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Microscopic observations of paper and parchment: the archaeology of small objects

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Abstract

The study of micro-objects, both inorganic and organic, which can be found associated to parchment or cellulose fibres of books and documents, or in the inks and the dirt, accumulated over the centuries between the pages, can unravel important information on the manufacture of the ancient documents, on their past vicissitudes or on the causes of aging and deterioration of the materials they contain. The results of a critical observation of micrometric objects and structures with a diagnostic or archaeological significance have in some cases been surprising. Apart from the curiosity that certain findings may or may not raise, what needs to be discussed is the fact that some chemical components, usually attributed to the manufacture of materials or the inks may instead have a particular origin and nature, and therefore lead, if not properly highlighted, to some erroneous diagnostics. Examples are given where the information obtained by means of scanning electron microscope imaging and Raman microscopy were coupled with dispersive X-ray microanalysis and Fourier Transform Infrared Spectroscopy.

Keywords: SEM–EDS, Parchment, Paper, Phytoliths, Raman, Manufacture, Clay, Silicon, Metals, Impurities

Introduction

The cultural role played by parchment and paper in human history has been immensurable: manuscripts, ancient books and archival materials are the most important repositories of our recorded history from early times. The awareness of the historical information that the ancient documents can still transmit has increased in the last decades along with a deeper collaboration among researchers, scientists, conservators and heritage managers. Archaeological investigation of written heritage, especially ancient and medieval books can, in fact, uncover valuable clues for a proper understanding of the historical, social and political context, in which they were made, circulated and were exchanged, and the people who read and owned them. This is one, among the many reasons, why it is important, even in the digital age, to preserve the materiality of archival and book heritage [1–5].

Books, documents, and manuscripts are predominantly made up of paper, parchment or both. The covers or protective parts can contain leather, wood, fabrics, plastic materials and metals, but generally represent a small part of the objects. There are, however, also many minor ingredients or compounds accidentally or purposely used or deposited in the materials that can be highlighted with new or particularly accurate analytical techniques, and which might give information on the history, manufacturing or conservation state of the objects [4, 6, 7].

This work aims to show the importance of the study of micro-objects or singular compounds, both inorganic and organic, which can be found among parchment or cellulose fibres of books and documents, objects that can tell a story about the materials or the vicissitudes of the same documents over the centuries.

A further objective of this work is also to emphasize the value of comparative studies carried out by conservation, chemistry and biology laboratories. The complexity in

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the interpretation of the results of some diagnostic analyses highlights the need of a close collaboration between the different competences, since often only the complementarity of knowledge leads to new findings [8–10].

Materials and methods

Analysed samples

The images and data reported in this study come from a selection of paper and parchment samples and manuscripts that have been analysed over a period of at least 10 years at the "Istituto centrale per il restauro e la conservazione del patrimonio archivistico e librario (Icrcpal)" in Rome. The considered case studies are reported below:

1. Chinese paper made from fibres of rice straw and bamboo from a 1732 printed book (Wang Yaoqu's Combined Explanations of Tang Poetry).
2. Parchment samples from an Ethiopian manuscript from the "Accademia Nazionale dei Lincei e Corsiniana" Conti Rossini Et. 70 (Psalter, eighteenth to nineteenth century).
3. Ethiopic manuscript made of parchment (Crypt. AET.7, second half of seventeenth century) belonging to the Library of the Abbazia di Grottaferrata, Italy.
4. Paper samples from an Italian eighteenth century printed Bible.
5. Japanese paper removed from the back of a drawing on paper (Pio Semeghini ca.1930).
6. Arabic paper from a manuscript dated back to 1293 from La Spezia's Notaries Public Archive, Italy.
7. Spanish paper from fourteenth century.
8. Modern A4 copy-paper sized with calcium carbonate.
9. 16th century parchment archival manuscript from the collection "Le Grazie" (Venice, Italy), volume n. 15.

Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS)

Paper and parchment samples or, when possible, whole pages, were examined uncoated, using a variable pressure SEM (EVO50, Carl Zeiss AG, Germany) equipped with a backscattered electrons (BSE) and detector. The paper and parchment fragments, when not repositionable in the original object and ranging between a few mm and less than 1 cm, were placed on a sulphur-free carbon adhesive (Spectrotabs AGG3358, Agar Scientific) glued onto an aluminium stub (1.25 cm diameter, Agar Scientific). Whole pages and large fragments were fixed reversibly on cardboard supports (Additional file 1: Figure S1). Chemical analysis was performed by means of EDS (INCA 250, Oxford Instruments). The SEM was fitted with a tungsten filament and operated at 20 keV, with an average working distance of 12.5 mm, with a chamber pressure between 30 and 150 Pa. The electron beam current was adjusted (~ 400 pA) to generate sufficient X-rays to allow reliable identification of EDS peaks, minimise spectral artefacts and achieve detector dead times < 20%. The beam spot size was 5 to 30 nm in diameter. Acquisition live time for single analysis was set at 50 s. In these conditions, we did not observe beam damage in most of the analysed spots. Conventional ZAF correction (for atomic number Z, absorption and fluorescence) was applied, integrated into the Oxford INCA 250 microanalysis software used [11–14].

Statistical analysis of EDS chemical data

The EDS measurements were analysed using statistical tests to evaluate the significance of the differences between objects and background. One-way analysis of the variance (ANOVA) was applied and the significance of the differences was tested at 95% confidence. ANOVA was followed by a post hoc analysis using Tukey's t-test to determine the critical value for significance. ANOVA, was performed using XLSTAT 2017 software (Addinsoft, Paris, France).

Raman analysis

Measurements were performed by means of a Renishaw In-Via Reflex Raman microscope equipped with a Renishaw diode laser at 785 nm (nominal output power 300 mW). Neutral filters were used to avoid sample degradation, reducing the power on sample from 0.03 to 0.3 mW, depending on the characteristics of the sample. The backscattered light was dispersed by a 1200 line/mm grating and the Raman signal was detected by a Peltier cooled (−70 °C) deep depletion charge-coupled device (CCD RD-VIU, 578 × 384 pixel) optimized for near-infrared and ultraviolet. The nominal spectral resolution obtained for the measurements was about 3 cm^{-1}. The system, equipped with a Leica DMLM microscope to focus the laser on the sample and a colour video camera, allows for the positioning of the sample and the selection of a specific region for the investigation. Spectral acquisitions (1–5 accumulations, 50 s each) have been performed with a 50× objective (N.A. 0.75), with a laser spot measuring about 20 $\mu\mathrm{m}^2$. Samples were analysed without any preparation. The analyses were performed on different areas of the samples.

Fourier Transform Infrared Analysis (FTIR)

The Fourier Transform Infrared (FTIR) spectroscopy measurements were performed in a micro-destructive way: tiny sample fragments from the cover not repositionable in the original object (about 0.5 mg each) were embedded in KBr tablets and measured with a Nexus Nicolet interferometer, equipped with a KBr beam splitter and Deuterated TriGlycine Sulfate (DTGS) detector with KBr window. The measurements were accomplished in the 4000–450 cm^{-1} range with a resolution of 4 cm^{-1}, averaging 400 acquisitions on each sample.

Results and discussion

Both paper and parchment contain, in variable percentages, inorganic compounds. Salts coming from manufacturing processes, sizing minerals or impurities, like metals from inks made with Cu and Fe, mineral compounds related to the use of pigments (i.e. orpiment, cinnabar, white lead, etc.) or from different origins are present in most of the library materials. The presence of minerals embedded in an organic matrix can create a patchy microenvironment where localised chemical reactions can produce a complex range of unwanted discolorations and damage, difficult to analyse and manage [4, 8, 15, 16]. The presence of mineral compounds potentially harmful to paper or parchment materials can be ascertained with various quantitative techniques [1]. However, the nature and origin of certain minerals, as well as their degree of dispersion in the organic matrix can be difficult to evaluate and, sometimes, represents an important and decisive element with respect to the interpretation of deteriorating phenomena. Among the diagnostic tools particularly valid in the study of additives and impurities in paper and parchment is the scanning electron microscope equipped with a "backscattered" electron detector (BSD), which reconstructs a sample image based on the number atom of the chemical elements present in it [2, 3, 8]. This represents a significant advancement for the observation of the surface of writing media, since the obtainable image accounts for both the topography of the fibres used for manufacturing and the material charge which, having a different composition, is highlighted through the contrast of atomic number. This allows an immediate localization of mineral particles that can be characterized by their elementary chemistry composition through microanalysis (EDS) [11, 12].

The presence of metals in paper can be due to many causes, from the use of metal-contaminated water to the handling of rusty moulds or the sizing with minerals like kaolin or carbonates containing iron, copper or other chemically reactive elements [6, 17, 18].

To know if the metallic impurity has been present since the cellulose pulp preparation or if it comes from successive phases of manufacture can have a certain importance. With SEM observations it is possible to know, for example, if the mineral is mixed with fibres or juxtaposed to them, if it is uniform or composite, if it is present in a dispersed form or whether it is concentrated in single isolated points. Figure 1 shows three examples of large (> 100 μm) metallic particles in paper. The particle in Fig. 1a contains mainly Cu and is completely embedded by cellulose fibres, suggesting that is was present in the pulp, Fig. 1b shows a cluster of mineral material containing mainly iron but also calcium and silicon. It is a material juxtaposed with the fibres and pressed onto them, presumably deriving from phases following the preparation of the sheet, and not present in the pulp. The large mineral in Fig. 1c is incorporated in the sheet and is made of Fe and Mn in the form of flat crystals. The metallic component was found to be associated to Ca and Si material, perhaps purposely added to the sheet as sizing treatment. The use of sizing minerals rich in metal impurities is often at the origin of severe damage occurring to paper [15, 16]. However, the fact that the mineral is embedded and partly covered by cellulose fibres suggests that its presence is to be associated with the preparation of the cellulose mixture and the making of the sheet, rather than to the accidental superficial deposition or gluing, subsequent to its manufacture.

In paper manufacturing, many compounds are added to increase opacity or whiteness of the support. Kaolin, $Al_2Si_2O_5(OH)_4$, is the most common form of clay added to paper as a filler. The first documented use of clay in European papermaking is reported in England in 1807 [6]. It can also be used in coating with particles that are less than two micrometres in diameter. According to the locations where clay is extracted the quality can vary considerably and can contain impurities, that should be carefully removed, in particular iron compounds, to avoid the deterioration of paper caused by chemical factors [15].

Ferruginous and titano-ferrous minerals are the common impurities found in kaolin and iron can be present in clays used in papermaking as oxides, hydroxides, oxy hydroxides, sulphides and carbonates. The relationship between the total iron content in the kaolin, the structural order of the iron species and the surface reactivity of kaolin can represent important variables that determine the potential damage to paper.

However, the silica present in the paper does not always come from the filler material. Figure 2 shows some curious origins of silicon-based compounds that can be found in paper. They range from siliceous frustules of diatoms, as in Fig. 2a, a species of unicellular algae in the class of Bacillariophyceae [19], coming either from the water used in manufacturing or from sizing material composed of diatomaceous earth [20]. Silicon can also be present in abundance as a component of the plant fibres themselves. Many herbaceous plant species, used in the manufacture of paper, have strongly silicified cells, such as rice straw and bamboo, widely used in Chinese paper [21], as shown in Fig. 2b, c. Phytoliths are considered as

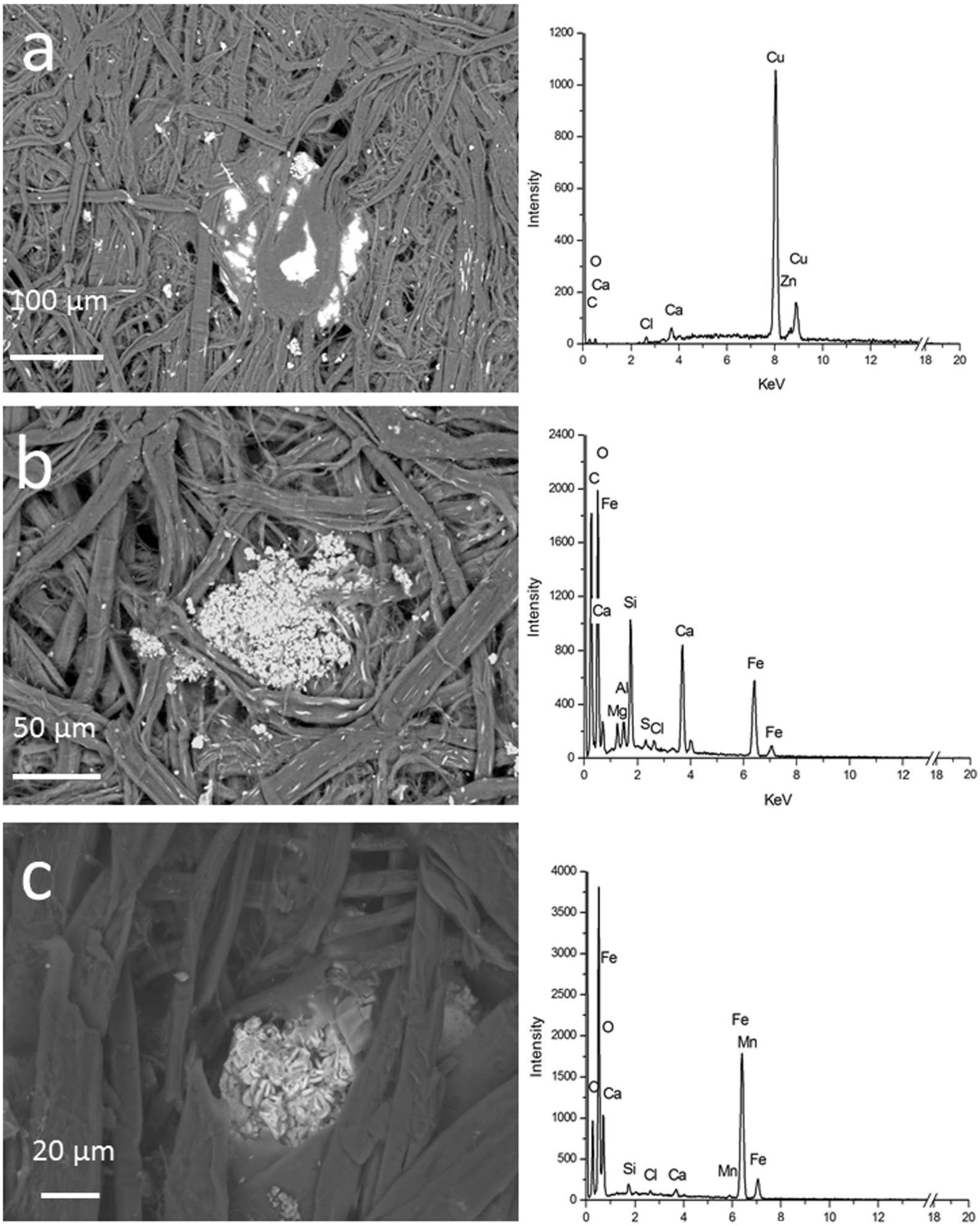

Fig. 1 SEM-BSD image (left) and EDX spectrum (right): **a** Paper samples from an Italian 18th century printed Bible; **b** Spanish paper from 14th century; **c** Japanese paper from the back of a drawing on paper (Pio Semeghini ca.1930)

promising for identifying and distinguishing different sources of fibre in handmade papers. In Chinese traditional papers the presence and diversity of phytoliths can be diagnostic of both the use of specific plant material and the processes used in papermaking [22]. Relatively large particles of rocks containing silicon, magnesium, aluminium and often Fe can be found also in ancient paper where no mineral sizing was used. The manufacture of Arabic paper for example required in the past the preparation of a glossy surface which was achieved through burnishing the sheets with smooth stones, often made of agate or glass [23]. The fibres were compacted reducing the air within the fibres thus creating a smooth, quite reflective surface. This treatment could result in the

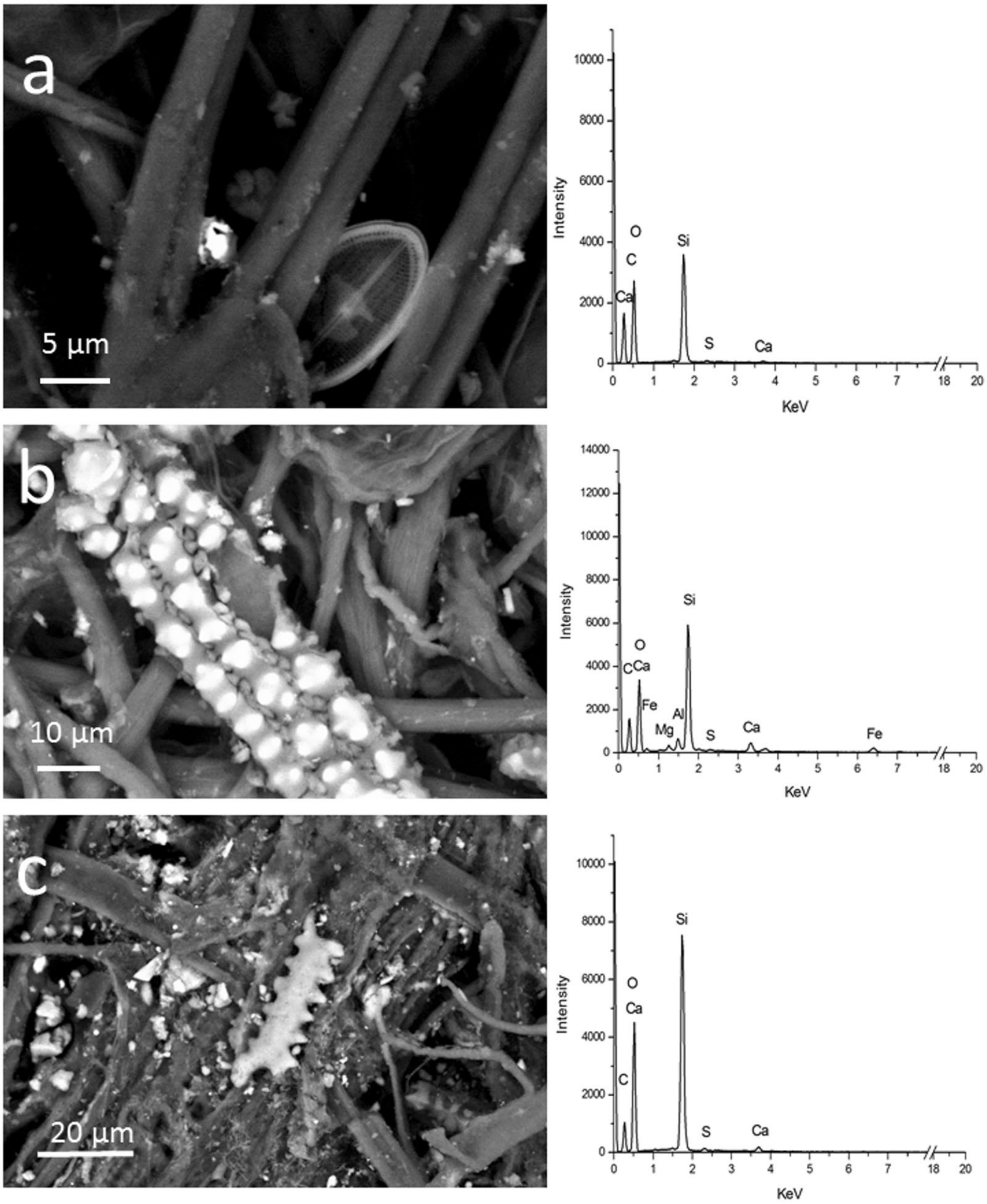

Fig. 2 SEM-BSD image (left) and EDX spectrum (right). Images from different samples of Chinese paper made from fibres of rice straw and bamboo fibres from a 1732 printed book Poetry Tang period (Wang Yaoqu's Combined Explanations of Tang Poetry), **a** the siliceous shell (frustule) of a diatom (unicellular algae), **b** the silicified epidermis of bamboo leaves, used in paper manufacture, **c** the silicified epidermis of rice leaves, used in paper manufacture

release of rock fragments into the paper, as showed in Fig. 3a, b, where the visibly flattened surface of the Arabic paper embeds aluminosilicate stones that have also some iron in their composition.

Another widely used filler material is calcium carbonate [17]. Even in this case, however, from the chemical analysis of a sheet of paper it is generally not easy to trace the type of material added in the manufacture or its origin. Figure 4 shows some examples of calcium-based minerals present in paper. Apart from natural or synthetic calcite, commonly added in modern paper as a filler, it is possible to find paper manufacturers that used also carbonates from natural sources, such as fossil sedimentary rocks (i.e. biomicrite) in which it is still possible

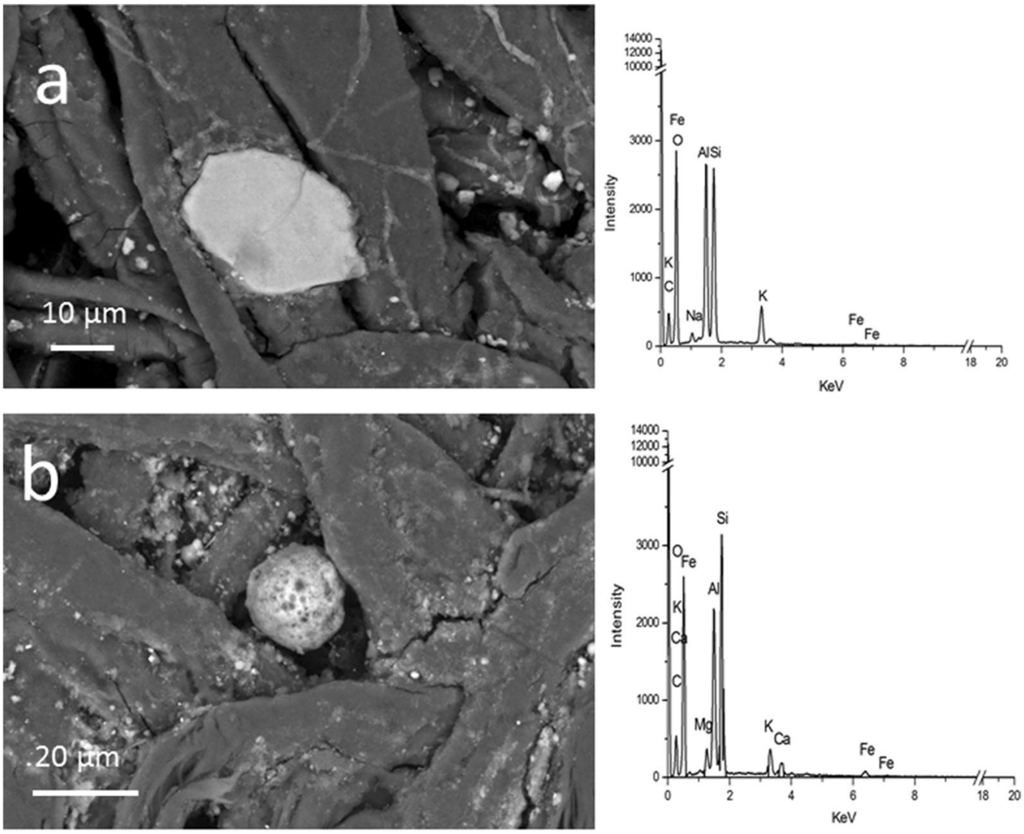

Fig. 3 SEM-BSD image (left) and EDX spectrum (right). Arabic paper from a manuscript dated back to 1293, **a** a stone embedded in the fibres after the smoothing of paper's surface with a stone or other tool, **b** residues of rocks used for surface smoothing among paper's fibres

to recognize some microfossils (Fig. 4a). Calcium, like silicon, can also be constitutive of the plant fibres used as cellulose sources [21]. Figure 4b shows ordered rows of druses of calcium oxalate among the fibres of paper (mulberry, *Morus* sp.). Calcium oxalate crystals occur in several higher plant families including gymnosperms and angiosperms [24]. The shape, size and number of crystals show variations among taxa and they have been classified into five main groups based on their morphology: as prism, druses, styloids, raphides and crystal sand. These crystals can be located in specific tissues such as epidermis, cortex, phloem, xylem and pith. Calcium oxalate crystals in plants can have different roles (storage of calcium, removal of excess metabolic oxalate, metal detoxification, tissue support, protection against insects and foraging animals). The druses of Fig. 4b seem produced by the plant in the xylem, as a system to block the passage of water [24]. These biogenic minerals are very stable and resist to pulping processes during paper manufacture.

Oxalate crystals in paper can have also a very different origin (Fig. 4c). Fungal interaction with the carbonates added to paper, for example, produces at a micrometric scale a replacement of the original minerals with newly formed crystals [25]. Oxalate production is caused by the metal-binding capacity of the organic acids generated by the fungi. These, together with the presence of hydrogen ions, cause substrate mineral hydrolysis and new mineral formation through metal–acid complexation. The action of these acids is combined with the action of fungal respiratory CO_2, which generates carbonic acid in the growth environment. It is hypothesized that the combined action of these two acid groups produce the dissolution pattern observed on the calcium-containing materials, and that a subsequent spontaneous re-crystallization of fungal oxalic acid occurs with the Ca^{++} ions. The effects of calcium oxalate precipitation among cellulose fibres, and the consequent diminishing of the alkaline reserve in paper can represent a cause of chemical and mechanical damage [26]. The presence of not well identified "crystals" on ancient paper from manuscripts was described by some authors as associated to biodeteriorated materials [27, 28] suggesting that this phenomenon occurs naturally on library materials when spoiled by fungi that produce organic acids.

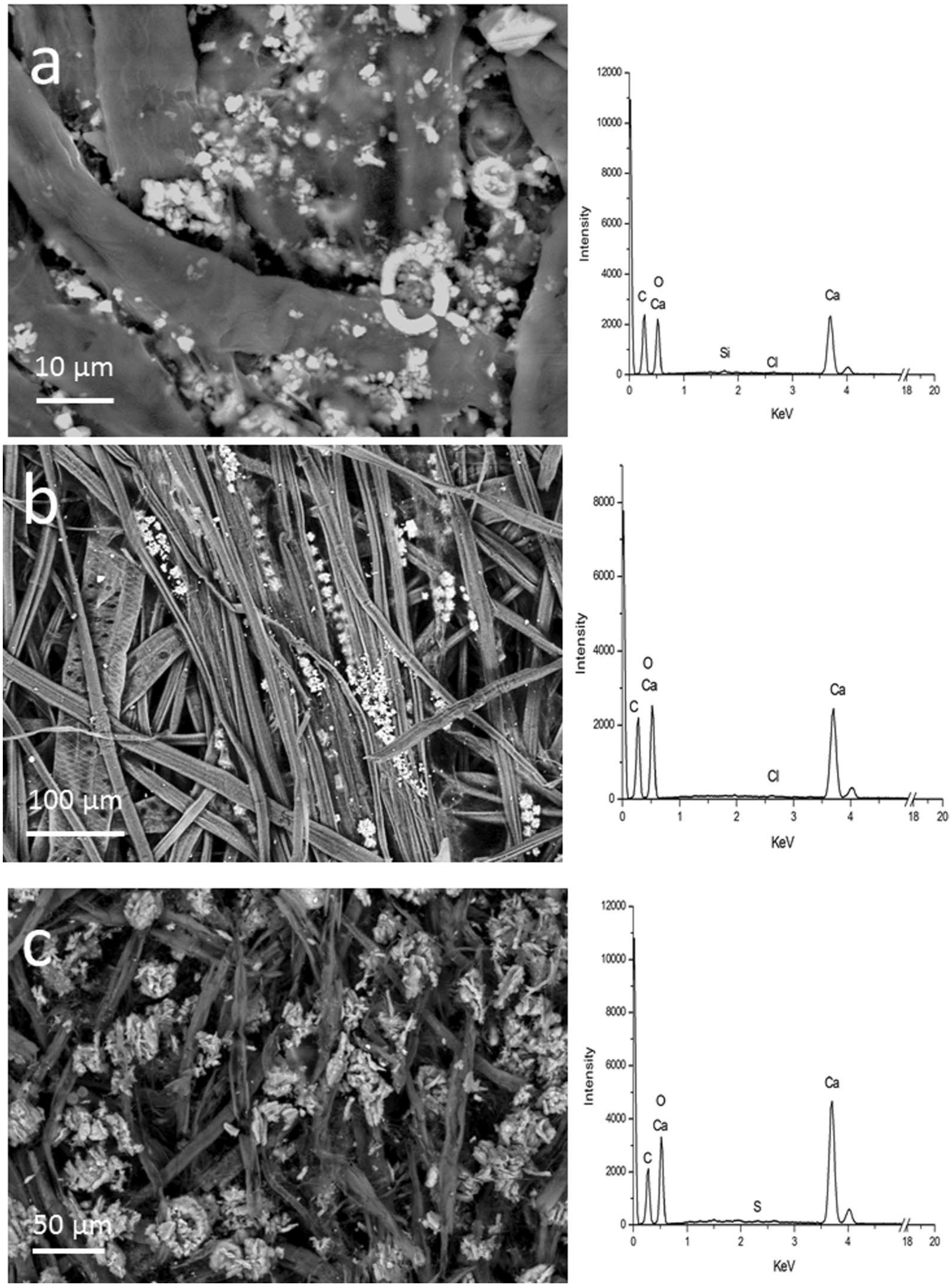

Fig. 4 SEM-BSD image (left) and EDX spectrum (right) of the bright mineral objects. **a** Modern A4 copy-paper sized with calcium carbonate from biomicrite or other fossil sedimentary rock, **b** Japanese paper made of mulberry fibres and containing calcium oxalates in the vessels, **c** Modern A4 copy-paper sized with calcium carbonate, after a fungal attack that tuned the carbonate into oxalate

The study at a microscopic scale of manuscripts and documentary materials holds surprises also when they are not made of paper, but of parchment. Parchment is prepared from animal hides [7, 29] with alkaline salts like lime, and NaCl. The main constituent of parchment is collagen, a structural organic polymeric protein whose structural unit is a triple-helix. During parchment manufacture, animal skin undergoes a series of procedures resulting in a product composed of the dermal skin layer only [30]. Finished parchments contain also other compounds that derive from the same skins (lipids, waxes, other peptides) and from manufacturing processes (salts and minerals, such as Al, Si, Mg, K, S, etc.).

After the fourth century dehairing was achieved by immersing the animal skins in a calcium hydroxide solution [31]. Calcium-based compounds were also used to make the surface good for writing, with lime, flour, egg whites and milk added on the surface to make it smooth. From the recipes reported by ancient texts and from the studies carried out so far, we know today different types of parchment preparations. Each culture has introduced variants that have led to quite different materials. Ethiopian parchment, for example, has morphological features that differentiate it from Western or Jewish parchments (Fig. 5).

The Ethiopian manufacturing process [32–34] do not involve lime bath or different chemical treatments: skins were only scraped on both sides, after they were stretched on a frame. The flesh side, wet or dry depending on the production areas, was cleaned with a knife and a stone, as well as the hair side. The last step involved washing and cleaning treatments on both sides, using knives, pumice, calcite, other stones, and vegetal soaps (mainly obtained from the berries of *Phytolacca dodecandra*, commonly known as African soapberry) until the surfaces were smooth and white as paper [35–37]. The different manufacturing method and absence of huge amounts of inorganic compounds on the surface is evidenced by the SEM images in Fig. 5, where a Western parchment is compared to an Ethiopian one. In order to obtain a shiny surface and to increase the parchment's resistance to microorganisms, sometime castor oil was added to the extract of *Phytolacca*.

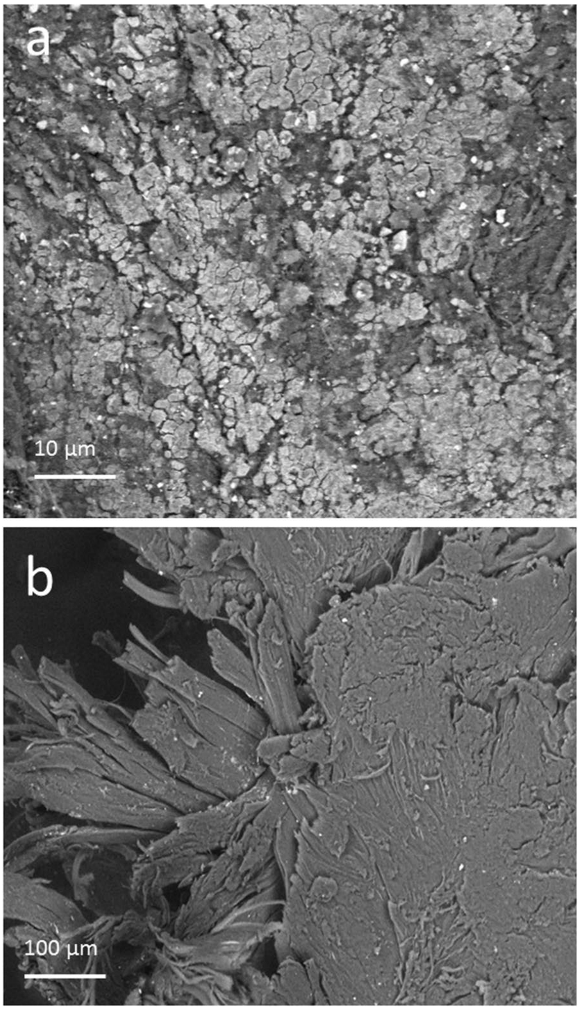

Fig. 5 SEM-BSD images. Comparison between **a** Western and **b** Ethiopian parchments, SEM images. The Western sample was from a 16th parchment archival manuscript (sample n. 9), while the Ethiopian parchment sample was from the seventeenth century sample Crypt.AET.7 (sample n. 3)

An Ethiopic manuscript (Crypt.AET.7, second half of seventeenth century) belonging to the Library of the *Abbazia di Grottaferrata* was analysed at the Icrcpal chemistry laboratory. Raman analyses were performed with a Renishaw In-Via Raman spectrometer with laser excitation in the near infrared (λ = 785 nm), but they were not indicative, being all spectra affected by a strong fluorescence background, induced by the shiny surface of the parchment, that had been treated with some organic compound. The images collected under the Raman microscope identified the presence of a yellow compound deposited on the surface (inset in Fig. 6), which has been recognized by FTIR, as an admixture of saponins and castor oil. This confirms the literature on the finishing surface treatments to which Ethiopian parchments were subjected. Figure 6 shows the typical FTIR spectrum collected from the analysed manuscript, arising from the superimposition of the parchment substrate, saponins [38], and castor oil. The attributions of the peaks to a specific vibration and the different compounds responsible for the vibration are reported in Table 1. Peaks are assigned in accordance with references [39–42].

Still in an Ethiopian parchment, but coming from a collection of manuscript belonging to the "Accademia Nazionale dei Lincei e Corsiniana" micro-objects with a spherical shape have been observed in fair amounts, clustered in drops of a few millimetres in diameter (Fig. 7a, b), made of P, Mg, Ca and K (Fig. 7a). These were recognised as spherulites which are biogenic stones that form in the gut of insects as a result of their catabolism and are indicators of insects' droppings.

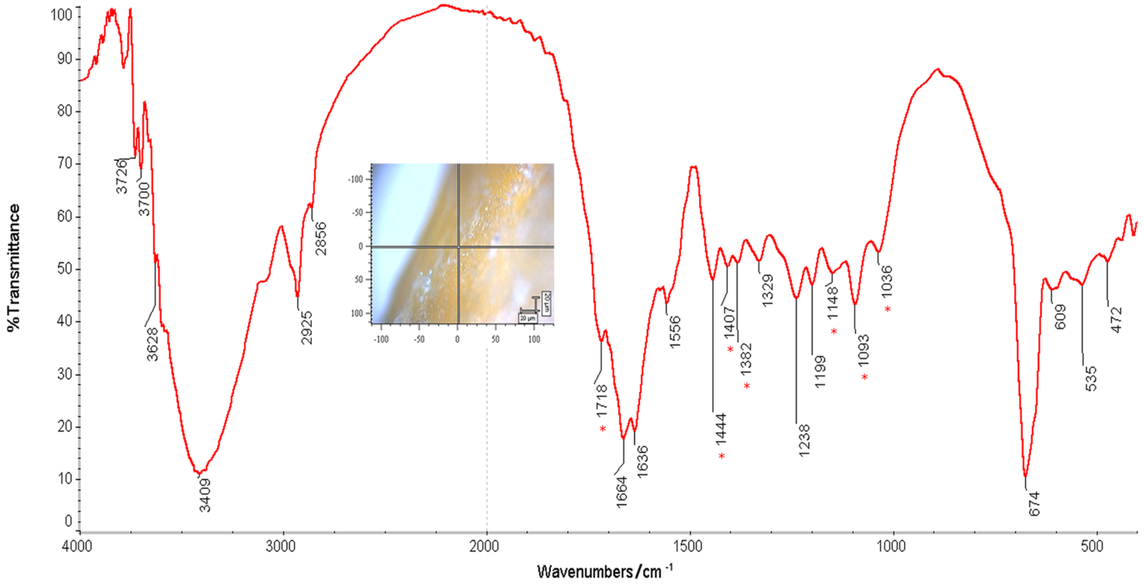

Fig. 6 FTIR spectrum in KBr of a parchment sample from the Crypt.AET.7 manuscript. The inset reports one image collected under Raman microscope of the yellowish compound deposited on the surface of the parchment. The most important peaks related to the presence of saponins and castor oil, assigned in Table 1, are marked with asterisks

Table 1 Possible assignment of the FTIR peaks of spectrum in Fig. 6

Wavenumber [cm^{-1}]	Group vibration	Compound responsible for vibration
3726; 3700	Free OH stretching	Inorganic compound (iron clay)
3409	OH bonded	Parchment, saponins, castor oil
2925, 2856	CH_3, CH_2 stretching	Parchment, saponins, castor oil
1718	C=O stretching	Saponins
1664	Amide I β sheet	Parchment
1636	Amide I α sheet	Parchment
1556	NH bend coupled with CN stretching	Parchment
1444, 1407, 1382	Scissoring of, O–C–H, C–C–H, CH_2, C–OH, CH_2 wagging, bending C–H	Parchment, saponins, castor oil
1328	Amide III	Parchment
1238	NH bending Amide III, C(O)–O stretching	Parchment, castor oil
1199	C–H bending	Parchment
1148	OH bending	Parchment, saponins, castor oil
1093, 1036	C–O stretching	Parchment, saponins, castor oil
674, 609, 535, 472	Not attributed	Skeletal vibration and inorganic compound related to free OH stretching

Spherulites typically show concentric laminations composed of compact and loosely packed strata alternately. Although the exact composition of these minerals could not be detected, since some of the elements were present also in parchment (like Ca and Mg), they showed a significantly higher content of P, Mg, Ca and K compared to the background (Table 2). Though EDX was inadequate to determine the accurate chemical formula of the spherulites, the molar ratios of Ca/P and Mg/P allows to hypothesise that they are mainly made of calcium phosphate and magnesium phosphate, with potassium as an additional but secondary cation.

These spherical micro-objects are used in archaeology as markers of the presence or use of animals' dung [43]. Among the treatments used in the past for depilating the skins there were also the so-called enzymatic treatments

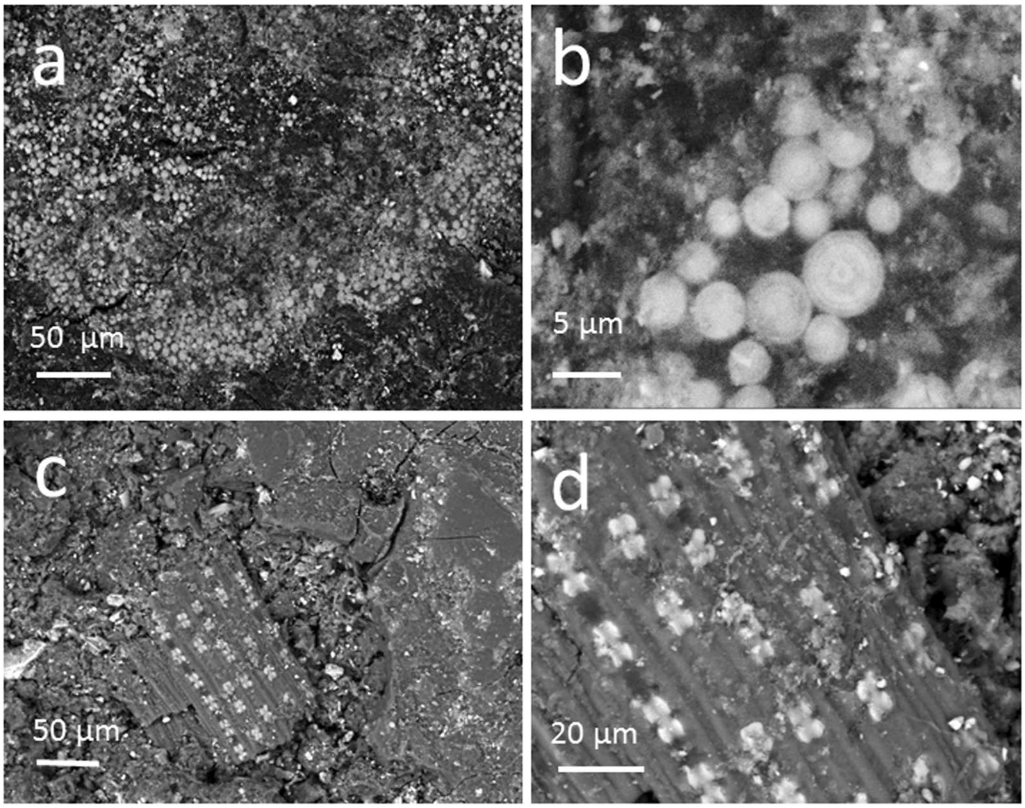

Fig. 7 SEM-BSD images from an Ethiopian manuscript from the "Accademia Nazionale dei Lincei e Corsiniana" Conti Rossini Et. 70 (Psalter, eighteenth to nineteenth century), **a**, **b** spherulites mainly containing P and Ca (Table 2), **c**, **d** portion of the epidermis of a plant leaf belonging to the Poaceae family, with embedded siliceous phytoliths. Figures b and d are enlarged views of specific areas of figures a and b

Table 2 Concentration (weight %) of major chemical elements in the spherulites and in the background

Element	Spherulites	Background	*p*
C	47.75	58.52	0.0379
O	43.10	38.95	0.2675
Mg	2.41	0.24	*0.0002*
Al	0.45	0.31	0.1568
Si	1.21	0.85	0.1075
P	3.01	0.26	*< 0.0001*
S	0.05	0.16	0.0825
K	0.61	0.14	*0.0035*
Ca	1.20	0.48	*0.0016*
Fe	0.22	0.08	0.1838

that included the use of animal's manure and dung [31]. It cannot therefore be excluded that the spherulites can represent a trace of the manufacturing treatments used in the making of that Ethiopic parchment.

The repeated EDS measurements on spherulites and parchment background were compared with ANOVA and shown in Table 2, where the elements in italic are present in significantly different concentration ($p < 0.005$) between background and main objects (spherulites).

Among the microscopic objects found on the Ethiopic parchment from Corsiniana Library there were also bilobate phytoliths that have been found in several places and on different pages of the manuscript, almost always associated with the epidermis of the leaves or stem of the respective herbaceous plant from the Poaceae family (Fig. 7c, Table 3). According to Ref. [43] the combination of spherulites and phytolith can be a reliable proxy for the inference of dung presence in archaeological contexts. This fact could therefore confirm that the Ethiopian parchment was treated with the dungs of herbivorous animals in a phase of its manufacture, presumably in the process of depilation. However, a second hypothesis cannot be completely excluded, namely that the fragments of herbaceous material containing the phytoliths comes instead from the ink, that resulted to be made of carbon and therefore, perhaps, produced by burning local weeds. In fact, the fragments of herbaceous material have been

Table 3 Concentration (weight %) of major chemical elements in the phytoliths and in the background

Element	Phytoliths	Background	*p*
C	43.91	61.47	0.0010
O	40.37	31.84	0.0041
Na	0.00	0.38	< 0.0001
Mg	0.38	0.26	0.3268
Al	0.37	0.30	0.8264
Si	10.82	1.70	0.0008
P	0.33	0.37	0.8206
S	0.23	0.46	0.1857
K	1.89	2.20	0.5813
Ca	0.91	0.79	0.7344
Fe	0.80	0.24	0.3726

The repeated EDS measurements on phytoliths and background were compared with ANOVA and are shown in this table where the elements in italic are present in significantly different concentration ($p < 0.005$) between background and main objects (phytoliths)

repeatedly found in correspondence of the writing. However, given the difficulties of observation (the pages of the manuscript were introduced entirely into the microscope chamber as shown in Additional file 1: Figure S1) it was not possible to accompany the observations of the phytoliths with a statistical treatment of the data.

Conclusions

This contribution is certainly not exhaustive of the kinds of unusual objects that are possible to find among the pages of documents and manuscripts, but it gives an idea of the type of findings that can be done observing surface details at a microscopic level or analysing chemical and structural aspects which are not immediate or obvious. Apart from the interest that certain findings may or may not raise, what should be discussed is the fact that some chemical components, often attributed to the manufacture of supports or inks may instead have a particular origin and nature, that can lead, if not properly highlighted, to some erroneous attributions. For example, if the silica present on a paper is attributed to the minerals used in the sizing, while instead it is constitutive of the fibres, as in the case of strongly silicified cells of some plants, it is easy to arrive at a misdiagnosis on paper manufacture. In light of the new diagnostic techniques and the power that some microscopic details may have, some considerations on the outcome that certain cleaning and consolidation protocols may have on the informative content of ancient documentary materials are now mandatory. Though it is very hard to establish rules in the area of diagnostics, since valuable heritage exists in the form of unique objects that cannot be the subject of any kind of standardisation, a new type of archaeology of ancient artefacts could arise from a wiser collection and conservation of the waste resulting from restoration treatments, or from a skilful combination of cleaning and sampling techniques.

Additional file

Additional file 1: Figure S1. SEM analysis of a dismounted page from the Ethiopian manuscript from the "Accademia Nazionale dei Lincei e Corsiniana" Conti Rossini Et. 70. When the dimensions of the objects under study allows it, the environmental SEM operated at atmospheric pressure can be used as a not-invasive technique to observe whole samples or parts that conservators can then reassemble.

Abbreviations

SEM: scanning electron microscopy; EDS: energy dispersive X-ray spectroscopy; ANOVA: analysis of variance; CCD: deep depletion charge-coupled device; FTIR: Fourier transform infrared (spectroscopy); DTGS: Deuterated TriGlycine Sulfate.

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Authors' contributions

FP and MB planned the experiments and took the lead in writing the manuscript. PB and PC contributed to carry out the experiments and the interpretation of the results. All authors provided critical feedback and helped shape the research, analysis and manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Datasets supporting the results and hypotheses reported in the article, along with further SEM images and EDX spectra on the same samples, are available to scholars on motivated request to the authors.

Competing interests

The authors declare that they have no competing interests.

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