

Original article

The hidden arsenic legacy in mid-19th century papered microscope slides

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ABSTRACT

This study investigated the presence of arsenic in Victorian-era microscope slides, particularly those containing green pigments. X-ray fluorescence (XRF), Raman, Surface Enhanced Raman Spectroscopy (SERS) and Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) were employed to analyse the elemental composition of pigments on the slides. The analysis identified arsenic primarily in green pigments containing emerald green but also in some red pigments.

To assess the prevalence of slides potentially containing arsenic, a separate analysis was conducted examining the colour of slides in existing collections. Across two data sources, focusing on slides with accompanying images, around 10 % of slides were identified to contain green pigment. Extrapolating this prevalence to UK collections suggests a potential for hundreds of thousands of slides to contain arsenic, posing potential health risks during handling and raising concerns about proper disposal practices.

This research highlights a previously unrecognised occupational and environmental hazard associated with historical slides. The study emphasises the importance of responsible handling and disposal practices for slides containing arsenic pigments, particularly those coloured green and red, to minimise health and environmental risks. We propose guidelines for safe handling and disposal practices, alongside outreach activities to raise awareness among stakeholders.

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1. Introduction

1.1. The birth of the microscope slide

The first microscope slides in the 17th century were made with basic materials such as cork and mica. Unstandardised methods of creating microscope slides continued until the 19th century when a shift towards glass slides began. Glass offered a clearer view and was better suited for the evolving needs of microscopy. By the mid-1800s, Germany emerged as a leader in microscope slide production, with leading companies establishing quality standards and standardisation of size (around 76 × 26 mm) ensuring compatibility with different microscope manufacturers [1].

The 20th century brought advancements in glass quality. Soda lime float glass became the preferred material for its clarity and uniformity. Pre-cleaned and ready to use slides became widespread, saving researchers time.

1.2. Amateur market in the 19th century

The early 19th century witnessed a surge of interest in microscopy, fuelled by advancements in achromatic lenses that offered clearer images. However, commercially produced microscope slides were still in their nascent stages. This gap led to a vibrant and informal amateur market for aspiring microscopists [17]. While general commercial glass production had advanced rapidly during this period, serving a wide range of applications, an established commercial supply of microscope slides was limited and the demand for slides was therefore often served by craftsmen and scientists who cut thin slivers from broken windowpanes or procured small glass squares from glaziers. These were then painstakingly ground and polished to achieve a suitable level of thinness and clarity.

Enthusiasts used readily available materials like water, honey, or even melted animal fat to mount specimens on their slides. These homemade media had limitations in terms of preservation and image quality, but they sufficed for basic observations [1]. Mounting techniques without proper adhesives often lead to specimen movement or degradation over time. Without standardised sizes, these amateur slides would not always fit perfectly within the stage

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Table 1
Microscope slide collections (specifying collection size) in selected UK institutions as of June 2024.

Institution	Size	URL
Natural History Museum	2.5 million	https://www.nhm.ac.uk/our-science/services/collections/digital/programme/microscope-slide-digitisation.html
Kew Gardens	150,000	https://www.kew.org/science/collections-and-resources/collections/microscope-slide-collection
Royal College of Surgeons	50,000	https://www.rcseng.ac.uk/library-and-publications/library/blog/very-large-collection-of-very-small-things/
Grant Museum of Zoology	20,000	https://www.ucl.ac.uk/culture/grant-museum-zoology/micrarium
Hunterian Museum	15,000	https://hunterianmuseum.org/collections/microscope-slide-collection
The Museum of Medicine and Health of Manchester University	15,000	https://herbologymanchester.wordpress.com/tag/microscope-slides/
Distributed System of Scientific Collections (DiSSCo) reports the number of microscope slides across 88 UK participating institutions	8.8 million	https://know.dissco.eu/

holders of microscopes, leading to potential damage or difficulty in observation. Scientific societies like the Royal Microscopical Society (founded 1830) played a crucial role in disseminating information on amateur slide preparation. They published journals with articles detailing techniques and materials for aspiring microscopists. These societies also fostered a culture of exchange, where members could share their experiences and innovations in slide preparation [21]. While widespread commercial production had not arrived yet, a few companies catered to the growing demand for microscopy supplies. Additionally, some scientific instrument shops offered basic tools like polishing stones or small glass plates for amateur enthusiasts.

1.3. Papered microscope slides: a touch of colour

During the middle of the 19th century, alongside the rise of standardised glass slides, a unique practice emerged: the use of papered microscope slides. These slides incorporated a decorative sheet of paper placed on the back and front of the glass slide, often acting as a label or a way to identify the preparer. Black paper was a popular choice, offering a stark contrast that made handwritten white labels stand out clearly. Red paper was another common colour, particularly associated with slides prepared by Charles Morgan Topping of London, a renowned Victorian microscopist. Some slides featured more elaborate designs, utilising marbled paper with swirling patterns in various colours [17]. Occasionally, slide manufacturers used paper featuring their company logo or branding elements.

Two primary functions of the paper were to help retain specimens in place and to provide a surface for writing labels with information about the specimen on the slide. This could include the type of specimen, date of preparation, or even the preparer’s name. For some preparers, the coloured paper might have been a way to add a personal touch or a decorative element to their slides. Additionally, some marbled paper patterns could have offered a subtle background that enhanced the viewing experience [20].

While papered microscope slides were aesthetically interesting, they had some drawbacks. The paper could obscure the edges of the specimen under the microscope, hindering observation. Additionally, paper was prone to deterioration over time, especially when exposed to moisture or chemicals used in some mounting techniques. By the late 19th century, advancements in labelling techniques including frosted ends on glass slides and the wider adoption of Canada balsam as a mounting medium (which required a clear view of the specimen) led to the decline of papered microscope slides [17]. However, these colourful slides remain a fascinating reminder of the early days of microscopy, offering a glimpse into the practices and aesthetics of Victorian era microscopists.

To this day, numerous museums and universities hold collections of period microscope slides, some dating back to the 19th

century. These slides offer valuable insights into historical medical practices, disease diagnosis, and biological understanding of the past. In addition, because of their aesthetic appeal, many collections cherish colourful and decorated papered microscope slides amongst their collections. As can be seen in Table 1, several UK institutions hold many period slides and potentially a proportion of papered microscope slides are included in those collections.

Beyond the extensive collections of papered microscope slides housed in museums, a considerable market for such period slides persists. For example, a simple search on a common online platform using the keywords “antique microscope slide” yields 715 individual results (as of May 2024, in the antique category of eBay UK). These results range from individual slides to sets of up to 720, with many featuring green papered mounts.

Thanks to modern practices in Heritage Science, many museums have started the process of digitalising their slide collections. Notably, the Natural History Museum and Kew Gardens have embarked on major programmes of digitisation and online access to the databases. These processes ensure that information about the slides alongside images will be readily available online when the projects are completed. However, despite their sometimes striking colours, digitised collections do not provide information on the colour of the slides, since no fields appear to have been created to input its colour or whether the slide is the so called “papered” microscope slide category.

Green emerges as a particularly striking colour among these papered microscope slides. While there is a lack of historical data on the prevalence of green-coloured slides, a superficial internet search suggests green is among the most dominant colour in surviving specimens. Notably, as anecdotal evidence, the main webpage (<https://www.nhm.ac.uk/our-science/services/collections/digital/programme/microscope-slide-digitisation.html>) of the Natural History Museum’s slide digitisation project features an image of a person holding four slides, two of which have green paper mounts (Fig. 1).

Given the prevalence of green hues in online listings and the materials used for green pigments in the middle of the 19th century, a connection can be drawn to the specific pigment employed during this period. Emerald green, or copper acetoarsenite, stands out as a readily available and inexpensive option. Historical records document the widespread use of arsenic containing pigments in Victorian era materials, including glazed confectionery paper, school supplies, theatre and concert tickets, playing cards, boxes, clothing, textiles, artificial flowers, toys, lampshades, wallpaper, curtains, furniture coverings, tin plates, blinds, and carpets [24]. Recent studies have also revealed the presence of emerald green in the covers of many surviving green books within public libraries [18].

Crucially, to our knowledge, there is no existing record of the specific pigment materials used to manufacture papered microscope slides, nor has any documented scientific research inves-

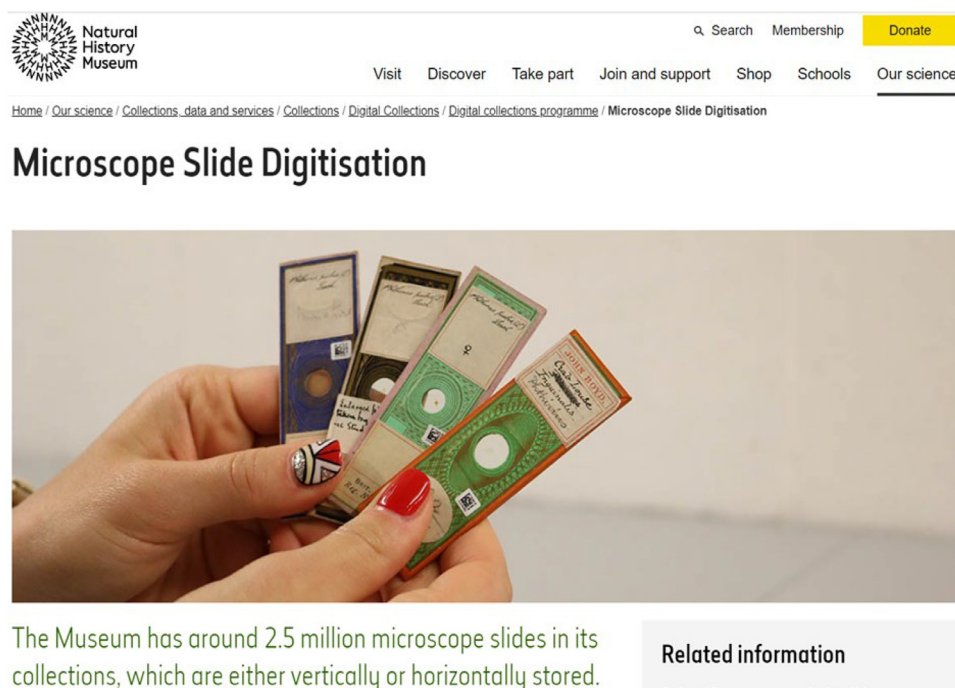


Fig. 1. Screen capture taken from the Natural History Museum slide digitisation project main website. (<https://www.nhm.ac.uk/our-science/services/collections/digital-programme/microscope-slide-digitisation.html>). Accessed August 1 2024, photographer of main image unknown.

tigated the pigments employed in Victorian papered microscope slides. Given the hitherto unreported presence of arsenic or other components in such slides it is therefore understandable that potentially sub-optimal handling as depicted in Fig. 1 may historically have occurred which will be discussed further in Section 5.4 in light of our findings.

2. Aim

To identify pigment materials used to make so called papered microscope slides prevalent in the second half of the 19th century with particular emphasis on estimating prevalence of arsenic-containing compounds, and to consider any potential health risks and environmental implications.

3. Materials and methods

3.1. Samples for chemical analysis

All analysed papered microscope slides were sourced from a private collection in the UK and permission was given to take microsamples. There are no records of their provenance, long term storage conditions or previous use. However, based on slide content, condition and features (e.g. named mounters) the authors have no reason to doubt that the 27 obtained slides are authentic and representative of the historical period of interest. All slides were immediately placed inside plastic zip bags upon receipt. Pictures of the front and reverse of the slides can be seen in Fig. 2.

3.2. Elemental analysis. X-ray fluorescence (XRF)

XRF analysis was performed using a portable Niton XL2 XRF (Thermo Scientific) with camera. An integration time of 20 s was used for all samples. The instrument is fitted with a 50 kV X-ray tube (100 μ A, 2 W maximum) and a silicon drift detector (SDD) with an active area of 5 mm² fitted with a polymer window (MOX-TEK AP 3.3 film). The X-ray beam's focal-spot size is about 3 mm in

diameter. SpectraGryph software was used to integrate the Kb line of arsenic at 11.73 KeV and Kb line of rhodium at 22.72 KeV. These lines are known to be free of interference if lead presence is suspected in the samples. Since the primary goal of this manuscript was to identify arsenic signals, and the software automatically labelled other elemental lines, we did not correct the automatic labelling. The uncorrected XRF data are presented in the supplementary information.

3.3. Elemental analysis (ICP-OES)

Small portions of the slide wrapping paper were taken using a scalpel (average mass taken: 2.5 mg). The samples were weighed using an electronic balance and the paper area was measured using a Nikon ShuttlePix P-400R Metrology microscope. Samples were then digested using the EPA 3051A (2007) method. Briefly, samples were transferred to Teflon® tubes, 9 mL of concentrated nitric acid were added and the mixture was digested in a MARS XPRESS microwave digestion system (temperature rise to 175 ± 5 °C in approximately 5.5 ± 0.25 min and retained at 175 ± 5 °C for the 10 min digestion period). The digested sample was transferred and made up in 25 mL volumetric flasks with deionised water (18M Ω). Total arsenic was analysed using an ICAP 6500 duo ICP-OES (Thermo Scientific) using a standard glass concentric nebuliser and a cyclonic spray chamber. The analysis was done in radial mode using the 189.042 nm arsenic emission wavelength, with an average of 3 readings per sample. Calibration curves were made using certified arsenic standards (1000 mg/L, Fisher Scientific).

3.4. Molecular analysis Raman microspectroscopy

Confocal Raman microscopy was performed using a Labram Aramis (Horiba Scientific) using a 532 nm laser (10 % power), a 50X objective, 600 l/mm grating, 50 μ m pinhole, 0.1–5 s integration time and average of 40 spectra accumulations, with spectra baseline corrected. The instrument was calibrated using a silicon

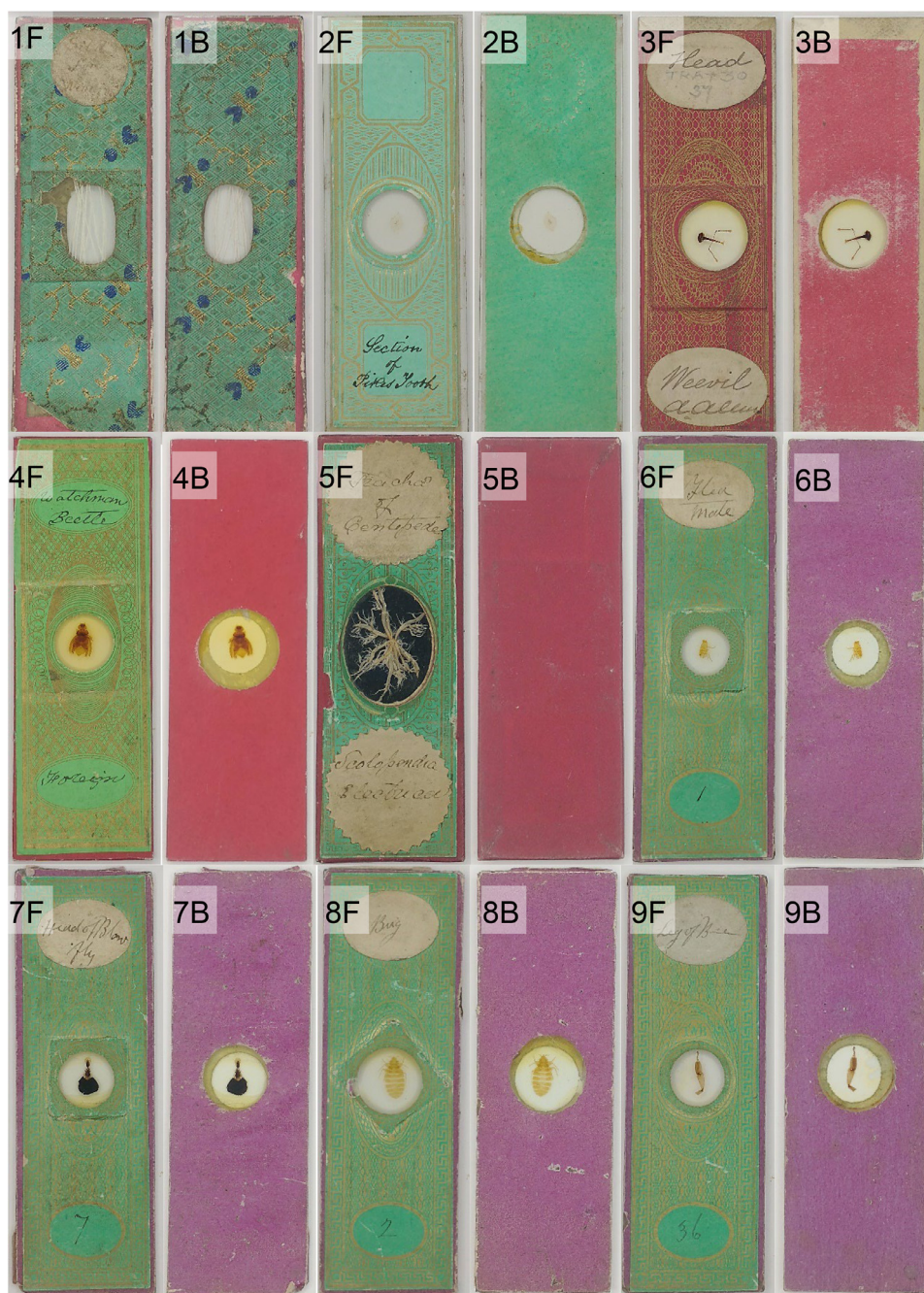


Fig. 2. Set of period microscope slides used for elemental and pigment analysis (F= front and B= back).

wafer and automatic software correction (Labspec 6). All reference spectra were taken from the Soprano, Marucci et al. and Sigma Aldrich Raman databases [7,12]. All Raman spectra show intensity in arbitrary units on the y-axis.

3.5. Surface enhanced Raman spectroscopy (SERS)

SERS spectra were acquired using a protocol from Cesaratto, Lombardi and Leona [4]. Briefly, silver nanoparticles were obtained by microwave reduction of Ag_2SO_4 in the presence of glucose and sodium citrate. The nanoparticles were concentrated 5 times (5X) by centrifugation and reconstitution to a smaller volume. Freshly prepared nanoparticles were deposited directly into the slide paper (4 μL), then left to dry to perform the analysis. The addition of an aggregating agent was not required [4].

3.6. Online catalogue search for visual analysis

Microscope slide information was obtained from two sources, focusing only on slides with accompanying images. The total number of slides, as well as those featuring green paper covers or labels, were recorded.

- Microscopical Mounts and Mounters by Brian Bracegirdle: This book contains a section showcasing photographs of 941 microscope slides [3].
- Natural History Museum Data Portal (May 2024): A search was conducted using the keywords “microscope slide” and “slides” with the “Gallery” filter applied to retrieve results with images. As some entries contained multiple slides, unique slides were counted, not the total number of entries [13].



Fig. 2. Continued

4. Results

4.1. Prevalence of green paper in microscope slides collections

To assess the prevalence of green-coloured paper, a search was conducted across two data sources, focusing on slides with accompanying images. Both fully green-covered slides and those containing green labels were considered positive results for the potential prevalence of arsenic. Fig. 3 provides examples of slides categorised as positive based on the presence of green paper elements.

The Microscopical mounts and mounters book (Brian Bracegirdle) contains 941 microscopic slides in total, from which 94 are green and 119 are fully papered [3]. The Natural history museum subsample consisted of 1,071 slides in total and 121 were consid-

ered positive for green with only 19 slides being fully papered. Ten percent of slides in the book were therefore positive for green pigment presence whereas 11.3 % slides from the Natural History Museum sample were considered positive for green [13].

4.2. Elemental and molecular analysis of period slides

A set of 27 period microscope slides, representing a variety of colours, was analysed using elemental and molecular methods (XRF, Raman and SERS). Digital images of both sides of each slide were captured (Fig. 2). This analysis revealed a range of light green shades amongst the slides, alongside one distinct dark green sample, with the remaining red, blue and yellow.



Fig. 2. Continued

Raman spectroscopy confirmed the pigment in all light green slides as emerald green (supplementary information, Raman spectra section). Additionally, elemental analysis confirmed the presence of arsenic and copper in these slides (Table 2). Arsenic in emerald green pigment exists in oxidation state +3, known to be one of the most toxic forms of arsenic. Conversely, the dark green sample was determined to contain chrome green pigment through Raman analysis (slide 25). This specific slide contained lead (Pb), chromium (Cr), iron (Fe), and arsenic (As), as confirmed by elemental analysis (supplementary information, XRF section). While the Raman spectra of some slides suggest the presence of carbon black, this material was not the focus of this study and was not investigated further.

Table 2 reveals the unexpected presence of arsenic signals in several red and purple coloured slides. XRF analysis has a pene-

tration depth ranging from micrometres to millimetres, depending on the sample matrix. Unfortunately, the tested microscope slides varied in thickness, and information regarding their glass or mica composition was unavailable. Furthermore, some slides exhibited green colouration on the front and red, purple, or yellow on the back. This raised the possibility of arsenic from the front green pigment (emerald green) influencing the XRF signal when analysing the reverse side. To address this concern, a negative control experiment was conducted. The clear section of Sample 22 was analysed by XRF, producing a minimal peak for the arsenic Kb line. Subsequently, this Sample 22 was overlaid on top of the front of Sample 17 (confirmed as emerald green with a strong arsenic Kb line). This experiment confirms that the XRF technique primarily detects elements within the top layer of the analysed samples, as demonstrated in Fig. 4 (Sample C).



Fig. 3. Natural History Museum slides considered positive for green pigment presence [13].

Table 2
Sample information and XRF, Raman and SERS analysis results.

Sample	Mounter	Colour -F	Colour -B	XRF -F	XRF -B	Raman -F	Raman -B
Slide 1	N/A	L green	L green	Cu, As	Cu, As	Em green	Em green
Slide 2	Charles Fletcher	Green	Green	Cu, As	Cu, As	Em green	Em green
Slide 3	N/A	Red	Red	Cu, As, Zn, Ca	As, Ca	Unknown	New Fuchisine
Slide 4	N/A	L green	Red	Cu, As	As, Ca	Em green	New Fuchisine
Slide 5	N/A	L green	Red	Cu, As	Cu, As	Em green	New Fuchisine
Slide 6	J.W. Bond	L green	Purple	Cu, As	As, Ca, Fe	Em green	Rosaniline
Slide 7	J.W. Bond	L green	Purple	Cu, As	As, Fe, Ca	Em green	Rosaniline
Slide 8	N/A	L green	Purple	Cu, As	As, Fe, Ca	Em green	Rosaniline
Slide 9	J.W. Bond	L green	Purple	Cu, As	As, Fe, Ca	Em green	Rosaniline
Slide 10	N/A	Red	Red	Cu, Zn, As	As, Ca, Fe	New Fuchisine	New Fuchisine
Slide 11	J Wilson	Red	Red	Cu, As, Fe, Ca	Ca, Fe	Unknown	Unknown
Slide 12	Possibly John Barnett	Blue	Blue	Cu, Zn, As	As, Fe, Ca	Ultramarine	Ultramarine
Slide 13	N/A	L green	Red	Cu, As	As, Ca, Fe	Em green	Unknown
Slide 14	Edmund Wheeler	Red	Yellow	Cu, As, Ca, Fe, Zn	Pb, Cr	Unknown	Chrome yellow
Slide 15	Possibly John Barnett	Red	Purple	Cu, Zn, As, Ca	As, Fe, Ca	Unknown	Unknown
Slide 16	Newton & Co	L green	Red	Cu, As	As, Sr, Ca, Fe	Em green	Unknown
Slide 17	N/A	L green	L green	Cu, As	Cu, As	Em green	Em green
Slide 18	C.M. Topping	Red	Red	Cu, Zn, As, Ca	As, Ca, Fe	Unknown	Unknown
Slide 19	Newton & Co	L green	Red	Cu, As	As, Fe, Ca	Em green	Unknown
Slide 20	Newton & Co	Red	Red	Cu, As, Fe, Ca	As, Ca, Fe	Unknown	Unknown
Slide 21	Smith, Beck & Beck	L green	Clear	As, Ca, Fe	As, Ca	N/A	N/A
Slide 22	N/A	Pink	Clear	Ca, Fe	Ca	N/A	N/A
Slide 23	G. F. Kent	White	Clear	As, Ca	As, Ca	N/A	N/A
Slide 24	Edmund Wheeler	White	Clear	As, Sr, Ca, Fe	As, Ca	N/A	N/A
Slide 25	N/A	D green	D green	As, Fe, Pb, Cr	As, Fe, Pb, Cr	Chrome green	Chrome green
Slide 26	N/A	Purple	Yellow	Cu, As, Ca, Fe	As, Ca, Fe	Rosaniline	New Fuchisine
Slide 27	Richard Suter	Pink	Clear	Ca	Ca	N/A	N/A

Front of the slide = F and Back of the slide = B, N/A = not available, L green = light green, D green = dark green, Em green = Emerald green.

It is important to acknowledge that the XRF technique employed in this study provides semi-quantitative results only. Several factors can influence the absolute arsenic content per unit of mass or area, including the thickness of the paper, the amount of pigment used, and potential degradation over time. The historical use and handling of the slides could also contribute to variations in arsenic concentration. For example, testing the centre of a slide might yield different results compared to the edges, which are more prone to wear and tear. Given these confounding factors, a full quantitative analysis would be necessary for a deeper understanding of factors affecting concentration distribution. However, despite the semi-quantitative nature of the data reported herein, valuable comparisons can still be made based on the observed sig-

nal strengths and corroborated by the strong correlations observed on a sub sample measured with the ICP-OES method (see Fig. 7). The area under the arsenic Kb line (at 11.73 KeV) for each sample was plotted in Fig. 4. This analysis revealed the highest arsenic content in slides containing emerald green pigment (Slide 5 front and 17 back and front). Interestingly, the red pigment in Sample 5 back, displayed a significantly stronger arsenic signal compared to some emerald green containing slides (Slides 1, 13, and 16). Subsequent SERS analysis of Sample 5 back, confirmed the presence of new fuchisine, also known as basic Violet 2. Other red and pink coloured slides were found to contain rosaniline using SERS (Fig. 5). The SERS spectra were confirmed by comparing with the data produced in the literature [4].

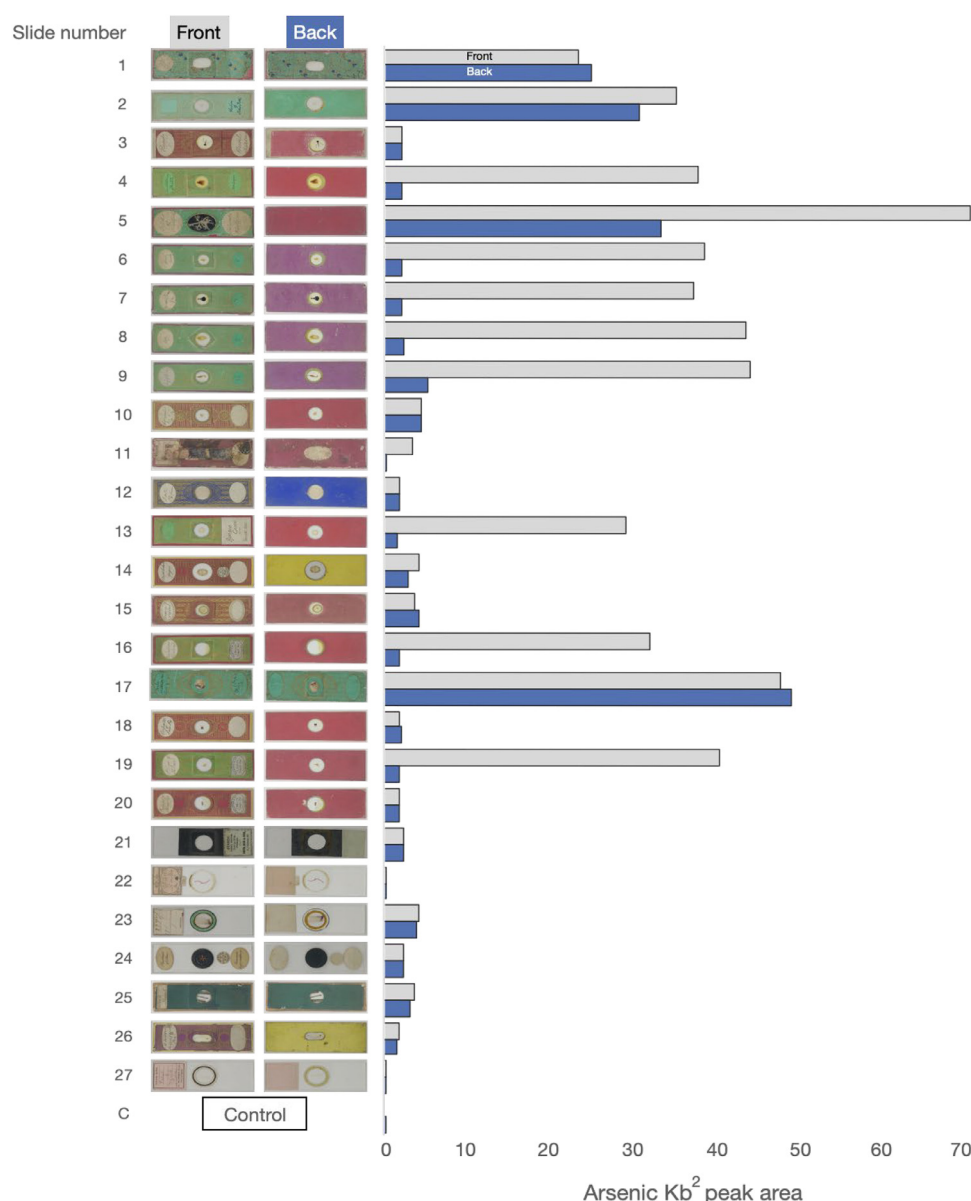


Fig. 4. Elemental analysis of the microscope slides, bars represent the integration of the area of the XRF Kb arsenic peak at 11.73 KeV. Control sample (C) is a clear slide 22 on top of slide 17 front.

The average area under the arsenic Kb line for emerald green containing pigments was 35.01 (SD = 10.32). Notably, all other samples positive for arsenic (excluding Sample 5) exhibited an average area of 2.53 (SD = 1.00). This suggests that slides other than green still contain, on average, approximately 7 % of the arsenic content typically found in emerald green pigmented slides.

The amount of arsenic found in different subsamples taken from the paper covering the slides (green and red) after acid digestion was compared to the areas (Fig. 6 and supplementary information, measurement of area section) under the curve derived by using the XRF spectrometer as can be seen in Fig. 7.

There is a very strong correlation between the amount of arsenic derived using acid digestion and ICP-OES and the signal generated using XRF ($R^2=0.9943$). It was therefore deemed reasonable to use the results obtained from the non-invasive XRF technique to calculate the amount of arsenic in the samples in per area unit (mg/cm^2). Using this information, we calculated that the average green paper slide contains $0.61 \text{ mg}/\text{cm}^2$ ($72.3 \text{ mg}/\text{g}$) of arsenic (SD = 0.19) ranging between 0.34 to $1.15 \text{ mg}/\text{cm}^2$ of arsenic.

5. Discussion

5.1. Prevalence of green paper in microscope slides collections

This study undertook a desktop investigation of a subsample from one of the world's most extensive microscope slide collections, revealing that approximately 11 % of the examined slides potentially contained paper with emerald green pigment. In addition, examination of Bracegirdle [3] who showcased amateur microscopists' slides also exhibited a similar prevalence (10 %) of slides potentially containing emerald green. However, it is important to acknowledge limitations in data from these sources given that information about the slides' provenance, storage conditions, and manufacturers was often unavailable, unrecorded or incomplete. Additionally, for the museum samples, details regarding the slide content were intentionally disregarded by us during analysis. Similarly, Bracegirdle [3] focused solely on slide mounter/manufacturer identification, omitting information about the slide content itself. Despite these limitations, it can reasonably be inferred from the

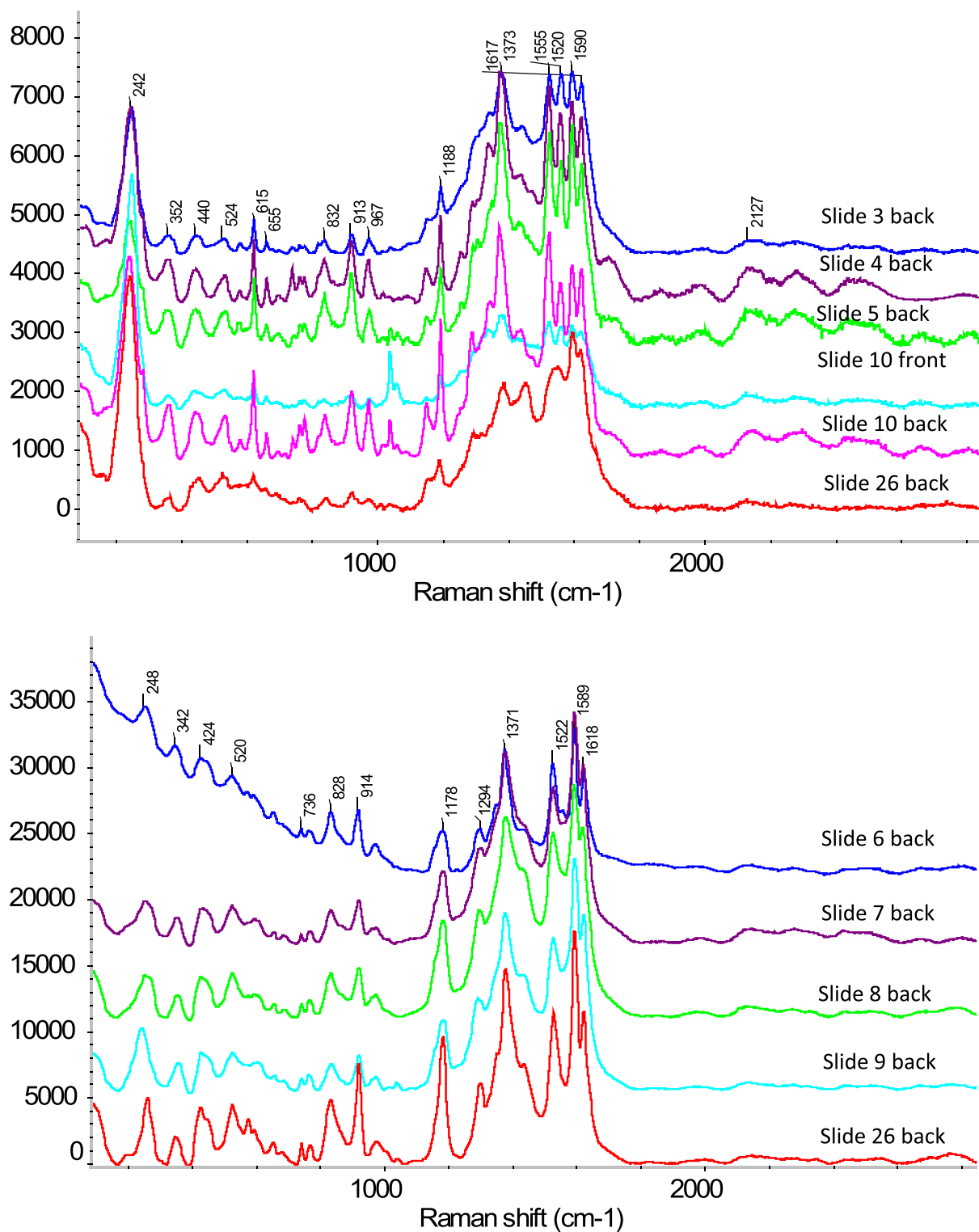


Fig. 5. Raman SERS spectra, slides 3, 4, 10 and 26 back as well as slide 10 front, corresponds to new Fuchsin. Slides 6, 7, 8 and 9 back as well as slide 26 correspond to rosaniline front, matched according to Cessaratto, Lombardi and Leona, 2016.

visual analysis conducted that the reported proportions could represent a fair estimate of the true number of arsenic-containing microscope slides in UK collections.

According to the Distributed System of Scientific Collections UK (DiSSCo), there are 8.8 million microscope slides in participating UK collections [16]. Considering the samples tested as potentially representative of the entire collections within the UK, our findings

suggest that approximately 882,000 slides across UK institutions might contain emerald green pigments. Since we have only considered UK collections in this study, the number of arsenic containing slides internationally is of course likely to be far higher.

It is notable that other papers used in the Victorian era besides green coloured paper might also contain significant arsenic levels. This is further supported by our observation that several analysed



Fig. 6. Measured area taken for elemental analysis from microscope slide 5, front.

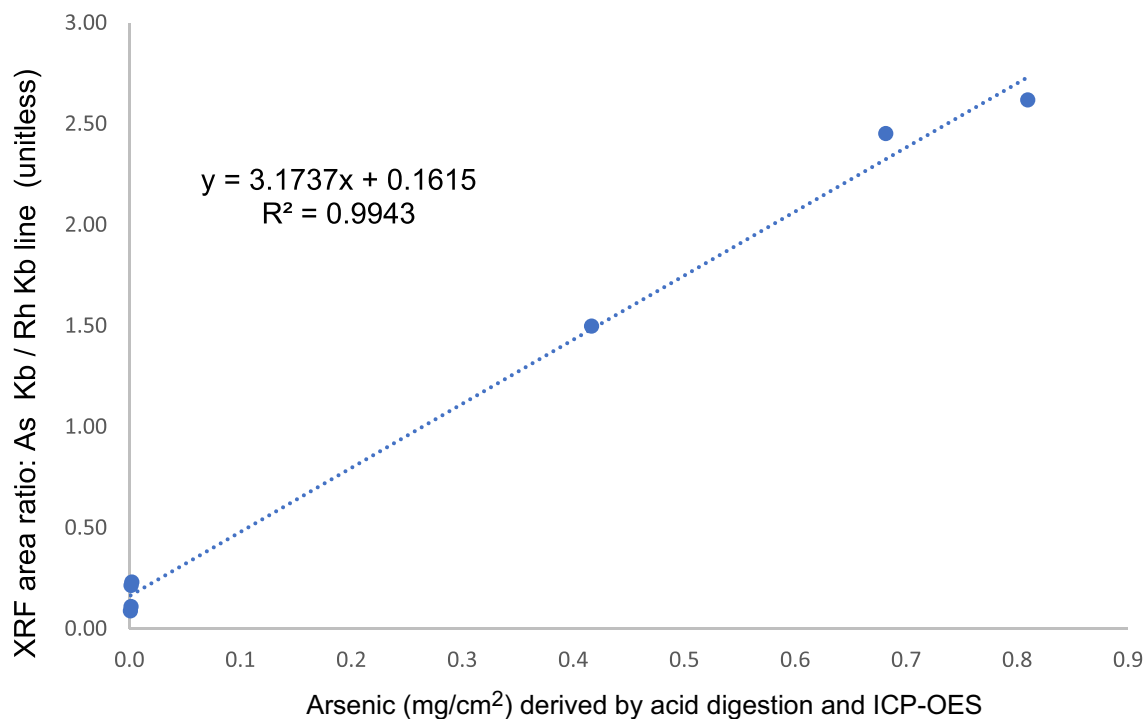


Fig. 7. Correlation between the total concentration derived from acid digestion ICP-OES analysis and the area ratio of the arsenic Kb and rhodium Kb line derived using a portable XRF (calculating the ratio is a way to correct for variations in the tube signal).

slides displayed different colours on their front and back surfaces, with corresponding variations in arsenic content. This finding highlights potential omissions in existing slide digitisation projects, such as neglecting to record slide colour or include images of the reverse side, potentially overlooking hypothetical arsenic content. Similarly, the “Microscopical Mounts and Mounters” book [3] did not capture pictures of the back of the slides.

This widespread lack of awareness regarding the materials, particularly pigments, used in Victorian era papered microscope slides that exists today is a concern. In stark contrast, a heightened awareness of arsenic pigments used in other Victorian products, such as the infamous arsenic laden wallpapers, already existed by the late 19th century. Evidence from historical studies supports this disparity. An 1884 American study by Wood [24] reported the

presence of arsenic in various coloured wrapping papers, with red and blue papers often containing even higher levels than green ones. The study also highlighted the inconsistency within colours, with some seemingly identical papers containing arsenic while others did not. Wood’s work concluded with the stark warning that “papers of any colour and of any shade may be arsenical”. The same study found that arsenic in red paper ranged from 0.069 to 0.690 g/m², whereas arsenic in green papers ranged from 1.760 to 4.341 g/m². A waste product of the aniline manufacturing industry, known as London purple was also tested and was found to contain up to 7.5 % arsenic w/w [24].

Similar observations were made in a 1904 study by Haywood and Warner [10] on wallpaper samples from various locations, where red and green again emerged as the predominant colours

containing arsenic [10]. More recently, research by Tedone and Grayburn [18] on historical book covers revealed that 10 % of green book covers analysed contained arsenic and copper, with an average arsenic content of 14.2 g/m² [18]. These findings further reinforce the broader concern about arsenic in coloured materials used during the Victorian era. The present investigation revealed both similarities and differences in arsenic content between book covers, wallpaper reported from the literature, and paper used in microscope slides. Something in common across these materials is the presence of arsenic in both green and red colours. Literature data suggests that the amount of arsenic in green pigments ranges from 0.176 to 1.42 mg/cm², with variations likely due to the amount of pigment applied, object age, and the degree of delamination [19,23]. Similarly, the arsenic content in red pigments is influenced by these factors, along with the dye's purification level. Generally, more expensive and purified dyes contained less arsenic compared to cheaper alternatives. Our study also observed a trend of lower arsenic concentration in red materials compared to green ones. Interestingly, the average arsenic content in red and other coloured papers in our analysis was approximately 7 % of that found in green slides. However, it is important to note that one red sample in our study exhibited a higher arsenic level than some green ones, highlighting the potential for significant arsenic content in certain red dyes, as documented in historical literature (up to 7.5 % w/w arsenic) [24].

A key distinction lies in the use patterns of book covers and microscope slides, which translates to potential health risks. There is a general consensus that primary health concerns arising from handling arsenic-bearing materials may be facilitated by potential delamination of pigment layers followed by accidental ingestion [14,18]. Microscope slides are typically secured using metal clamps that exert pressure on the edges, potentially causing friction and delamination with repeated handling. In contrast, book covers are not subjected to such physical stress through contact with sharp objects.

Assuming a standard microscope slide size of 75 × 25 mm (with a double-sided area of 37.5 cm²) and an average arsenic content of 0.61 mg/cm² in emerald green paper, each green slide could potentially contain on average 22.9 mg of arsenic, ranging from 12.8 to 43.1 mg of arsenic. Based on estimates of museum collection sizes and the proportion of arsenic-containing slides, this translates to a potential cumulative storage average mass of 20,198 g ranging from 11,290 to 38,014 g of total arsenic in known UK collections, DiSSCo [16]). While this study shows for the first time the presence and estimates of arsenic from a new source, further work is clearly warranted to establish routes of human exposure e.g. friability of paper and modes of transmission during handling.

5.2. Selection bias and representativeness

This study employed an observational approach to assess the prevalence of arsenic containing slides in museum collections and among amateur microscopists. While our analysis revealed a significant presence of emerald green slides it is important to consider selection biases both historically and in our sampling from current archived material.

We lack definitive historical evidence suggesting a widespread preference for green slides in Victorian microscopy practices. However, it has been suggested that vibrant colours capture attention more readily compared to muted tones, potentially leading to a higher likelihood of green slides being documented, preserved and displayed [9]. It is possible therefore that museums and collectors might be more inclined to preserve and document visually striking features like emerald green paper, potentially skewing the perception of their true historical and current prevalence. It is therefore

also possible that our estimates of their prevalence in UK collections could overstate their numbers based on a preference to exhibit them.

The journey of many collections often involves antique dealers who, driven by commercial interests, might have prioritised slides with visually appealing features like vibrant green paper. These slides likely hold greater appeal for potential buyers, including museums and collectors. This interplay of commercial factors and human colour preference could contribute to a higher survival rate of green papered microscope slides [6] as well as more a greater likelihood of them been handled and/or unwittingly exposed to private collectors.

We deliberately chose samples for analysis that represented a wide range of colours and from various manufacturers. While our deliberate approach to colour selection helps address selection bias concerns, future studies seeking a more representative picture of historical slide usage and arsenic prevalence should incorporate a broader range of slide materials and collections. This may involve:

- Including a wider variety of colour variations.
- Expanding analysis to encompass a larger sample size across diverse museum and private collections.
- Consulting further historical records and documentation on slide usage practices during the Victorian era.

By acknowledging the limitations of our study and outlining potential strategies for future research, we aim to contribute to a more comprehensive understanding of the prevalence of arsenic in historical microscope slides. Further fully quantitative analyses involving full digestion of whole slide coverings would also be warranted to provide accurate mass estimates of total arsenic content although our preliminary investigations (see Fig. 7) suggest non-invasive methods are useful for this purpose.

5.3. Beyond emerald green: the case of red pigments

While our study focused on the prevalence and potential health risks associated with arsenic containing green pigments, red coloured slides also warrant attention. We observed that many slides having red pigments on the reverse of the slides are often unrecorded and disregarded. This seemingly insignificant detail takes on greater significance when considering the historical use of arsenic based red dyes.

Shortly after William Perkin's discovery of mauveine in 1865, Edward Chambers Nicholson and his collaborators patented a method for synthesising fuchsine using arsenic acid. This method offered a significantly higher yield (25 % to 42 %) of Fuchsine hydrochloride compared to Perkin's 5 % yield for mauveine. However, the primary product was actually Fuchsine arsenite, which was then converted to Fuchsine hydrochloride using sodium chloride. Despite attempts at purification, the final product could still contain up to 6 % arsenic in the form of soluble arsenite [15]. As previously noted, arsenite is one of the most toxic forms of arsenic.

Despite the well documented toxic nature of arsenic for centuries (including its use as a poison for pests), this arsenic based synthesis of Fuchsine remained popular with manufacturers throughout the 19th century. This historical context highlights the potential presence of arsenic in red coloured slides beyond the more commonly recognised green pigments.

5.4. Health risks and recommendations

While handling a single slide may pose minimal risk for a healthy adult, the situation is different for children. Their developing bodies are more susceptible to the toxic effects of arsenic [22]. This is particularly concerning in the context of microscopic

societies that organise outreach events for children handling period slides. Any human chronic exposure through prolonged handling of numerous slides, especially if the paper is damaged or delaminating, could lead to chronic arsenic poisoning. Exposure to arsenic can cause a variety of health problems, including skin lesions, gastrointestinal issues, and even cancer [11].

Drawing upon recent recommendations for handling arsenic containing materials like green book covers [18], with some key adaptations for microscope slides, we propose the following guidelines:

- Minimise contact and exposure: whenever possible, limit direct contact, inhalation, and ingestion of or in close proximity to slide materials.
- Prioritise arsenic free alternatives: once catalogued and whenever feasible, facilitate the use of arsenic free alternatives where the content is identical. For example, when two slides, one plain and other papered are available for viewing the same specimen type, the arsenic free alternative should be used.
- Restrict borrowing: discourage the borrowing of emerald green slides to minimise potential exposure outside of controlled environments.
- Digitise where possible: encourage researchers to utilise digitised surrogates whenever possible in lieu of handling the original slides.
- Controlled handling procedures: when handling original emerald green slides is essential, implement the following procedures:
 - When opening up boxes of archived slides, conduct handling within a controlled environment, such as a fume hood.
 - Ensure all staff are trained for handling activities.
 - Utilise nitrile gloves when handling slides.
 - Employ hard, wipeable surfaces for handling slides.
 - Develop a protocol for routine cleaning of microscopes after use.
- Store slides in clear zip-top bags for visibility and added protection.

Based on our preliminary findings, it is appropriate to raise awareness among museum staff, researchers, microscopy societies, and amateur microscopists regarding the potential health risks associated with the handling of papered microscope slides. Implementing these recommendations could significantly reduce exposure risks and ensure the safe handling of these valuable historical artifacts.

In terms of identification/recognition of potentially problematic slides, an approach similar to the Winterthur Museum, Garden & Library and the University of Delaware partnership (known as the Poison Book Project) could be employed. They use an emerald green coloured bookmark for book cloth colour identification in their collaborative survey of Victorian-era publishers' bindings (<https://sites.udel.edu/poisonbookproject/resources/>). A similar approach could be used to provide a match to the characteristic hue of emerald green paper used in microscopy slides. In addition, a previously identified emerald green containing slide in a closed zip bag can be used for identification. However, it is crucial to emphasize that colour matching alone is not a definitive indicator of arsenic presence. Further analysis using techniques like XRF is recommended for confirmation.

A similar approach should be taken for red colour slides where a definitive indicator of arsenic presence is an elemental analysis technique such as XRF.

We understand some concerns regarding the potential for mould growth in sealed plastic bags and the subsequent volatilisation of arsenic. However, it is important to clarify some misconceptions that have permeated popular culture (e.g. with respect to

mobilisation of arsenic by this mechanism from mouldy Victorian wallpaper).

- It is important to highlight that the volatile form of arsenic produced by moulds is trimethylarsine (TMA) and not arsine gas as many believe.
- While some moulds have the ability to methylate arsenic, this process is often inefficient, thus producing very small amounts of methylated species [5].
- Unlike mercury, where methylation increases toxicity, arsenic becomes less toxic in its methylated forms [2]. For example, (TMA), a potential product of mould methylation, has a much higher LC50 (Lethal Concentration for 50 % test organism) compared to inorganic arsenic. [5].

5.5. Public outreach and awareness

The potential presence of arsenic in green and red papered microscope slides provides public outreach opportunities targeting various audiences:

Museums: Museums can develop engaging and informative educational materials, both physical and digital, to raise awareness among visitors. These materials could include:

- Interactive exhibits showcasing historical microscope slides with clear explanations about potential arsenic content.
- Short educational videos highlighting the dangers of arsenic and safe handling practices, particularly when handling slides with children present.
- Social media campaigns using eye catching visuals and clear messaging to spread awareness across common social media platforms.
- Collaborations with AI chatbot developers to create informative chatbots that can answer visitor questions about arsenic containing slides.
- Facilitate donations of potentially arsenic containing slides since awareness of their content may instigate some private collectors to dispose of them. Collaborate to develop standardised protocols for safely accepting donations of potentially arsenic containing microscope slides from the public (to avoid improper disposal). This could involve:
 - Creating clear guidelines for donation, including information on how to identify and package these slides safely.
 - Establishing designated drop-off locations or mail in procedures for receiving donations.
 - Partnering with other museums or relevant organisations to share resources and expertise in handling these materials.

Microscopy societies: Microscopy societies have a crucial role to play in educating their members through:

- Workshops and seminars focused on the identification and safe handling of arsenic containing slides.
- Dedicated online resources and forums for members to share information and best practices.
- Social media campaigns, utilising targeted hashtags and collaborating with prominent microscopy influencers to reach a wider audience.

Amateur microscopists and public collectors: Engaging amateur microscopists and the public with antique collections requires a multifaceted approach:

- Educational brochures and pamphlets distributed at relevant events and society meetings.
- Online resources and articles on popular science websites and microscopy forums.
- Public webinars hosted by experts, accessible through on-line platforms.

- Citizen science initiatives encouraging responsible handling and reporting of potentially arsenic containing slides to relevant institutions.

By utilising a combination of traditional and innovative tools, including social media, AI chatbots, and citizen science initiatives, public awareness can be effectively raised about the potential risks associated with arsenic containing microscope slides and promote safe handling practices across all audiences.

5.6. Environmental concerns

Beyond the direct health risks associated with handling arsenic containing microscope slides, there are also potential environmental concerns to consider, particularly regarding improper disposal practices. Amateurs who are unaware of the potential arsenic content in their slides might dispose of them through household rubbish. Landfills are not designed to contain hazardous materials like arsenic, and improper disposal could lead to contamination of soil and groundwater. The potential environmental impact of improperly disposed arsenic containing microscope slides arises from the long-term persistence and leaching of arsenic into the surrounding environment. This process can occur over decades or even centuries, depending on various factors including soil properties and groundwater characteristics mainly regarding pH and redox potential as well as microbiological activity [8].

6. Conclusion

This study reveals for the first time the presence of arsenic in Victorian-era microscope slides, particularly those containing green pigments like emerald green. However, arsenic was also detected in some red pigments, suggesting a wider presence in collections. Visual analysis of existing slide collections suggests that a significant proportion (10 % to 11.3 %) could contain arsenic, potentially translating to hundreds of thousands of slides within UK collections alone. The presence of arsenic raises potential health risks during handling of these slides. It emphasises the importance of responsible handling practices for curators, collectors, and anyone working with historical slides as well as documenting practices that include the pigment materials used to fabricate the slides and the back of the slide. Improper disposal of these slides poses a previously unrecognised environmental hazard. Arsenic leaching from improperly disposed slides could contaminate soil and groundwater resources. This study highlights the need for further research on the prevalence of arsenic in historical microscope slides. Additionally, it emphasises the importance of developing and disseminating guidelines for safe handling and disposal practices. Outreach activities are crucial to raise awareness among curators and collectors about the potential health and environmental risks associated with arsenic-containing slides.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used Gemini / Google service in order to improve readability. After using

this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.culher.2024.11.002](https://doi.org/10.1016/j.culher.2024.11.002).

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