at 15 kV under high vacuum conditions.

#### DNA extraction, PCR amplification and DNA sequencing

Before proceeding with DNA extraction, a 7 day culture grown in liquid medium (MEB) was repeatedly washed in distilled water to remove any residual culture medium. DNA extraction was performed using CTAB (cetyltrimethylammonium bromide) combined with enzymatic (proteinase K) and mechanical steps (freeze and thaw cycles), followed by organic extractions and isopropanol precipitation of DNA. The result of the extraction was visualized on agarose 0.8% gels by ethidium bromide staining and UV illumination.

Amplification of the ribosomal internal transcribed spacer regions ITS1 and ITS2 and the 5.8S rRNA gene situated between them, was done using universal primer ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al. 1990). PCR were performed in a TPersonal Thermal Cycler (Biometra, Germany). The cycling profile consisted of an initial step at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and elongation at

72 °C for 45 s and with a final extension step of 10 min at 72 °C. PCR product was electrophoresed on 1.5% agarose gel with 100 bp DNA ladder, stained with ethidium bromide and photographed with a Kodak EDAS 290 camera.

Sample was then purified with the kit for purification of Qiagen PCR and sequenced by the BMR Genomics, Padova (Italy) following the manufacturer's protocol.

The sequence was analyzed using BioEdit software (Hall 1999) and run against NCBI's BLAST (Basic Local Alignment Search Tool) database (<http://www.ncbi.nlm.nih.gov/blast/>) in order to assess the similarity with published sequences, belonging to identified fungal species.

Preparation of paper samples simulating storage conditions of 18th century book and contaminated with *A. versicolor*

Given the high value of the book and in order to assess the effect of MW heating on artworks, two degradation processes (exposure to wet atmosphere and to acidic attack) contaminated by *A. versicolor* isolated from 18th century book on commercial MOTIFCOPY paper were simulated.

#### Exposure to acidic attack

The exposure to acidic attack was simulated by treating paper samples ( $2 \times 7 \text{ cm}^2$ ) with  $\text{H}_2\text{SO}_4$  solution 72% for 5 s. Paper samples were then washed with deionized water (pH 5.0) and then dried. Morphological analysis by SEM and DP were performed. UNI 8282 method was used to determine the cellulose DP in cupriethylenediamine (CED).

#### Exposure to wet atmosphere

The exposure to moisture was simulated as reported by Cerchiara et al. (2009) exposing samples to vapors deriving from a water bath at 60 °C and 70% relative humidity (R.H.) for 15 days. Morphological analysis by SEM and DP were performed. UNI 8282 method was used to determine the cellulose DP in cupriethylenediamine (CED).

#### Fungal inoculation

Paper samples exposed to acidic attack and to wet atmosphere respectively were placed in Petri dishes with no addition of any nutritional substance, in order to ensure microbial growth at the expense of the paper (De Filpo et al. 2016). Inoculation was performed by deposition of conidia solution over the sample surface. All samples in petri dishes were covered by plastic lids and kept at 30 °C in a thermostatic cell with a relative humidity of 80% for 5, 15 and 25 days. DP and morphological analysis were performed.

#### Microwave heating on paper samples contaminated with *A. versicolor*

Both paper samples with fungal inoculation were directly placed flat in a commercial microwave oven (Moulinex—Ultimys Duo Grill, 5140—modello AFW1) at 300, 600 and 900 W for 2 min and 50 s. Paper samples were observed by optical microscopy after 10 and 30 days. Temperature of paper samples was carry out using Fluke 52 K/J Thermometer and DP was determined according to viscosimetric technique with respect the standard UNI 8282. The DP was measured for cellulose extracted from paper samples before and after microwave heating. Morphological analysis by SEM was performed.

#### Statistical analysis

All experiments were performed at least in triplicate. Results are expressed as mean  $\pm$  SD. *t* tests were used to determine statistical significance of results. Differences were considered significant for values of  $P < 0.05$ .

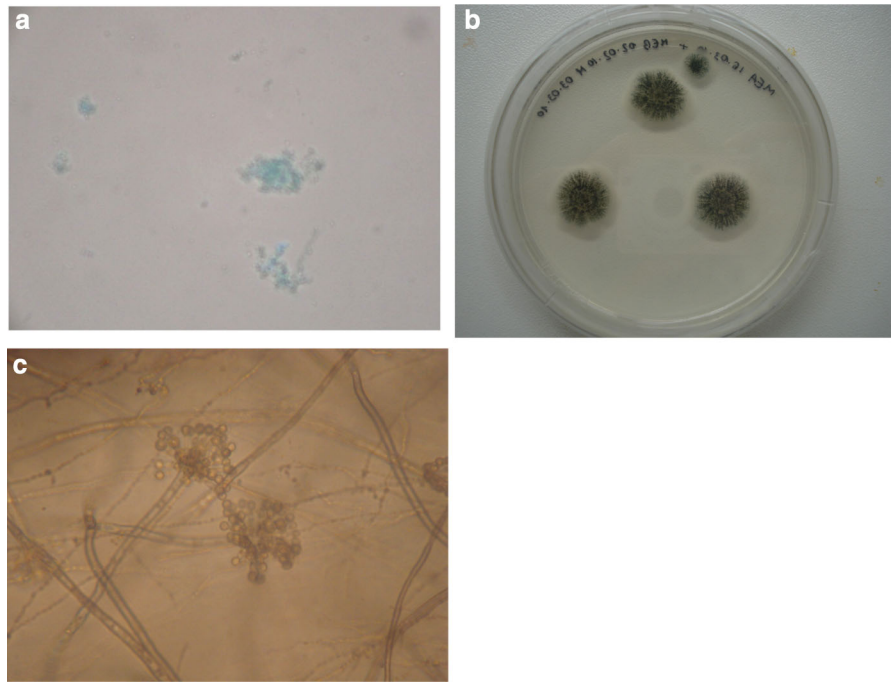
## Results and discussion

Isolation and molecular identification of fungal species from 18th century book damaged by foxing.

#### Microscopy analysis

##### *Light microscopy*

Figure 2a shows chains of spores growth on the samples and isolated by adhesive tape from the book and coloured with green malachite. In addition, we



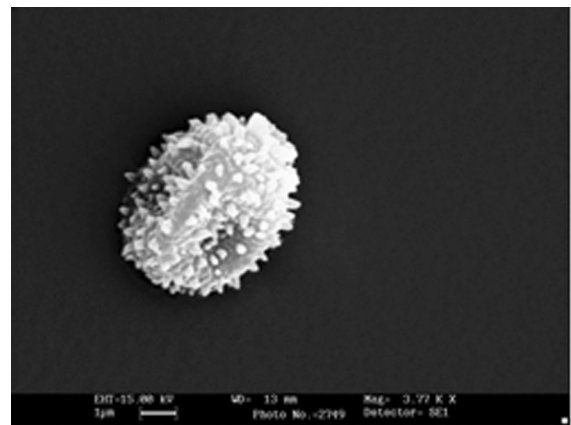
**Fig. 2** a Spores of *Aspergillus* coloured with Green Malachite solution (20 $\times$ ) b colonies of *Aspergillus* on MEA c light microscopic examination of *Aspergillus* conidiophores (50 $\times$ )

observed colonies on MEA at 25 °C grew rapidly (Fig. 2b), reaching 24–26 mm in diameter by the 7th day, centrally rising, floccose, radially sulcate. They showed regular margins, and exudates are absent. The variation in color was as follows: white at first, later becoming grayish yellow to blue green with age, reverse yellowish. Conidiophores from substrate and aerial hyphae were colorless, smooth, and thick-walled. At first observation with light microscopy the conidial heads (Fig. 2c) appear radiated and vesicles ellipsoid. Morphological observations by light microscopy lead to the morphology of the genus *Aspergillus*.

### SEM

SEM was used to examine the conidia and in Fig. 3 is reported the SEM micrograph of isolated conidia. The conidia are jar shaped with low ridged ornamentation in a cerebellate pattern and are usually isolated or in small clusters, not in chains.

The observed conidia were conspicuously roughened. Based on the surface texture of conidium, the conidium was found to be echinulate, which is a



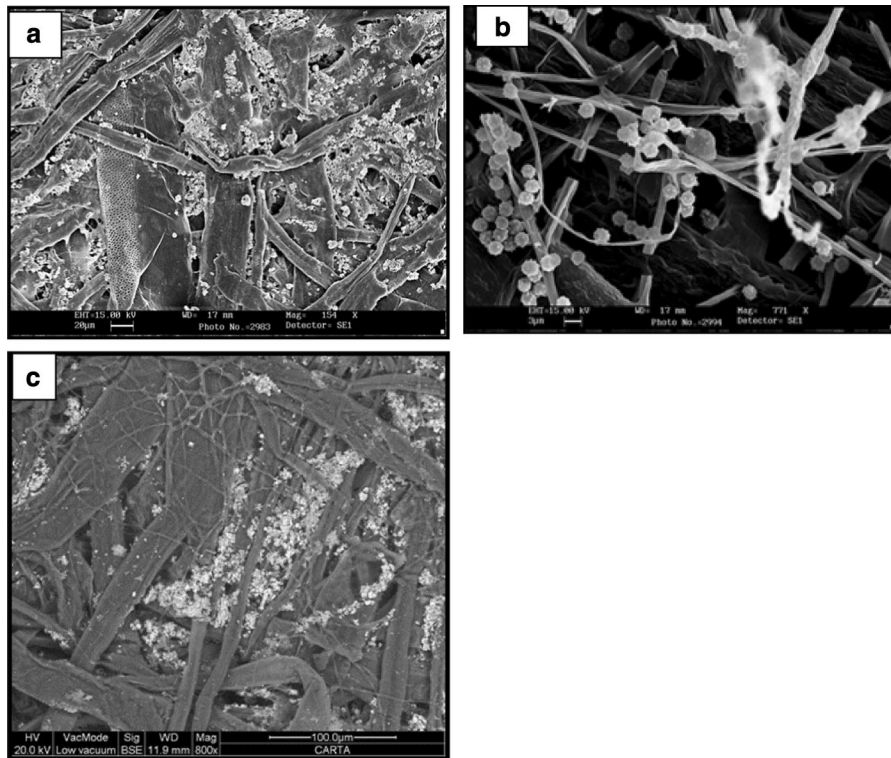
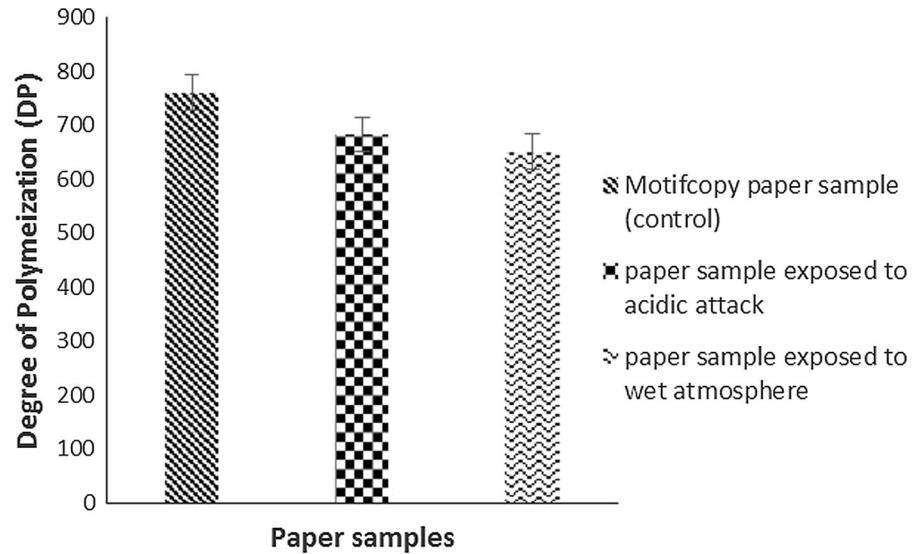
**Fig. 3** SEM micrograph of *Aspergillus* conidia

characteristic of the echinulate category of *Aspergillus* genus (Kozakiewicz 1989).

### Molecular identification

Amplification of ITS regions generated a PCR product of 564 bp and the sequence obtained was compared in GenBank database. The sequence was also deposited in GenBank (accession numbers JN997427).

**Fig. 4** DP of paper samples exposed to degradative processes

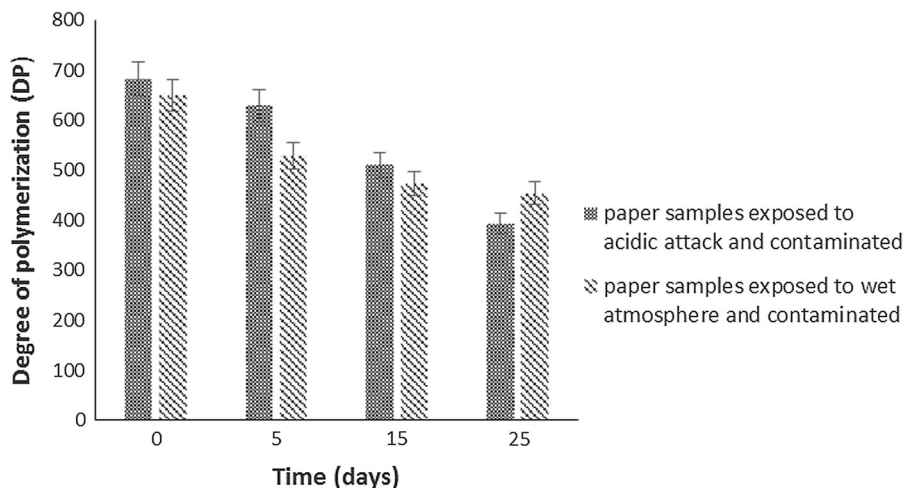


**Fig. 5** Scanning electron microscopy (SEM) micrograph of **a** MOTIFCOPY paper samples, **b** paper samples exposed to acidic attack, **c** paper samples exposed to wet atmosphere

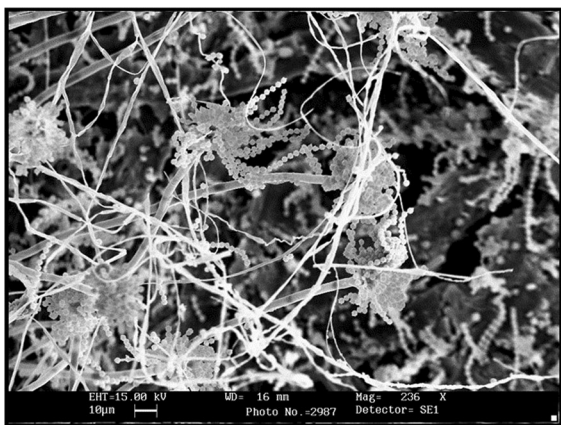
Identification of the unknown sample was made using the highest bit score of listed species with analogy of 100 and 99%. The following species: *Aspergillus versicolor*, *A. carneus*, *A. sydowii* and *A. flavus*, all biodeteriogens paper, were chosen.

Alignment of ITS regions showed that the unknown sample had high homology with respect to *A. versicolor*.

The ITS region has been frequently used, and with good results, for the identification of fungal organisms



**Fig. 6** DP of paper samples exposed to degradative processes and contaminated with *A. versicolor* after 5, 15 and 25 days



**Fig. 7** Scanning electron microscopy (SEM) micrograph of conidial germination of *A. versicolor* on paper samples exposed to acidic attack

that contaminate documents and art objects (Michaelson et al. 2006). This region is widely used for the identification of organisms and for phylogeny studies. In fact, it satisfies many of the demands of universal marker since this region can be amplified for many fungi, is present as multiple copies in the fungal genome and has the additional advantage that a large number of sequences for this locus are deposited in GenBank (<http://www.ncbi.nlm.nih.gov>) making easy a quick comparison of the sequence from an unknown isolate (Balajee et al. 2009).

There is considerable consensus regarding the use of ITS sequencing as the initial step in mold identification. An international *Aspergillus* working group

recommended the use of the ITS region for sub-genus/section-level identification for the genus *Aspergillus* (Balajee et al. 2007). Also, the International Subcommittee on Fungal Barcoding has proposed the ITS region as the prime fungal barcode or the default region for species identification (<http://www.allfungi.com/its-barcode.php>).

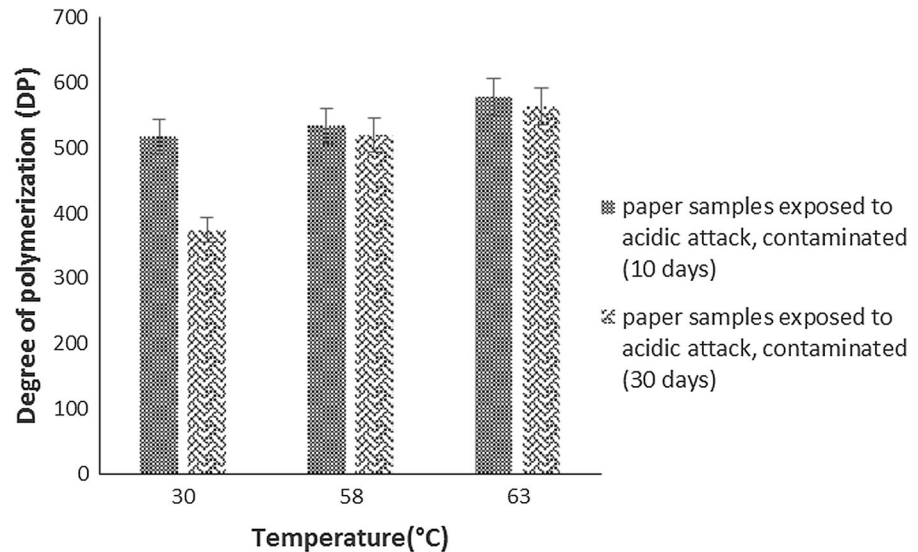
In this work, the sequences we obtained from the total ITS region, together with the morphological analysis, allowed the identification of the species *A. versicolor*. This species is usually found as biodeterioration agent in many different habitats and materials, including those considered as representative of historical and cultural heritage (Abrusci et al. 2005). In particular *A. versicolor* is an indicator organism of moisture problems in cultural heritage confirming the storage conditions where the book of 18th century was left for many years.

#### Characterization of paper samples after simulation of storage conditions of 18th century book

Two different degradative processes (exposure to wet atmosphere and to acidic attack) have been considered for simulating storage conditions of 18th century book. The deterioration state of the samples was monitored with cellulose DP and SEM.

Figure 4 shows DP of paper samples before and after degradation process. DP of paper samples exposed to degradation processes (exposure to wet atmosphere and to acidic attack) was lower than control. In particular, DP of paper samples exposed to

**Fig. 8** DP of paper samples exposed to acidic attack and contaminated with *A. versicolor* after microwave heating at different temperatures



wet atmosphere was slightly lower than exposed to acidic attack. Probably, it can be attributed to difference of exposure time of paper sample to degradation processes: 5 s to sulphuric acid for acidic attack and 15 days at 60 °C with 70% RH for wet atmosphere.

Moreover, the deterioration state of paper samples was also analyzed by SEM. The SEM image of the control paper is shown in Fig. 5a. Results indicated closely packed cellulose fibers with an interwoven network without any deformation. Cellulose fibers of different size are made from cotton linters. The effect of acidic attack on paper sample is shown in Fig. 5b. Fibers after exposure to acidic attack show several transversal breaks, indicating an effective degradation process induced on cellulose by acid environments (Cerchiara et al. 2009). Conversely, no degradation of the fiber structure have been observed on paper samples after exposure to wet atmosphere (Fig. 5c).

Characterization of paper samples exposed to degradative processes and contaminated with *A. versicolor*

Even in this case, DP determination and morphologic analysis using SEM were carried out to characterize paper samples degraded and contaminated with *A. versicolor*. DP was determined on paper samples after 5, 15 and 25 days of *A. versicolor* inoculum. As seen in Fig. 6, DP of paper samples decreased with increasing time indicating enzymatic hydrolysis of cellulose caused by evident fungal growth. In fact, Fig. 7 shows conidial germination of *A. versicolor* on

paper samples exposed to acidic attack. Similar results were obtained on the paper samples exposed to wet atmosphere (data not reported).



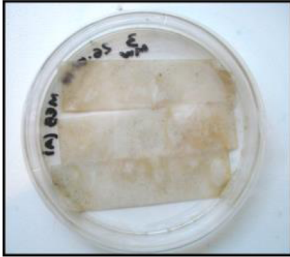
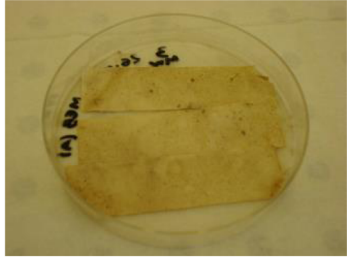




MW heating on paper samples exposed to acidic attack and contaminated with *A. versicolor*

In the conservation context, MW heating is one of the physical methods proposed to ensure protection against fungal growth. So, we studied the use of MW heating at three different temperatures (30, 58 and 63 °C) on paper samples exposed to acidic attack and contaminated with *A. versicolor* setting MW power to 300, 600 and 900 W, respectively.

MW heating effects on paper samples were evaluated through DP determination as shown in Fig. 8. In fact, DP of paper samples exposed to 30 °C for 2 min and 50 s was lower than exposed to 58 and 63 °C indicating a clear influence of MW heating on the fungal vitality. As noted, when paper samples were exposed to low temperature (30 °C) an increase of viability of spores was observed, improving fungal growth. The growth of fungi was stopped without causing significant damage of paper such as cellulose depolymerization at higher temperature (58 and 63 °C, respectively).

Figure 9 shows images of inhibition fungal growth resulting from MW heating on paper samples. Five days after inoculation, the typical coloured spots were shown on the paper surface suggesting the presence of fungal colonization. After 10 days of MW heating (30 °C), it was observed on paper samples a biostatic/



Treatment Time's (days)	10	30
Untreated		
Paper samples exposed to acidic attack and contaminated with <i>A. versicolor</i> after microwave heating at 30 °C		
Paper samples exposed to acidic attack and contaminated with <i>A. versicolor</i> after microwave heating at 58 °C		
Paper samples exposed to acidic attack and contaminated with <i>A. versicolor</i> after microwave heating at 63 °C		

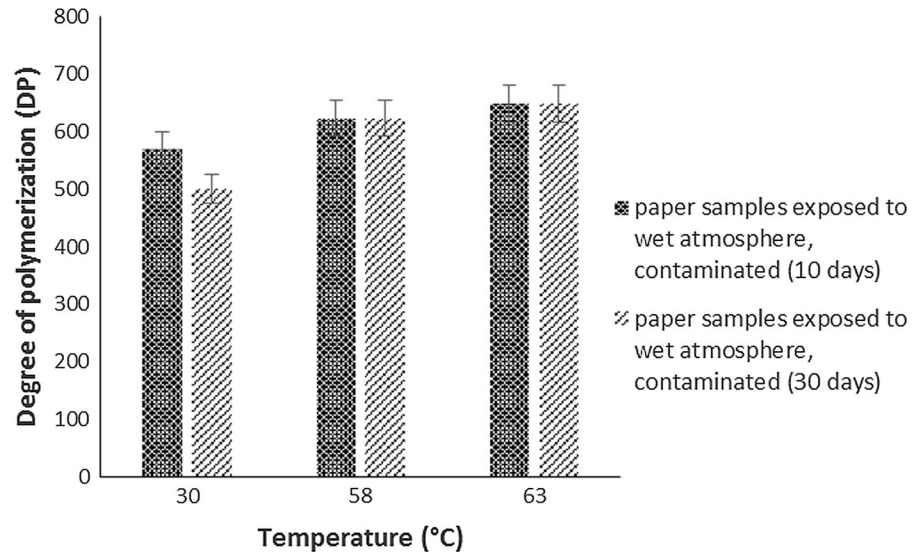
**Fig. 9** Images of paper samples exposed to acidic attack and contaminated with *A. versicolor* after microwave heating at different temperatures

biocidal activity, while after 30 days there was evident fungal re-growth as shown in Fig. 10. In fact, on paper samples there are many coloured spots.

The effects of MW heating at 58 °C after 10 and 30 days respectively led to a biocidal activity (no

fungal re-growth was observed) or at least, biostatic activity (no spore development) after the treatment (Fig. 9). Similar results were obtained exposing paper samples at 63 °C. In addition, no spots originating

**Fig. 10** DP of paper samples exposed to wet atmosphere and contaminated with *A. versicolor* after microwave heating at different temperatures



from *A. versicolor* were observed on paper samples suggesting the biocidal activity of MW.

MW heating on paper samples exposed to wet atmosphere and contaminated with *A. versicolor*

Even in this case, results obtained for MW effects on paper samples exposed to wet atmosphere and contaminated with *A. versicolor* are similar to those above described for paper samples exposed to acidic attack and contaminated with *A. versicolor*.

Fungal re-growth was observed exposing to MW heating at 30 °C after 10 and 30 days respectively, as confirmed by DP reported in Fig. 10. Whereas, no fungal re-growth was evident after MW heating at 58 and 63 °C respectively, suggesting the ability of MW to stop growth of fungi. Paper samples exposed to 58 and 63 °C after 10 and 30 days exhibit higher DP than exposed to MW heating at 30 °C as shown in Fig. 10 indicating no cellulose degradation occurred.

Images of paper samples after the MW treatment show no fungal re-growth on paper samples exposed to MW heating at 58 and 63 °C after 10 and 30 days (data not reported).

## Conclusions

The book of 18th century “Trattato delle acque minerali” written by Niccola Andria was biodeteriorated by *A. versicolor* as confirmed the morphological analysis and molecular biology techniques.

Molecular studies have demonstrated that a method where the ITS region is sequenced and the result is analyzed by phylogenetic methods, is a robust strategy for fungal species identification.

Identification of *Aspergillus* species using nucleic acid sequence analysis of the ITS 1 and 2 regions in combination with a BLAST analysis is a reliable and efficient method that provides earlier identification than standard culture methods. Indeed, to confirm there is a reduction in analysis time and the opportunity to use cultural small portions.

*A. versicolor* is widespread in terrestrial ecosystems from polar to southern latitudes (Domsch et al. 1993), it can survive for a long time in natural environments also in unfavorable condition (Fomicheva et al. 2006). Finally, our research is supported by several articles in the literature that confirm the presence of *A. versicolor* as a ubiquitous paper biodeteriogen. Taken into account it, we used *A. versicolor* as biodeteriogen to contaminate paper samples exposed two degradation processes (exposure to wet atmosphere and to acidic attack) simulating storage conditions of 18th century book.

Finally, we investigated the application of MW as physical method to protect biological attack due to fungi in paper artworks. MW heating at three different temperatures (30, 58 and 63 °C) was applied on different types of paper samples affected by spots originating from *A. versicolor*. DP of paper samples and SEM microscopy were evaluated after prefixed times of fungal attack. The best results in stopping

fungi growth's were obtained with MW heating paper samples at 58 and 63 °C for few minutes ensuring that there was no damage to the cellulose. Experiments performed proved that the use of MW heating could help to effectively protect artworks contaminated with *A. versicolor*.

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

### References

- Abrusci C, Martìn-Gonzalez A, del Amo A, Catalina F, Collado J, Platas G (2005) Isolation and identification of bacteria and fungi from cinematographic films. *Int Biodeterior Biodegrad* 56:58–68. <https://doi.org/10.1016/j.ibiod.2005.05.004>
- Andreuccetti D, Bini M, Ignesti A, Gambetta A, Olmi R (1994) Microwave destruction of woodworms. *J Microw Power Electromagn Energy* 29(3):153–160. <https://doi.org/10.1080/08327823.1994.11688244>
- Balajee SA, Gribskov J, Brandt M, Ito J, Fothergill A, Marr KA (2005) Mistaken identity: *Neosartorya pseudofischeri* and its anamorph masquerading as *Aspergillus fumigatus*. *J Clin Microbiol* 43:5996–5999. <https://doi.org/10.1128/JCM.43.12.5996-5999.2005>
- Balajee SA, Houbraken J, Verweij PE, Hong SB, Yaghuchi T, Varga J, Samson RA (2007) *Aspergillus* species identification in the clinical setting. *Stud Mycol* 59:39–46. <https://doi.org/10.3114/sim.2007.59.05>
- Balajee SA, Borman AM, Brandt ME, Cano J, Cuenca-Estrella M, Dannaoui E, Guarro J, Haase G, Kibbler CC, Meyer W, O'Donnell K, Petti CA, Rodriguez-Tudela JL, Sutton D, Velegriaki A, Wickes BL (2009) Sequence-based identification of *Aspergillus*, *Fusarium*, and *Mucorales* species in the clinical mycology laboratory: where are we and where should we go from here? *J Clin Microbiol* 47(4):877–884. <https://doi.org/10.1128/JCM.01685-08>
- Canhoto O, Pinzari F, Fanelli C, Naresh M (2004) Application of electronic nose technology for the detection of fungal contamination in library paper. *Int Biodeterior Biodegrad* 54:303–309. <https://doi.org/10.1016/j.ibiod.2004.04.001>
- Cerchiara T, Chidichimo G, Gallucci MC, Ferraro R, Vuono D, Nastro A (2009) Use of Spanish broom (*Spartium junceum* L.) canvas as a painting support: evaluation of the effects of environmental conditions. *J Cult Herit* 10:396–402. <https://doi.org/10.1016/j.culher.2008.12.002>
- Cuzman OA, Olmi R, Riminesi C, Tiano P (2013) Preliminary study on controlling black fungi dwelling on stone monuments by using a microwave heating system. *IJCS* 4:133–144
- De Filipo G, Palermo AM, Tolmino R, Formoso P, Nicoletta FP (2016) Gellan gum hybrid hydrogels for the cleaning of paper artworks contaminated with *Aspergillus versicolor*. *Cellulose* 23:3265–3279. <https://doi.org/10.1007/s10570-016-1021-z>
- Domsch KH, Gams W, Anderson TH (1993) *Compendium of soil fungi*, vol I. IHW-Verlag, Alemanha, pp 1–672
- Florian MLE, Manning L (2000) SEM analysis of irregular fungal fox spots in an 1854 book: population dynamics and species identification. *Int Biodeterior Biodegrad* 46:205–220. [https://doi.org/10.1016/S0964-8305\(00\)00062-7](https://doi.org/10.1016/S0964-8305(00)00062-7)
- Fomicheva GM, Vasilenko OV, Marfenina OE (2006) Comparative morphological, ecological and molecular studies of *Aspergillus versicolor* (Vuill.) tiraboschi strains isolated from different ecotopes. *Microbiology* 75(2):186–191. <https://doi.org/10.1134/S0026261706020123>
- Gorny RL, Mainelis G, Wlazo A, Niesler A, Lis DO, Marzec S, Siwinska E, Ludzen-Izbinska B, Harkawy A, Kasznia-Kocot J (2007) Viability of fungal and actinomycetal spores after microwave radiation of building materials. *Ann Agric Environ Med* 14:313–324
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98
- Hinrikson HP, Hurst SF, Lott TJ, Warnock DW, Morrison CJ (2005) Assessment of ribosomal large-subunit D1–D2, internal transcribed spacer 1, and internal transcribed spacer 2 regions as targets for molecular identification of medically important *Aspergillus* species. *J Clin Microbiol* 43:2092–2103. <https://doi.org/10.1128/JCM.43.5.2092-2103.2005>
- Hong SB, Go SJ, Shin HD, Frisvad JC, Samson RA (2005) Polyphasic taxonomy of *Aspergillus fumigatus* and related species. *Mycologia* 97:1316–1329. <https://doi.org/10.1080/15572536.2006.11832738>
- Kozakiewicz Z (1989) *Aspergillus* species on stored products. *Mycol Pap* 161:1–188
- Magan N (1997) Fungal adaptation to environmental stress. In: Wicklow DT, Soderstrom B (eds) *MYCOTA, environmental and microbial relationships*, vol IV. Springer, Berlin
- Mesquita N, Portugal A, Videira S, Rodriguez-Echeverria S, Bandeira AML, Santos MJA, Freitas H (2009) Fungal diversity in ancient documents. A case study on the Archive of the University of Coimbra. *Int Biodeterior Biodegrad* 63:626–629. <https://doi.org/10.1016/j.ibiod.2009.03.010>
- Michaelsen A, Pinzari F, Ripka K, Lubitz W, Pinär G (2006) Application of molecular techniques for identification of fungal communities colonizing paper material. *Int Biodeterior Biodegrad* 58:133–141. <https://doi.org/10.1016/j.ibiod.2006.06.019>
- Montemartini Corte A, Ferroni A, Salvo VS (2002) Isolation of fungal species from test samples and maps damaged by foxing and correlation between these species and the environment. *Int Biodeterior Biodegrad* 51:167–173. [https://doi.org/10.1016/S0964-8305\(02\)00137-3](https://doi.org/10.1016/S0964-8305(02)00137-3)
- Pinzari F, Fanelli C, Canhoto O, Magan N (2003) Electronic nose technology applied to the detection of fungal infections in libraries and archives. In: *Proceedings of moulds, health and heritage conference*, Braunschweig, Germany
- Pinzari F, Pasquariello G, De Mico A (2006) Biodeterioration of paper: a SEM study of fungal spoilage reproduced under

- controlled conditions. *Macromol Symp* 238:57–66. <https://doi.org/10.1002/masy.200650609>
- Plaza PJ, Zona AT, Sanchis R, Balbastre JV, Martinez A, Munoz EM, Gordillo J, de Los Reyes E (2007) Microwave disinfection of bulk timber. *J Microw Power Electromagn Energy* 41(3):21–36
- Rakotonirainy MS, Heude E, Lavédrine B (2007) Isolation and attempts of biomolecular characterization of fungal strains associated to foxing on a 19th century book. *J Cult Herit* 8:126–133. <https://doi.org/10.1016/j.culher.2007.01.003>
- Riminesi C, Olmi R (2016) Localized microwave heating for controlling biodeteriogens on cultural heritage assets. *Int J Conserv Sci* 7:281–294. ISSN: 2067-533X
- Sterflinger K (2010) Fungi: their role in deterioration of cultural heritage. *Fungal Biol Rev* 24(1–2):47–55. <https://doi.org/10.1016/j.fbr.2010.03.003>
- Summerbell RC, Levesque CA, Seifert KA, Bovers M, Fell JW, Diaz MR, Boekhout T, de Hoog GS, Stalpers J, Crous PW (2005) Microcoding: the second step in DNA barcoding. *Philos Trans R Soc B Biol Sci* 360:1897–1903. <https://doi.org/10.1098/rstb.2005.1721>
- Szczepanowska H, Cavaliere AR (2000) Fungal deterioration of 18th and 19th century documents: a case study of the Tilghman Family Collection, Wye House, Easton, Maryland. *Int Biodeterior Biodegrad* 46:245–249. [https://doi.org/10.1016/S0964-8305\(00\)00061-5](https://doi.org/10.1016/S0964-8305(00)00061-5)
- Urzi C, Albertano P (2001) Studying phototrophic and heterotrophic microbial communities on stone monuments. In: Doyle RJ (ed) *Methods in enzymology*. Academic Press, San Diego
- Urzi C, De Leo F (2001) Sampling with adhesive tape strips: an easy and rapid method to monitor microbial colonization on monument surfaces. *J Microbiol Methods* 44:1–11. [https://doi.org/10.1016/S0167-7012\(00\)00227-X](https://doi.org/10.1016/S0167-7012(00)00227-X)
- White TJ, Bruns T, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and applications*. Academic Press, Inc., New York, pp 315–322