Characterization of foxing stains in early twentieth century photographic and paper materials

Aurora Modica\textsuperscript{a}, Maurizio Bruno\textsuperscript{a}, Marco Di Bella\textsuperscript{b}, Maria Francesca Alberghina\textsuperscript{c}, Maria Brai\textsuperscript{c,d}, Dorotea Fontana\textsuperscript{c} and Luigi Tranchina\textsuperscript{d}

\textsuperscript{a}Department STEBICEF, University of Palermo, Palermo, Italy; \textsuperscript{b}Corso di Laurea in Conservazione e Restauro dei Beni Culturali (PFP 5), University of Palermo, Palermo, Italy; \textsuperscript{c}Department of Physic and Chemistry, University of Palermo, Palermo, Italy; \textsuperscript{d}Area Tecnica, Tecnico-Scientifica e Elaborazione Dati - ATeN Center, University of Palermo, Palermo, Italy

\textbf{ABSTRACT}

The subject of this present work is a group of nine historical pictures shot in Palermo by the Sicilian photographer E. Interguglielmi in 1912. They are nine matte-collodion prints mounted on the original cardboard supports and all of them show foxing stains affecting the paper surface. In order to characterise the chemical composition of the supports and investigate foxing spots, non-destructive and micro-destructive analysis were carried out. X-rays fluorescence (XRF) analysis was used to characterise the elemental composition of all the mounting boards, allowing a comparison between the foxing spots and non-affected areas. Laser-Induced Breakdown Spectroscopy was used to investigate the presence of lower atomic number elements, not detectable by XRF, while SEM imaging allowed the investigation of surface appearance and nature of original paper samples from the cardboards.

\textbf{ARTICLE HISTORY}

Received 17 December 2015
Accepted 12 April 2016

\textbf{KEYWORDS}

Foxing; fungi; XRF; LIBS; SEM

\textbf{CONTACT}

Maurizio Bruno  maurizio.bruno@unipa.it

Supplemental data for this article can be accessed at http://dx.doi.org/10.1080/14786419.2016.1180600.

© 2016 Informa UK Limited, trading as Taylor & Francis Group
1. Introduction

Foxing is the term generally used referring to a class of stains which differ in size, shape and colour, typically found on cellulose-based materials. Despite the vast literature on this subject, there’s still no full agreement on the real nature of this complex phenomenon. Several studies have been carried out since the first half of the twentieth century, trying to understand and characterise this particular type of discolouration and to find a proper treatment, without harm to the original supports.

Foxing has been recognised as caused by fungal activity, as a metal-induced degradation, or both. Whatever the origin, it results anyway in the oxidation of the cellulose chain, with consequent paper fibres weakening; even if it never results in the actual loss of material. Since the beginning of the studies, in the past century, the biological nature of paper staining has always been assumed, even though sometimes it is not easy to prove. There are hundreds of fungal species able to affect paper supports, but only some of them can be associated with foxing stains. Those micro-organisms, that can infect the paper during the manufacture or at later stages, are able to survive dormant for years, waiting for the right conditions to grow and reproduce. According to Arai’s researches on stains of induced foxing, organic acids, mainly malic acid, amino acids and glucose, produced by fungi, can react with paper cellulose causing the stains, because of some melanoidines due to the Maillard reaction. Several fungal species had been isolated by Beckwith et al. (1940), Arai (1987), Nol et al. (1983) and Cain et al. (1987) that, cultured specifically from foxing spots, create new stains when re-inoculated into paper under laboratory conditions (Table 1). However, it has been noticed that not all the areas of the samples in which fungal growth was detected showed the typical foxing stains; probably there is an association between the micro-organism vital stage and paper staining, meaning that the fungal hyphae produce those particular chemical substances only in the final stage of their life (Florian 1997).

Generally, a fungal colony can deteriorate the paper support with its extracellular enzymes, able to break long chain molecules, like cellulose, in smaller chemical units that can be absorbed into the cell through the cell membrane. The micro-organisms’ metabolism also causes the production of other substances, like butyric, lactic, citric and oxalic acid. Paper degradation early stages leads to the production of glucose and cellobiose that represent, together with water, an important source of nourishment for fungal growth and development. Furthermore, different metals have been recognised to be connected to fungal growth (Lulloff et al. 2004) and it has been underlined that fungi can activate the expression of several systems to enhance the uptake of zinc to satisfy their needed. (Staats et al. 2013)

| Table 1. Micro-organisms detected in foxing spots. |
| Beckwith et al. (1940) | Arai (1987) | Nol et al. (1983) | Cain et al. (1987) |
|------------------------|-------------|------------------|-------------------|
| Alternaria             | Aspergillus penicilloides | Aspergillus carneus | Aspergillus repens |
| Aspergillus            | Eurotium herbariorum       | Aspergillus flavus Link (with Sclerotia) |
| Byssoschlamys          |                           | Aspergillus flavus Link (lacking Sclerotia) |
| Chaetomium             |                           | Aspergillus fumigatus Thom |
| Fusarium               |                           | Aspergillus niger van Tieghem |
| Hormodendrum           |                           | Aspergillus terreus var. aureus Thom & Raper |
| Monilia                |                           | Aspergillus tamari |
| Mucor                  |                           | Gliocladium roseum (Lin k) Thom |
| Penicillium            |                           | Penicillium funiculosum Thom |
| Stemphylium            |                           |                   |
Metal contamination of paper has also been considered a potential cause of foxing stains. Metal particles can come from manufacture equipment or water, or simply settle on paper with dust. Those metal inclusions can stain the substrate due to oxidation reaction, causing ‘rusty’ stains, with a darker centre surrounded by a lighter halo; discoloration can be caused by the migration of soluble degradation products from the metal centre. (Daniels & Meeks 1988; Neevel & Mensch 1999; Reissland 1999; Bicchieri et al. 2001).

Considering that both fungal spores or structures and iron particles are usually found on paper supports it’s possible to suggest a combined effect which eventually lead to paper staining. Organic acids and other metabolic substances produced by fungi can react with paper iron trace, forming unstable organic iron salts that, once decomposed, can cause the typical rusty stains, due to the presence of iron oxides and hydroxides (Iiams & Beckwith 1935). Then, humidity can facilitate the combined effect, whether started by fungi or iron particles, gradually reducing substrate pH values and, eventually, causing the death of the fungus (Hey 1983). This theory could explain why the alteration always shows small stains and doesn’t cover the entire surface of the material.

In order to fully understand the nature of this particular phenomenon, it is very important to be able to recognise and properly classify with as much precision as possible the foxing stains. To do so, it proved fundamental to describe stains by providing as many information as possible, starting with stains’ visible appearance, specifying colour (yellow, orange or reddish-brown), dimensions, shape (well-defined edges, irregular edges, areas clearer than the surrounding paper) and if the stains spread all over the sheet, identical, in shape and colour, to the stains on the adjacent sheets, if they have black nucleus and concentric rings. Moreover, it is important to describe the stains’ appearance in UV light specifying, if present, the intensity, colour and shape of the fluorescence.

Original paper samples from a group of nine historical photograph’s backing cardboards from the Palermo Municipal Archive, affected by foxing, had been studied in order to investigate the nature of stains in this particular case. All of the nine matte-collodion prints, shot by the renowned photographer Eugenio Interguglielmi in 1912, are mounted on the original cardboard supports, bearing several historical information: the name of the photographer, the date and the subject.

2. Results and discussion

Both pictures and cardboards were affected by an extensive water damage that caused both biological and microbiological attacks, severe losses, stains and discolouration (Figure 1). Furthermore, the high content of humidity caused the delamination of the cardboard various layers, showing the difference between the inner layers and the surface paper, a common feature in photographic cardboards manufacture of the time. The bulk of the cardboard, clearly of poor quality, showed brittleness and discoloration, while the surface layer, better preserved and also lighter in colour, suggested a finer manufacture and some finishing treatment. It is interesting to notice that only the surface paper shows foxing stains (both on recto and verso of the cardboard).

Foxing stains observed on the surface paper of the cardboards appear very similar to each other, showing a homogeneous distribution, reddish-brown colour, quite regular roundish edges and a small diameter, from 1 to 3 mm (Figures 2(a) and 2(b)); some of the stains have
a darker nucleus. Observed under UV light, the stains show a light yellow fluorescent halo with irregular edges (Figures 2(c) and 2(d)).

Micro-chemical tests were carried out on detached paper fragments to identify the pulps’ nature of the two layers of the cellulosic supports: the sample from the cardboard’s surface...
showed a chemical pulp composition, while the sample from inner layers’ paper has been identified as groundwood pulp.

Considering the extensive damage and the discoloration affecting the cardboards and especially the inner layers, pH was measured to assess their condition. Both the sample from the surface paper, affected by foxing and the sample from inner layers’ paper had nearly neutral pH (7.01 and 6.48, respectively), showing no signs of major acidic degradation.

In order to characterise the chemical composition of the supports and investigate foxing spots, non-destructive and micro-destructive analyses were carried out on the original surfaces. X-rays fluorescence (XRF), being a non-destructive technique, was used to characterise the chemical composition of all the nine mounting boards, allowing a comparison between the foxing spots and non-affected areas. Laser Induced Breakdown Spectroscopy (LIBS) was used to investigate the presence of lower atomic number elements, not detectable by XRF; being a micro-destructive analysis, sampling was only allowed on a single small detached paper fragment.

Figure 3 shows two typical XRF measurement points and the related XRF spectra. Elements detected in the cardboards were: Si, K, Ca, Fe and Zn. Table 2 reports XRF results, expressed as counts at element emission energy, on both foxed and unfoxed areas. Ca, Zn and Fe are generally found in larger amount in the foxing stains than the not affected areas.

Figure 2c. Appearance under UV light of foxing stains typically found on all the nine cardboards.

Figure 2d. Foxing stains observed with digital OM – 50× in UV light.
LIBS results obtained on a fragment of N.I. 9 cardboard confirmed a higher intensity signal for calcium emission line (393.3 and 396.8 nm) compared with the non-degraded areas acquired in the same area. Moreover, the LIBS acquisition revealed the presence of sodium and magnesium in corresponding of foxing areas. These evidences are reported in Figure S1 that, showing the comparison between the stained and unstained paper sample areas, confirm the different detected intensity for calcium, magnesium and sodium, respectively (see Table 3 and section 3.4 in Supplementary Material). These chemical elements characterising of the areas of foxing could be related to the metabolic activity of micro-organisms (Bicchieri et al. 2002).

Figure 3. Cardboard N.I. 2. XRF spectra obtained in a non-affected paper area (red line) and in a foxing spot (black line). Pictures of measurement points are also shown.

Table 2. XRF signal intensities of detected elements from measurements performed on each sample. The f letter indicates measurements performed on foxing spots.

| Photo number | Si   | K    | Ca   | Fe   | Zn   |
|--------------|------|------|------|------|------|
| 1            | 278  | 198  | 150  | 695  | 126  |
| 1f           | 267  | 264  | 207  | 727  | 184  |
| 2            | 248  | 264  | 141  | 657  | 130  |
| 2f           | 237  | 171  | 244  | 716  | 232  |
| 3            | 305  | 190  | 120  | 712  | u.d.l. |
| 3f           | 209  | 182  | 371  | 731  | 199  |
| 4            | 298  | 223  | 145  | 648  | 128  |
| 4f           | 247  | 226  | 170  | 672  | 180  |
| 5            | 261  | 183  | 137  | 660  | u.d.l. |
| 5f           | 290  | 175  | 169  | 692  | u.d.l. |
| 6            | 294  | 172  | 134  | 705  | 172  |
| 6f           | 246  | 183  | 212  | 691  | 222  |
| 7            | 259  | 164  | 174  | 664  | u.d.l. |
| 7f           | 270  | 163  | 243  | 739  | 175  |
| 8            | 156  | 119  | 223  | 712  | 165  |
| 8f           | 171  | 149  | 401  | 961  | 250  |
| 9            | 291  | 166  | 150  | 708  | 151  |
| 9f           | 261  | 165  | 251  | 701  | 164  |
To analyse XRF results from a statistical point of view, a principal component analysis (PCA) has been performed. A qualitative estimation of elemental composition of samples and group differentiation has been obtained by performing PCA analysis.

Among the available techniques, PCA represents a useful tool for data variability reduction and classification. It is available in most, both commercial and free, statistical packages. Generally, the cluster distribution of observations (samples) as function of variables (elements amount), in the new $F_1$ vs. $F_2$ PCA space, reported in the bi-plot, made it possible to identify, more clearly, elements that characterise the chemical alteration which affect the analysed cardboards.

A review of PCA in Cultural Heritage field can be found in Alberghina (2014), Gioventù et al. (2011), Luciano et al. (2009), Tzeng and Berns (2005). Other applications of PCA are related to environmental analysis (i.e. for examining the spatial variability of contaminants) (Tranchina et al. 2008), to artwork provenance and classification along with weathering condition investigation (Moropoulou & Polikreti 2009), and spectroscopic imaging data analysis (Attas et al. 2003).

The main aim of PCA statistical technique is reducing data dimensionality transforming the original data-set to a new uncorrelated set of variables, called principal components (PCs) or factors (Fs). They are obtained as the eigenvectors of the variable correlation matrix and are therefore a linear combinations of the original variables. By definition, the new variables are uncorrelated. The factors are ranked according to the amount of the total variability of the data they are able to explain. A new variable set is derived from the above one diagonalising the Pearson similarity matrix. The XLStat 5.01 software by Addinsoft has been used for PCA analysis.

In our case, starting from semi-quantitative value for each detected chemical element, PCA results are shown in Figure S2 by plotting components vectors of all the samples along $F_1$ and $F_2$ and the projection of the corresponding Fs of each sample along the vector axes. This Figure shows that all foxing stains are characterised by an increment of Ca, Zn and Fe with respect to the corresponding paper. A right shift of points, with or without foxing, and belonging to the same sample, can be observed in this figure. Only the sample N.I. 5 did not show this behaviour, elemental composition of paper and of foxing area in this cardboard remains almost the same. The higher amount of Na, Mg, Ca and Zn revealed by XRF and LIBS integrated investigation in the foxing areas compared with the not degraded paper surface, suggests a biological nature of the stains, proved by SEM observations, that showed a significant difference between the foxing stains and unaffected areas: within the foxing spots globular structures, probably fungal spores and conidia wrapped in filaments similar to fungal hyphae were clearly visible. (Figure 4(a)) Associated with the paper fibres and fungal structures, calcium oxalate (CaOx) crystals or biogenic calcite were also detected (Figure 4(b)), consistently with fungal metabolism products (Pinzari et al. 2010; Piñar et al.

**Table 3.** Emission lines suitable for the detection of chemical element, chosen as markers for foxing monitoring, identified in corresponding of not degraded paper and of the foxing stains.

| Chemical element | Atomic emission lines (nm) |
|------------------|-----------------------------|
| Ca (I)           | 393.3, 396.8                |
| Mg (I), (II)     | 279.5, 280.2                |
| Na (I)           | 588.9, 589.5                |
Microbiological tests were carried out in order to verify the presence, over the cardboards’ surface, of living micro-organisms that could be related to this microscopic and analytical evidence. The absence of microbial growth suggests that the damage could have been caused by a previous fungal colonisation, now inactive due to environmental condition (like temperature, RH, pH) adverse to microbial life.

Figure 4a. SEM image of cardboard sample N.I. 9. Globular structures, probably fungal conidia and spores visible within the foxing stain. The image shows an ascomycete with flattened, collapsed and sometimes intact ascospores, attached to paper fibres through hyphae that could be morphologically related to the Chaetomium genus.

Figure 4b. SEM image of cardboard sample N.I. 9. Crystal structures attached to cellulose fibres and fungal structures.

These features weren’t detected in the unaffected areas of the sample. (Figures S3(a) and S3(b)).
Those first results seem to prove the XRF and LIBS data previously obtained, that showed a greater presence of calcium within foxing stains, probably referred to crystal formation related to micro-organism’s metabolism. Summarising, it is possible to explain the big amount of Na, Mg, Ca and Zn in the foxing spot by assuming that these elements are needed by fungi because of the metabolic role they play, hence they are accumulated during fungi life cycle where fungi colonised paper, and they remain in these places also after fungi death.

3. Experimental
All experimental details are given in the supplementary material.

4. Conclusion
This work shows data of the systematic study of nine cardboards affected by foxing through the elemental composition comparison between affected and unaffected paper areas.

The analytical evidences highlighted that some elements such as Na, Mg, Ca, Zn and Fe characterise the foxing spots in the cardboards. To reach these results, two spectroscopic techniques, namely XRF and LIBS, have been used. The PCA statistical technique has provided a synoptic scheme capable of improving XRF data interpretation concerning the elemental composition of the analysed surfaces, highlighting the difference in elemental distribution in foxed and not foxed areas. PCA results, coming from the analysis of a limited number of data, allowed evaluating the reliability of this analytical approach in interpreting the chemical behaviour of degraded paper surfaces. In the future further data, acquired in several new sample surfaces affected by foxing, will allow evaluating the typology of alteration and any variation over time.

The elemental data obtained by these integrated investigation, confirmed by SEM imaging, suggested the biological nature of these foxing stains.

These first results and procedures appear effective, allowing the investigation of original samples in non-destructive and micro-destructive ways, even though foxing is a complex phenomenon that certainly deserves further investigations, crucial to evaluate on a case-by-case basis the individual causes of the stains.

Acknowledgements
Authors thank Dr Francesco Napolitano, University of Naples, for the microbiological tests.

Disclosure statement
No potential conflict of interest was reported by the authors.

References
Alberghina MF, Barraco R, Basile S, Brai M, Pellegrino L, Prestileo F, Schiavone S, Tranchina L. 2014. Mosaic floors of roman Villa del Casale: principal component analysis on spectrophotometric and colorimetric data. J Cult Heritage. 15:92–97.
Arai H. 1987. On the foxing-causing fungi. ICOM Committee for Conservation: 8th Triennial Meeting; Sydney, Australia; September 6–11; Preprints Marina del Rey: The Getty Conservation Institute; p. 1165–1167.
Attas M, Cloutis E, Collins C, Goltz D, Majzels C, Mansfield JR, Mantsch HH. 2003. Near-infrared spectroscopic imaging in art conservation: investigation of drawing constituents. J Cult Heritage. 4:127–136.

Beckwith TD, Swanson WH, Tiams TM. 1940. Deterioration of paper: the cause and effect of foxing. Univ Calif Publ Biol Sci. 1:299–356.

Bicchieri M, Pappalardo G, Romano FP, Sementilli FM, De Acutis R. 2001. Characterisation of foxing stains by chemical and spectrometric methods. Restaurator. 22:1–19.

Bicchieri M, Ronconi S, Romano FP, Pappalardo L, Corsi M, Cristoforetti G, Legnaioli S, Palleschi V, Salvetti A, Tognoni E. 2002. Study of foxing stains on paper by chemical methods, infrared spectroscopy, micro-X-ray fluorescence spectrometry and laser induced breakdown spectroscopy. Spectrochim Acta Part B. 57:1235–1249.

Cain CE, Stanley MB, Roberts WH. 1987. Paper foxing: biochemical effects of fungal infections of paper. J Miss Acad Sci. 32:24–30.

Daniels VD, Meeks ND. 1988. Foxing caused by copper alloy inclusions in paper. Proc Symp, Ottawa. 88:229–233.

Florian ML. 1997. Heritage eaters. Insects and fungi in library collections. London: James & James.

Gioventù E, Lorenzi PF, Villa F, Sorlini C, Rizzi M, Cagnini A, Griffio A, Cappitelli F. 2011. Comparing the bioremoval of black crusts on colored artistic lithotypes of the Cathedral of Florence with chemical and laser treatment. Int Biodeterior Biodegrad. 65:832–839.

Hey M. 1983. The antiquarian book. Mon rev. 10:341. In Ardelean E, Melniciuc Puică N. 2013. Conservation of paper documents damaged by foxing. Eur J Sci Theol. 9:145–154.

Iiams TM, Beckwith TD. 1935. Notes on the causes and prevention of foxing in books. Libr Q. 5:407–418.

Luciano G, Leardi R, Letardi P. 2009. Principal component analysis of colour measurements of patinas and coating systems for outdoor bronze monuments. J Cult Heritage. 10:331–337.

Lulloff SJ, Hahn BL, Sohnle PG. 2004. Fungal susceptibility to zinc deprivation. J Lab Clin Med. 144:208–214.

Moropoulou A, Polikreti K. 2009. Principal component analysis in monument conservation: three application examples. J Cult Heritage. 10:73–81.

Neevel JG, Mensch CTJ. 1999. The behaviour of iron and sulphuric acid during iron-gall ink corrosion. 12th Triennial Meeting, Lyon; August 29–September 3; ICOM-CC (Vol. 2); London: James & James; p. 528–533.

Nol L, Henis Y, Kenneth RG. 1983. Biological factors of foxing in postage stamp paper. Int Biodeterior Biodegrad. 48:98–104.

Piñar G, Tafer H, Sterflinger K, Pinzari F. 2015. Amid the possible causes of a very famous foxing: molecular and microscopic insight into Leonardo da Vinci’s self-portrait. Environ Microbiol Rep. 7:849–859.

Pinzari F, Zotti M, De Mico A, Calvini P. 2010. Biodegradation of inorganic components in paper documents: formation of calcium oxalate crystals as a consequence of Aspergillus terreus Thom growth. Int Biodeterior Biodegrad. 64:499–505.

Reissland B. 1999. Ink corrosion: aqueous and non-aqueous treatment of paper objects – state of the art. Restaurator. 20:167–180.

Staats CC, Kmetzsch L, Schrank A, Vainstein MH. 2013. Fungal zinc metabolism and its connections to virulence. Front Cell Infect Microbiol. 3 Article. 65:1–7.

Tranchina L, Basile S, Brai M, Caruso A, Cosentino C, Micciché S. 2008. Distribution of heavy metals in marine sediments of Palermo Gulf (Sicily, Italy). Water, Air, Soil Pollut. 191:245–256.

Tzeng DY, Berns RS. 2005. A review of principal component analysis and its applications to color technology. Color Res Appl. 30:84–98.