Characterization of an early 20th century Chinese manuscript with foxing stains

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Abstract

Foxing spots are reddish-brown, brown or yellowish in irregular shapes, commonly discovered on paper materials. However, effects of the spots on degradation of Chinese papers have rarely been reported. In this study, a 20 th century Chinese manuscript with few foxing stains was explored. This manuscript is made of 80% chemical softwood pulp and 20% mechanical softwood pulp, on which foxing spots are surrounded by a clear rim of fluorescence illuminated by UV light. Paper areas with the foxing stains are more acidic than the un-foxed. However, no obvious difference of cellulose crystallinity identified by XRD (X-ray diffraction) were found between paper areas with and without foxing spots. Also, characteristic peaks of Fourier transform infrared spectroscopy (FTIR) spectra of paper areas with and without the spots do not show obvious difference. Fungal identification of the spots demonstrates that fungi belong to the genus Alternaria tenuissima and Alternaria solani . This research extends our understanding of foxing spots on Chinese archives and informs further preventive conservation.

1 Introduction

The term foxing originated from rusty red color of fox, and its use was firstly recorded in 1848 [1, 2]. Foxing spots in various sizes and shapes have a range of colors from yellow to black, commonly found on Chinese books, paintings and calligraphy [3, 4]. Chen and Xie indicated that foxing spots on Xuan paper (made of blue sandalwood and straw) [3, 5], were due to growth of fungi, i.e. Aspergillus and Penicillium. However, the fungal activity on other types of Chinese paper remains unclear.

Since the 1930s [6], attempts have been made to understand and describe color changes resulting from foxing spots on western papers, where three major explanations were proposed. Firstly, transition metals were detected from these spots, such as iron [7–10], tin [8], and copper [9–11], acting as catalysts for cellulose oxidation [11–13].

Fungal infection, reported by Pierre Sée [11], is another possible cause of foxing spots. It leads to acid-catalyzed hydrolysis of paper due to generated extracellular enzymes and metabolites from fungi such as butyric, lactic, citric and oxalic acid [11, 12]. Other organic acids, like amino acids and malic acid, could lead to yielding melanoidins in papers [14, 15]. Identified fungal species isolated from foxing spots were Penicillium, Cladosporium, Aspergillus and Eurotium [14, 16–19]. Other fungal species, such as C. globosum, E. purpurascens, Peziza ostracoderma, P. pomorum, Trichoderma spp. and U. botrytis, were found to have intense cellulolytic activities [20]. Florian [21] and Florian & Manning [22] suggested that autoxidation of lipids from conidia might be another possible explanation for discoloration of papers.

Thirdly, a dual activity between fungi and iron was assumed by Iiams and Beckwith (1935), indicating that these acids and metabolic substances produced by fungi could react with iron in paper, yielding unstable organic ferric substance [6]. Cain and Miller [7] found that snowflake-like foxing spots contain significantly higher concentration of iron than surrounding paper areas, where hyphae and occasional fruiting bodies were detected.

Traditional Chinese paper is made of non-woody fibers manually, such as fibers of hemp, ramie, jute, paper mulberry, bamboo, rice and wheat straw [23, 24], and papers made of kraft pulps, sulfate pulps, cotton pulps and wood pulps are commonly used in Europe [25]. From the 19th century on, machine-made papers began to be widely used in China, and wood pulp started to predominate the market [26]. Survey from study of Chen and Zhang [4] in 2012 showed that books with foxing spots (produced during1659–1982) reached 60% in Shanghai History Museum, and occurrence rate of the spots on machine-made papers were higher than that of handmade papers.

Certain sizing and fillers are capable of promoting fungal growth and paper degradation [27]. During the eastern Han dynasty (25–220 CE), sizing began to be applied to Chinese papers using starch, gelatin and gum. Occasionally, the gelatin was replaced by acidic rosin/aluminum in modern Chinese machine-papermaking [26]. However, due to catalytic ability of alum [28], alkaline and neutral sizing agents were in use in 1980s such as alkyl ketene dimer (AKD), alkenyl succinic anhydride(ASA) [29–33]. In addition, various fillers make papers smoother, flatter and less transparent [34]. Kaolin filler ($Al_2O_3.SiO_2.2H_2O$), was found in most of 19th-century printing papers [35]. From the 20th century on, other fillers like Calcite ($CaCO_3$), gypsum ($CaSO_4$) and white zinc oxide (ZnO) were mostly used, with occasional application of Talc ($3MgO•4SiO_2•H_2O$), titanium dioxide (TiO_2) and barium sulfate ($BaSO_4$) [17, 35, 36].

For better understanding of the foxing spots, their chemical and biological properties were explored on a 20th century Chinese manuscript as a case study. Due to sample availability, non-invasive instruments and tests requiring small sample quantity were used and conducted. Herzberg staining was employed for fiber identification, and paper fillers were analyzed by scanning electron microscopy coupled energy dispersive spectroscopy (SEM-EDS) and total reflection Fourier transform infrared spectroscopy (ATR-FTIR). Multiple analytical methods were used for the morphological and chemical characterization of foxed and un-foxed areas of the investigated archival paper, combined with microbiological study.

2 Methodology

2.1 Sample Description

A handmade manuscript (made in 1938) was used for all analysis, provided by Archives Centre, Xi'an Jiaotong University, Xi 'an in China. It presents irregular yellowish-brown spots in the upper left and lower right corners, and in the central part of the paper (Fig. 1a). The spots keep spreading and penetrating into underneath papers (Fig. 1b), leading to incomplete, fragile and brittle pieces. The method of papermaking was estimated based on 15 laid lines in 1 cm and chain lines with 17 mm interval.

2.2 UV examination

Color and morphology of the spots were observed by images taken with a camera (Leica D-LUX, Type 109, Germany), under the natural light. UV filters were used to observed the spots at 254 nm (Philips TUV 6W/G6T5, Poland) and 365 nm (Hitachi F6T5 6WATT, Japan).

2.3 Herzberg stain test

Following TAPPI T401 om-03 [37], Herzberg stain was made by mixing 25 ml saturated solution of zinc chloride in distilled water with iodine solution (0.25 g iodine and 5.25 g potassium iodide dissolved in 12.5 ml distilled water). The resulting solution was kept still over 6 hours, and then supernatant was decanted into a dark bottle to avoid excessive exposure to light and air. A small piece of iodine was added during storage [38].

Samples were taken from paper pieces with foxing spots (~ 2 × 2 mm^2), stained with Herzberg solution, and were observed and photographed using a XWY-VI paper fiber analyzer with 4 × and 10 × magnifications.

2.4 pH test

According to the cold extraction method TAPPI T529 om-14 [39], pH of random five points of foxed and un-foxed areas on the first page was measured using pH meter (Merrler Toledo FE28, China).

2.5 X-ray diffractometer (XRD)

Two small detached pieces of foxed and un-foxed paper areas (~ 5 × 5mm^2) for XRD analysis. An XRD spectrometer (PANalytical X'Pert^3 Powder, Netherlands) was used equipped with Cu target and Cu-Kα radiation. Angular range (2θ) was scanned from 10° to 50° at a step size of 0.013°, and the working voltage and current were 40 kV and 40 mA respectively.

2.6 SEM-EDS

The two same samples used for XRD analysis were placed on double sided sticky tape on SEM sample holders, in which a SEM-EDS (Hitachi SU3500, Japan) with variable pressure was used.

2.7 ATR-FTIR

ATR-FTIR spectrometer (Thermo Scientific Nicolet iS50, USA) was used coupled with a middle and far infrared diamond ATR module for analysis of filler. Spectra were acquired in the transmittance mode over the range of 4000–500 cm^{-1} and with 32 scans at 4 cm^{-1} resolution.

2.8 Fungal culture and identification

A sample was taken with a sterile inoculating loop from the foxed paper areas of first page, and then was inoculated in a Sabouraud dextrose agar (SDA) for fungal growth at 28 °C, RH 80% for 4–5 days. Then, all different colonies were picked and inoculated in Sabouraud dextrose broth (SDB) for obtaining pure cultures under the same condition. Fungi were stained with lactophenol cotton blue, observed with the microscope (Olympus BX41, Japan), and digitally recorded by a camera (Leica D-LUX, Type 109, Germany).

The pure cultures were inoculated in SDB and shaken mechanically at 28 ^{o}C to obtain fungal solution for DNA sequencing. The fungal strains were identified at the genus level by observation of macroscopic features of the colonies (texture and color) and hyphae and reproductive structures. The results were compared with Internal transcribed spacer (ITS) sequences in National Center of Biotechnology Information (NCBI) database.

3 Results And Discussion

3.1 Descriptive information of foxing spots

A clear rim of florescence surrounds each yellowish-brown spot under UV illumination at 365 nm (Fig. 2a – 2d). It is suggested that formation of these fluorescent species is an intermediate stage of paper degradation, eventually leading to the brown discoloration of paper [40, 41], due to increasing numbers of conjugated double bonds (C = C, C = O, or C = N) in cellulose molecules [42].

In Fig. 2e, fibers from un-foxed area show purple blue (~ 80%) and yellow (~ 20%) by the Hertzberg stain. The fibers present ribbon-like structures with pointed ends and pits in cross-fields, and also, vessels were not found (Fig. 2f). These characteristics indicate that raw materials of this paper consist of 80% chemical and 20% mechanical softwood pulp [43].

For identification of fillers, main elements detected from this manuscript are C, O, Al, Si, Ca, S, Mg (Table 1), whose EDS mapping is presented in Fig. 3. High amount of Ca suggests that calcium carbonate ($CaCO_3$) or/and calcium sulfate ($CaSO_4$) might be added. Presence of Al and Si, and Mg and Si presumably refer to kaolin (Al_2O_3•$2SiO_2$•$2H_2O$) and talc ($3MgO$•$4SiO_2$•H_2O).

In ATR-FTIR spectra, peaks at 1427 cm^{-1}, 1105 cm^{-1} and 518 cm^{-1} correspond to the stretching vibration of CO_3^{2-}, SO_4^{2-} and Si-O-Al, respectively (Fig. 5), further confirming that fillers of this manuscript contains $CaCO_3$, $CaSO_4$ and kaolin.

Table 1. EDS mapping summary of samples (%). Symbol '-' represents the element was not detected.

Sample	C	O	Al	Si	Ca	Mg	Na	S	N
Un-foxed	45	37	3	4	4	2	1	4	-
Foxed	55	30	3	4	3	2	2	-	1

3.2 Comparison of pH and crystallinity between paper areas with and without foxing spots

Un-foxed areas are slightly acid with average pH 5.68 (uncertainty 2%, n = 5). However, pH of foxed areas is much lower at 4.73 (uncertainty 3%, n = 5), which might be due to metals or acids from fungal

metabolites accelerating the oxidation of the cellulose chain, as Manso et al suggested [1].

XRD could determine the crystallinity and orientation degree of cellulose [44]. XRD pattern of cellulose I was characterized by a well-defined principal peak at 22.5° (2θ) and two secondary peaks at 14.5° and 16.3° (2θ). However, with cellulose degradation, the two secondary peaks firstly merge and then are destroyed, leading to a smaller principal peak [45], at 22.7° (2θ) attributed to (002) crystal plane (Fig. 4) [46]. The discernible difference of cellulosic crystalline structure was not found between samples with and without foxing stains.

From ATR-FTIR spectra in Fig. 5, an interesting peak at 1646 cm^{-1} can be attributed to amide I (1600–1700 cm^{-1}), suggesting the existence of proteinaceous material [17, 47], presumably indicating the sizing agent; however, the peak of amide II (~ 1545 cm^{-1}) is masked by a linear decrease of absorbance. This informs us that fungi identification is needed for further confirmation.

3.3 Isolation and identification of the fungi

Infections of fungi are widespread in libraries and archives, and the fungal species were identified to belong to four main genera, i.e. Aspergillus sp. (~ 30%), Penicillium sp. (~ 30%), Cladosporium sp., and Ulocladium sp.[17, 48–52]. Therefore, it is assumed that foxing stains of our sample might be caused by one of these species.

For verifying this hypothesis, three strains of fungi were selected, isolated and purified from the foxing spots on the manuscript, labelled as No.1, No.2 and No.3, taking morphological characteristics and culture properties into consideration [18, 53]. The isolated fungal colonies were flocculent and grew rapidly, during which hyphae were colorless or dark white at the beginning, and then turned brown (No.1 and No.2) or black brown (No.3), as shown in Fig. 6. Conidia mostly present obclavate shape, divided by both horizontal and vertical septa (Fig. 7).

After the DNA sequencing, obtained ITS sequences were compared with NCBI database. The results show that the three strains (No.1, No.2, No.3) all belong to Alternaria, in which homologies of No.1, No.2 and No. 3 fungus are attributed to sequences of Alternaria tenuissima (KX783391.1), Alternaria tenuissima (KX783385.1), and Alternaria solani (LC339938.1) respectively, and the homology of all fungus reaches 99% in their nucleotide. Although both No.1 and No.2 both are identified to be Alternaria tenuissima, different sequences of ITS-DNA should be considered as different species.

Genus Alternaria is one of the most common fungal conidia with strong adaptability to environment and matrix. The hyphae are slender with colors ranging from pale to brown. Most of them can parasitize plant, leading to plant diseases, such as stains of leaves and stems or fruit rot, and a few are saprophytic fungi living in soil. Cellulase can be produced from this species by metabolism process [53], to hydrolyze the β-1, 4-glucoside bond of cellulose.

4 Conclusion

By a series of characterization and fungi identification, this case study gives an insight into the source of foxing stains using a 20th century manuscript in a severely degraded condition. The following conclusions can be reached:

- It is found that foxing spots are able to grow on the paper made of 80% chemical softwood pulp and 20% mechanical softwood pulp. Fillers like kaolin ($Al_2O_32SiO_2{\cdot}2H_2O$), calcium carbonate ($CaCO_3$), calcium sulfate ($CaSO_4$) and talc ($3MgO{\bullet}4SiO_2{\bullet}H_2O$) were added during papermaking, while it is not found that these additives have any impact on generation of foxing spots.
- A clear rim of florescence surrounds each spot when irradiated by UV light, presenting bigger and more obvious foxed stains than the stains observed by the naked eye.
- pH test demonstrates that paper area with foxing spots has lower pH than the un-foxed. However, no obvious difference of crystallinity and spectra was present from paper areas with and without foxing stain by XRD and ATR-FT-IR.
- *Alternaria tenuissima* and *Alternaria solani* were identified from fungi identification, verifying that fungi are the main cause the foxing spots for this manuscript. Corresponding preventive conservation strategies could be formulated to inhibit fungal growth.

This research mainly gives the understanding of sources of the foxing spots on a Chinese manuscript. Due to sample limitation, we only selected one manuscript for analysis and conducted a preliminary research. More samples should be examined to learn about other factors affecting the generation of foxing spots, for better prevention of achieves with foxing spots in the future.

Abbreviations

XRD : X-ray diffraction; FTIR: Fourier transform infrared spectroscopy; AKD: Alkyl ketene dimer; ASA: Alkenyl succinic anhydride; SEM-EDS: Scanning electron microscopy coupled energy dispersive spectroscopy; ATR-FTIR: Total reflection Fourier transform infrared spectroscopy; SDA: Sabouraud dextrose agar; SDB: Sabouraud dextrose broth; ITS: Internal transcribed spacer; NCBI: National center of biotechnology information.

Declarations

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Authors' contributions

JL, YL and ZJ designed the research; JL, GT and PF performed the research and analyzed data. JL and YL wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

References

1. Manso M, Pessanha S, Figueira F, Valadas S, Guilherme A, Afonso M, Rocha AC, Oliveira MJ, Ribeiro I, Carvalho ML. Characterisation of foxing stains in eighteenth to nineteenth century drawings using non-destructive techniques. Anal Bioanal Chem. 2009; 395(7): 2029-36.
2. Meynell GG, Newsam RJ. Foxing, a fungal infection of paper. Nature. 1978; 274: 466-8.
3. Chen Y, Xie Y. The causes of foxing on the Chinese painting. Sci conserv archaeol 2002; 14 Suppl: 63-76 **(in Chinese)**.
4. Chen G, Zhang T. Study of the features of foxing in modern books(before 1950's). Sci conserv archaeol. 2012; 24(4): 66-70 **(in Chinese)**.
5. Mullock H. Xuan paper. The paper conserv. 1995; 19(1): 23-30.
6. Iiams TM, Beckwith TD. Notes on the causes and prevention of foxing in books. The library quarterly. 1935; 5(4): 407-18.
7. Cain CE, Miller BA. Photographic, spectral and chromatographic searches into the nature of foxing. AIC preprints. American institute for conservation 10th annual meeting, Milwaukee, Washington: 1982 , 54-62.
8. Kenjo T, Arai H, Suzuki T. Application of scanning electron microscope in the field of conservation science of cultural properties. JEOL News. Electron optics instruments/application. 1987; 25E(1): 13-7.
9. Tang LC. Determination of iron and copper in 18th and 19th century books by flameless atomic absorption spectroscopy. JAIC. 1978; 17(2): 19-32.
10. Tang L, Troyer MA. Flameless atomic absorption spectroscopy: a useful tool for direct determination of elements in library archival materials. Technol Conserv. 1981; 6(2): 40-5.

11. Rebrikova NL, Manturovskaya NV. Foxing: A new approach to an old problem. The library quarterly. 2000; 21(2): 85-100.
12. Bicchieri M, Ronconi S, Romano FP, Pappalardo L, Corsi M, Cristoforetti G, Legnaioli S, Palleschi V, Salvetti A, Tognoni E. Study of foxing stains on paper by chemical methods, infrared spectroscopy, micro-X-ray fluorescence spectrometry and laser induced breakdown spectroscopy. Spectrochim Acta B.2002; 57(7): 1235-49.
13. Bicchieri M, Pappalardo G, Romano FP, Sementilli FM, De Acutis R. Characterization of foxing stains by chemical and spectrometric methods. Restaurator: international journal for the preservation of library and archival material. 2001; 22(1): 1-19.
14. Arai H. Foxing caused by fungi: Twenty-five years of study. Int Biodeter Biodegr. 2000; 46(3): 181-8.
15. Arai H. On the foxing-causing fungi. ICOM committee for conservation: 8th Triennial meeting, Sydney, Australia: Preprints Marina del Rey: The getty conservation institute, 1987, 1165-7.
16. Nunes M, Relvas C, Figueira F, Campelo J, Candeias A, Caldeira AT, Ferreira T. Analytical and microbiological characterization of paper samples exhibiting foxing stains. Microsc Microanal. 2015; 21(1): 63-77.
17. Manente S, Micheluz A, Ganzerla R, Ravagnan G, Gambaro A. Chemical and biological characterization of paper: A case study using a proposed methodological approach. Int Biodeter Biodegr. 2012; 74: 99-108.
18. Wei J. Manual of fungal identification. Shanghai: Shanghai science and technology press. 1979 **(in Chinese)**.
19. Zotti M, Ferroni A, Calvini P. Microfungal biodeterioration of historic paper: Preliminary ftir and microbiological analyses. Int Biodeter Biodegr. 2008; 62(2): 186-94.
20. Montemartini Corte A, Ferronib A, Salvo VS. Isolation of fungal species from test samples and maps damaged by foxing, and correlation between these species and the environment. Int Biodeter Biodegr. 2003; 51(3): 167-73.
21. Florian M-LE. The role of the Conidia of fungi in fox spots. Stud Conserv. 1996; 41(2): 65-75.
22. Florian M-LE, Manning L. Sem analysis of irregular fungal fox spots in an 1854 book: Population dynamics and species identification. Int Biodeter Biodegr. 2000; 46(3): 205-20.
23. Pan J. The four great inventions of ancient China: Their origin, development, spread and influence in the world. Hefei: University of science and technology of China press. 2002 **(in Chinese)**.
24. Pan J. History of science and technology in China: Paper and printing. Beijing: Science Press. 1998 **(in Chinese)**.
25. Hubbe MA, Bowden C. Handmade paper: A review of its history, craft, and science. Bioresources. 2009; 4(4): 1736-92.
26. Wang J. The history of papermaking engineering technology in ancient China. Tai Yuan: Shanxi Education Press. 2006 **(in Chinese)**.

27. Beckwith TD, Swanson WH, Iiams TM. Deterioration of paper : The cause and effect of foxing. Publications of the university of California at Los Angeles in biological sciences. 1940; 1(13): 299-356.
28. Erhardt D, Tumosa C. Chemical degradation of cellulose in paper over 500 years. Restaurator. 2005; 26(3): 151-8.
29. Espy HH. The genesis of alkaline sizing and alkaline-curing wet-strength resins. Alkaline paper advocate. 1990; 3(3): 28-9.
30. Pinzari F, Pasquariello G, Mico AD. Biodeterioration of paper: A sem study of fungal spoilage reproduced under controlled conditions. Macromolecular symposia. 2006; 238(1): 57-66.
31. Area MC, Cheradame H. Paper aging and degradation: Recent findings and research methods. Bioresources. 2011; 6(4): 5307-37.
32. Song X, Chen F, Liu F. Study on the reaction of alkyl ketene dimer (AKD) and cellulose fiber. Bioresources. 2011; 7(1): 652-62.
33. Pinzari F, Zotti M, Mico AD, Calvini P. Biodegradation of inorganic components in paper documents: Formation of calcium oxalate crystals as a consequence of aspergillus terreus thom growth. Int Biodeter Biodegr. 2010; 64(6): 499-505.
34. Casoli A, Cremonesi P, Isca C, Groppetti R, Senin N. Evaluation of the effect of cleaning on the morphological properties of ancient paper surface. Cellulose. 2013; 20(4): 1-17.
35. Beazley K. Mineral fillers in paper. The paper conserv. 1991; 15: 17–27.
36. Manso M, Carvalho ML, Queralt I, Vicini S, Princi E. Investigation of the composition of historical and modern Italian papers by energy dispersive X-ray fluorescence (EDXRF), x-ray diffraction (XRD), and scanning electron microscopy energy dispersive spectrometry (SEM-EDS). Appl Spectrosc. 2011; 65(1): 52-9.
37. TAPPI T401 om-03, Fiber analysis of paper and paperboard, 2008.
38. Clark FC. Paper testing methods, microscopical, chemical, and physical processes described. New York: TAPPI Publishing Corporation. 1920.
39. TAPPI T529 om-14, Surface pH measurement of paper, 2009.
40. Rebrikova NL, Manturovskaya NV. Foxing – a new approach to an old problem. Restaurator. 2000; 21(2): 85-100.
41. Pedersoli JL, Ligterink F, Van Bommel MR. Investigation into fluorescence changes accompanying paper discoloration. ICOM-CC's interim meeting of the working group on graphic documents at EVTEK institute of art and design. 2001: 7-10.
42. Brandt NN, Chikishev AY, Itoh K, Rebrikova NL. ATR-FTIR and FT Raman spectroscopy and laser cleaning of old paper samples with foxings. Laser Phys. 2009; 19(3): 483-92.
43. Wang J. Papermaking raw materials of China an atlas of micrographs and the characteristics of fibers. Beijing: China light industry press. 1999 **(in Chinese)**.

44. Ma X, Huang L, Chen L, Cao S. Determination methods for crystallinity of cellulose. Paper sci technol. 2012; 31(2): 75-8 **(in Chinese)**.
45. Foner HA, Adan N. The characterization of papers by X-ray diffraction (XRD): Measurement of cellulose crystallinity and determination of mineral composition. J forensic sci soc. 1983; 23(4): 313-21.
46. Moropoulou A, Zervos S. The immediate impact of aqueous treatments on the strength of paper. Restaurator. 2003; 24(3): 160-77.
47. Derrick MR, Stulik D, Landry JM. Infrared spectroscopy in conservation science. Scientific tools for conservation. Los Angeles: The getty conservation institute. 1999.
48. Gallo F, Il biodeterioramento di libri e documenti. In: Centro di Studi per la Conservazione della Carta. ICCROM, Roma, 1992.
49. Nyuksha JP, Garg KL, Garg N, Mukerji KG. The biodeterioration of papers and books. Calcutta, India: 1993.
50. Zyska B. Fungi isolated from library materials: A review of the literature. Int Biodeter Biodegr. 1997; 40(1): 43-51.
51. Altibrandi MG, Il deterioramento di natura biologica. In: Chimica e biologia applicate alla conservazione degli archivi. Pubblicazione degli Archivi di Stato, Roma, Saggi, 2002,74, 367-79.
52. Mesquita N, Portugal A, Videira S, Rodríguez-Echeverría S, Bandeira AML, Santos MJA, Freitas H. Fungal diversity in ancient documents. A case study on the archive of the university of Coimbra. Int Biodeter Biodegr. 2009; 63(5): 626-9.
53. Zhang T. Alternaria. Beijing: Science Press. 2003 **(in Chinese)**.

Figures

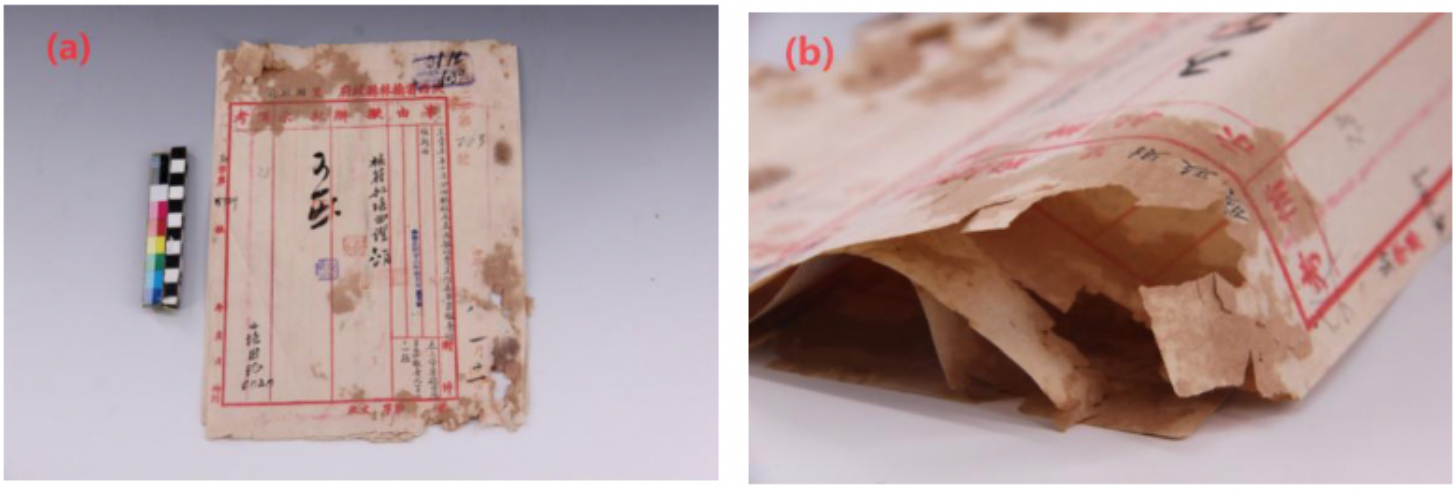

Figure 1

A Chinese manuscript with foxing spots

Figure 2

Fiber morphology of foxed area of the manuscript under different light illuminations (a and c: standard light; b and d: 365nm UV light). Morphological features of the fibers from un-foxed area of the manuscript (e: 40× and f:100×).

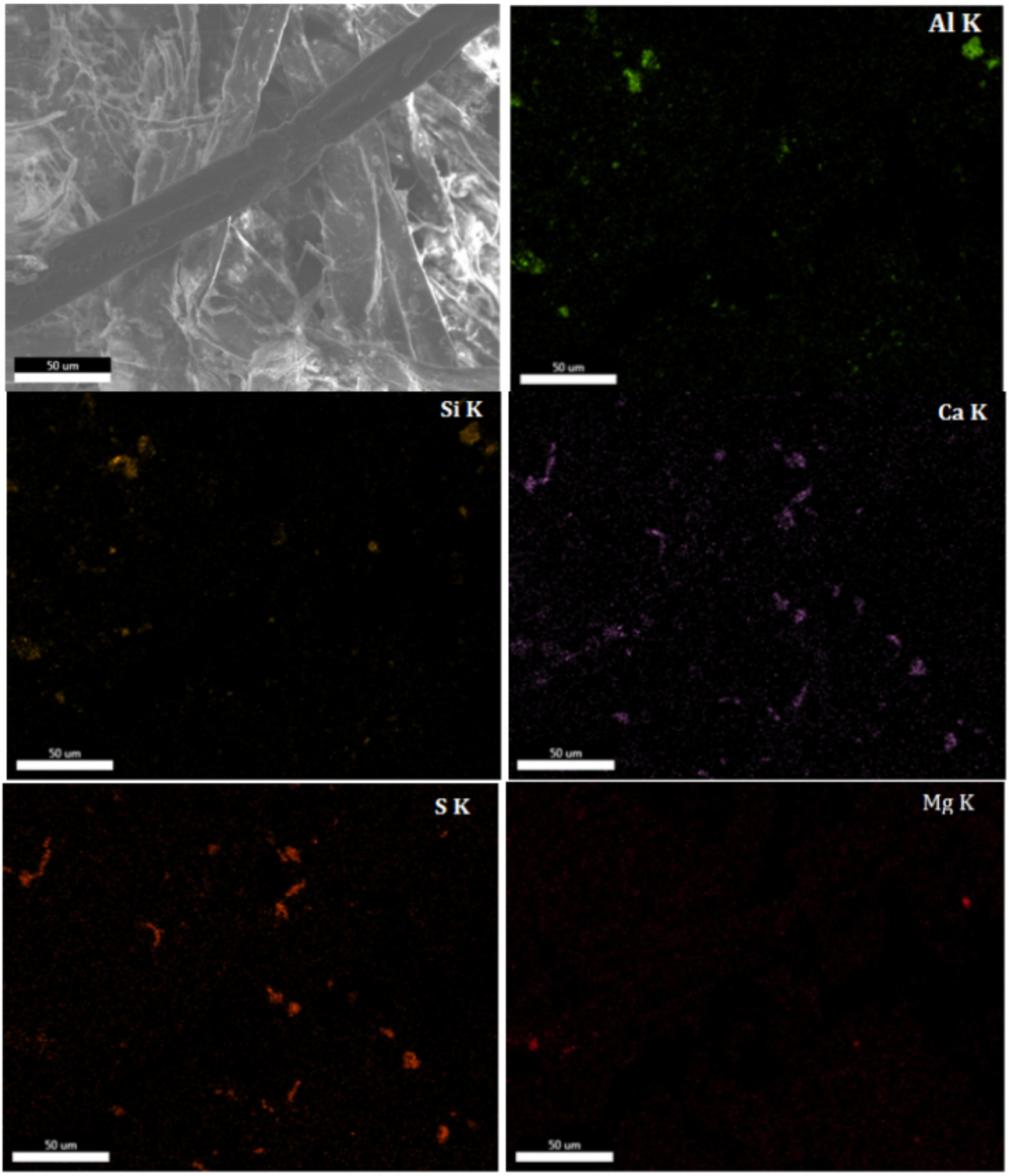

Figure 3

SEM images and elemental mapping (Al, Si, Ca, S, Mg) of un-foxed areas of the manuscript

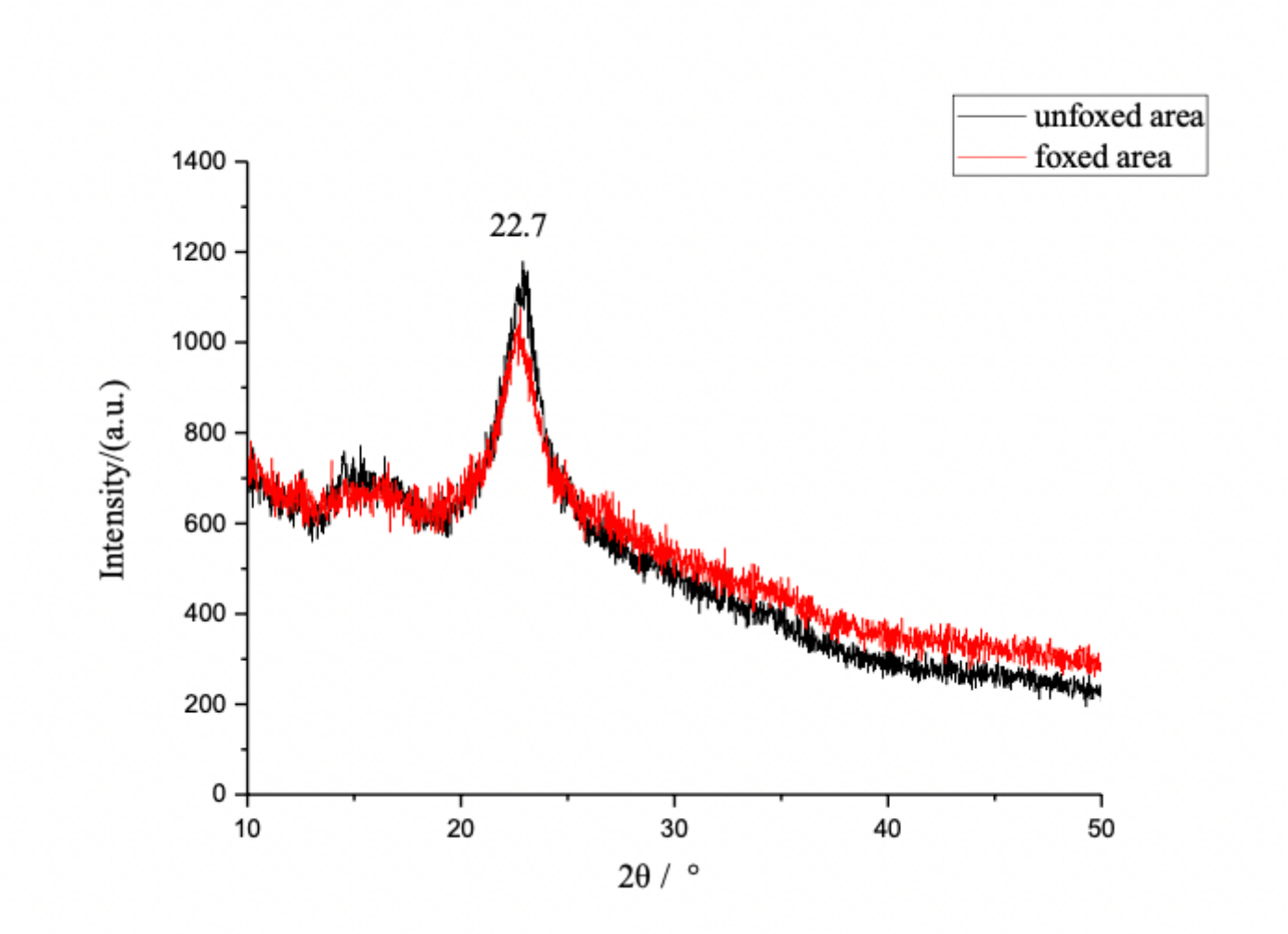

Figure 4

XRD pattern of the un-foxed area and the foxed area of the manuscript

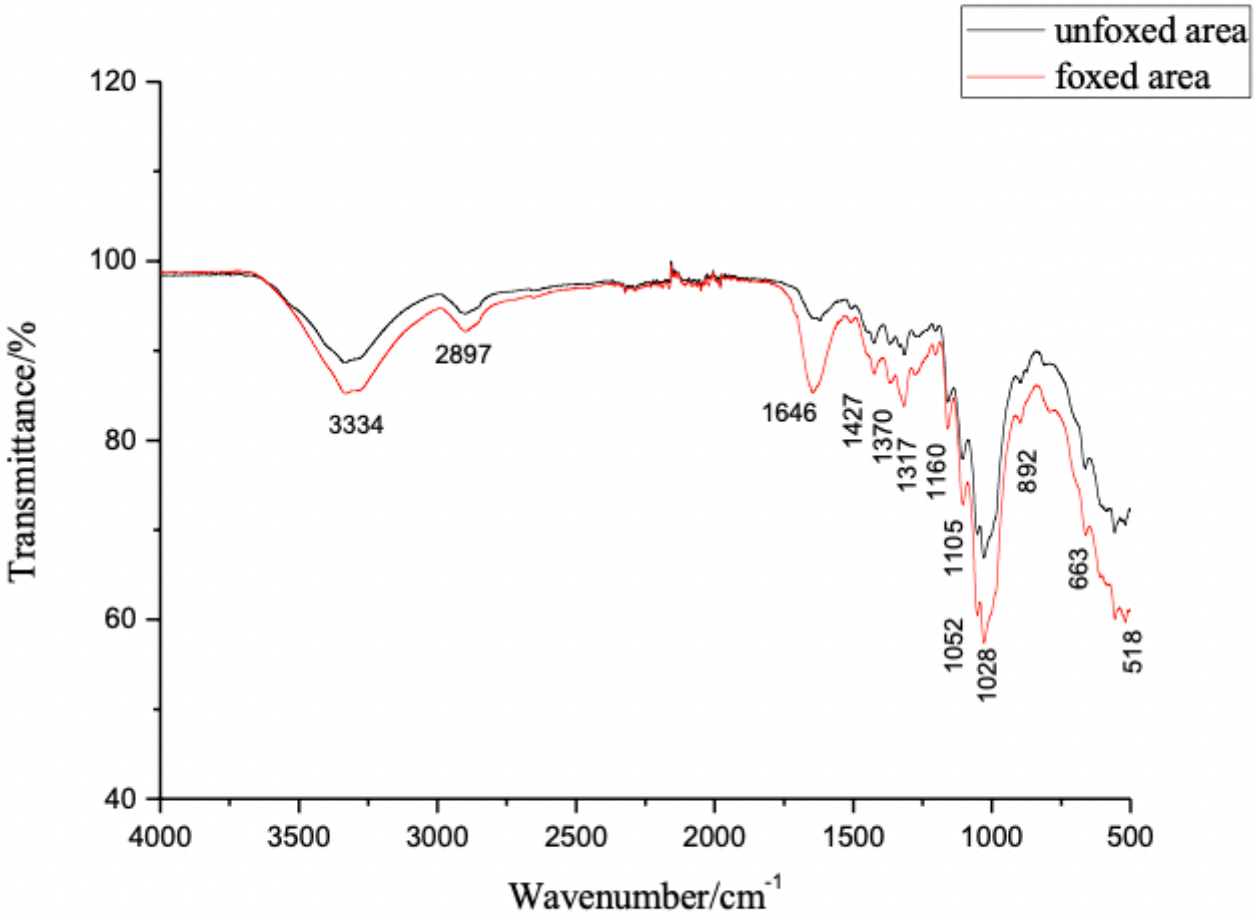

Figure 5

ATR-FT-IR spectra of un-foxed area and foxed area of the manuscript

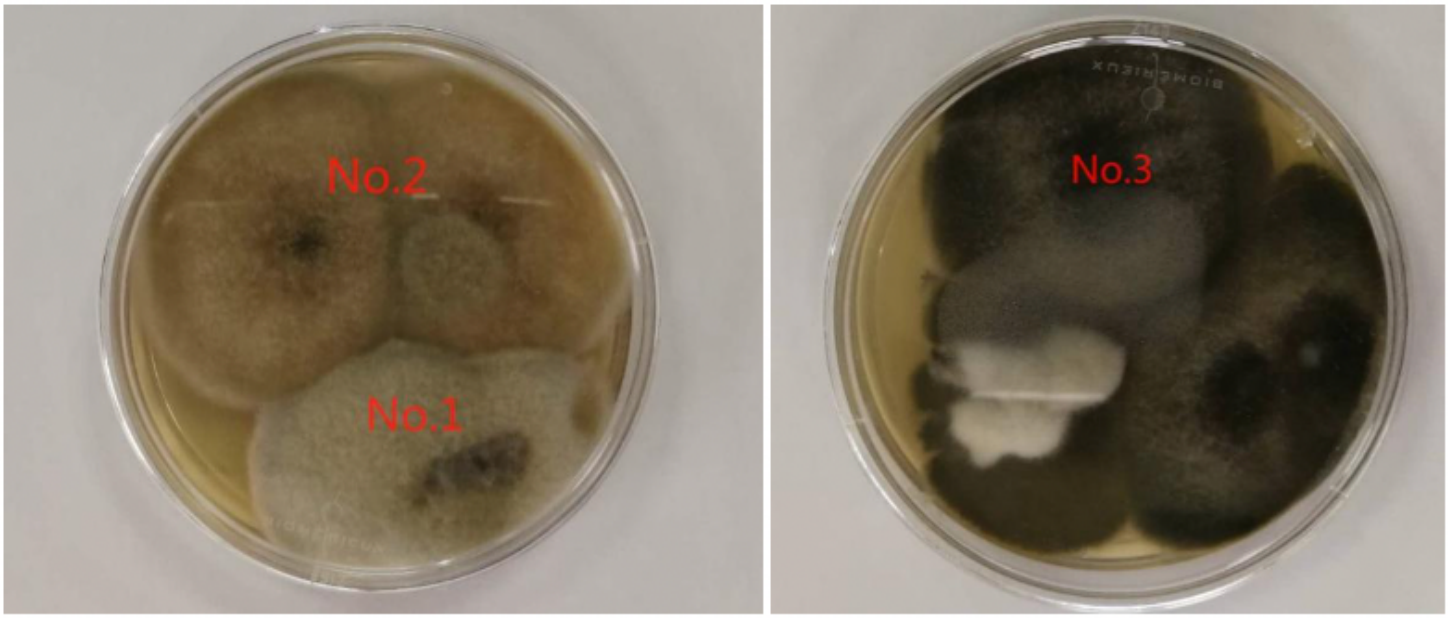

Figure 6

The final morphological characteristics of the isolated fungal colonies

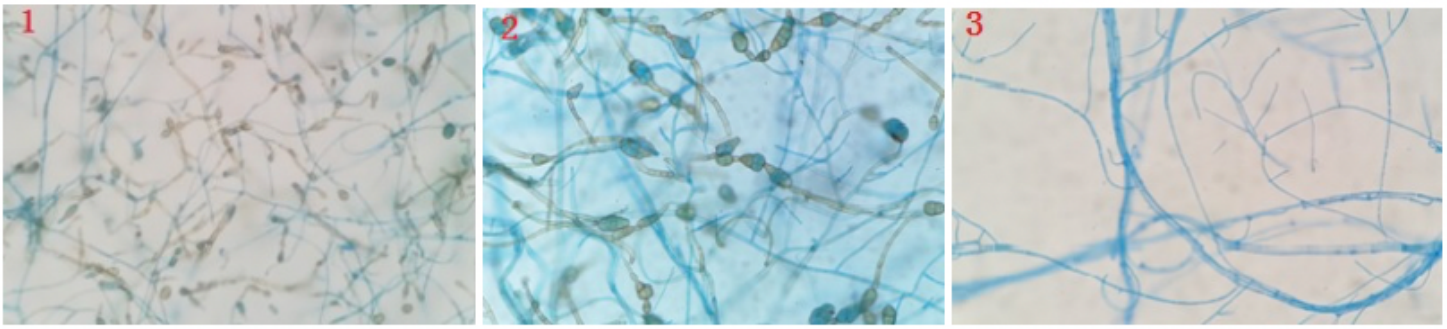

Figure 7

The microscopic morphological characteristics of the isolated and purified strains