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The Identification of Historic Biocide Residues on Herbarium Material at the National Museum of Wales

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Abstract. The National Museum of Wales (NMW), houses *c*.250,000 higher plant specimens, with material dating back to the 17th century. Herbaria have been a major source of botanical research and reference for centuries and the collections have increased over time from donations and through collecting.
Due to its organic content, botanical material is susceptible to insect and fungal attack. Even aged, dried material is a source of sugar and protein. Institutions and collectors have prevented such attacks through the application of pesticides. Treatments containing compounds of arsenic, lead and mercury were commonplace, and have remained stable over time. Consequently, present-day handling of these collections presents a potential health risk to staff and visitors through inhalation and skin absorption, particularly since the quantity and nature of the pesticide applied is unknown. Occasionally the residues are visible, but research has shown that herbarium sheets, which appear untouched, have been previously treated, and contain high concentrations of toxic metals.
The use of a UV hand-held lamp has helped to identify sheets that have been treated, even though treatment is not visible to the naked eye. The UV causes areas to fluoresce on the herbarium mount sheet. These areas were analysed by proton induced X-ray emission (PIXE), and have been found to correlate with pesticide applications.
This research has provided data for the identification and quantification of the applied pesticides. The information has enabled safe standard procedures to be implemented to protect personnel, and has also provided a rapid, effective method of identifying contaminated samples within the collections and provided a means to prioritise which collections require immediate re-mounting. This has enabled the removal of a large amount of hazardous chemical from the herbarium environment, and allowed for safe disposal.

Keywords: Herbaria, biocide residues, heavy metals, non-destructive analysis, PIXE elemental line mapping.

INTRODUCTION

The National Museum of Wales (NMW) herbarium houses *c*.250,000 higher plant specimens, with material dating back to the 17th century. Considering the organic nature of this material, the collection has survived remarkably well – even aged, dried material is a source of sugar and protein, providing an attractive food source for insects and fungi. The specimens in the collection are attached with linen tape to paper support sheets, with contemporary collection data attached alongside (Fig.1).

It has been generally accepted that zoological material was historically treated with hazardous chemicals to protect against insect and fungal attack. The residues of these biocides are still present today, and pose a potential health hazard to museum staff and visitors. Until recently, there has, however, been a general ambivalence towards similar threats from botanical material, despite an abundance of literature showing that herbarium collections have been similarly protected in the past [1–4].

There is a range of biocides that have been used on botanical specimens; both organic (naphthalene, *p*-dichlorobenzene) and inorganic (mercuric chloride, lead hydrogen arsenate, arsenic trioxide, barium fluoro-silicate). Application mechanisms varied according to the biocide being used, but included fumigation, dipping, brushing, spraying, sprinkling of powders, and the continuous application through sublimation [5].

FIGURE 1. A typical example of a NMW herbarium specimen (*Salix myrsimifolia*), showing mounting technique and mount sheet beneath.

Mercuric chloride (corrosive sublimate) was the most common biocide used on botanical specimens, dissolved in phenol and methylated spirits, and applied by brushing, spraying or dipping the specimen. Although the earliest reference to its use is in 1770 [6], a Leonard Plukenet specimen of *Hippuris equisetus* in the NMW collection, dated *c*.1687, indicates earlier use – this specimen has traces of mercury metal present, presumably as a result of the reduction of an historic mercuric chloride application. The practice of using mercuric chloride as a biocide on botanical material continued in the UK until 1982 [4].

Unfortunately, records relating specimen to the type and method of chemical treatment applied, have rarely survived, if indeed they ever existed. This has unfortunately left a legacy of hidden hazards within the museum collection.

Following the cessation of mercuric chloride use as a biocide for herbaria, some collectors (notably The Royal Botanic Gardens, Kew, and Cambridge University Herbarium) began to monitor levels of mercury vapour in the air [7], and the potential health hazards to workers and visitors alike, became apparent.

With the potential risk in handling the specimens, due to the presence of toxic residues [8, 9], the NMW was keen to determine which specimens within the collection were most contaminated. With such a large collection, it is impossible to decontaminate and/or re-mount the entire collection in one go, but a methodology that would allow specimens to be prioritised, according to the level of contamination, would be invaluable. Needless to say, quantitative analysis of each sample was also out of the question, from both time and economic restraints. Furthermore, certain historically significant collections, such as Lord Bute's collection from the 18th century, can neither be re-mounted, nor destructively analysed, posing additional problems.

During visual inspection of a number of specimens, it was noted that, under UV illumination (366 nm), certain areas on the backing sheets fluoresced. These areas were not apparent under visible light, apart from the occasional foxing stain. The observed fluorescence ranged from cream to orange in colour.

The main aim of this work is to determine whether a quick survey of the collection, with a hand-held UV lamp, will offer sufficient information to determine whether botanical specimens have been exposed to historic toxic metal biocide treatments, thus informing the prioritisation of a decontamination programme.

EXPERIMENTAL

Sample Preparation

Fluorescent areas on the backing sheets of 32 specimens[1] were identified under UV light (366 nm), and the perimeter of each area marked with a pencil. One hundred fluorescent areas were identified in this way, and the sheets cut down to a small size for convenient handling and mounting for analysis by proton induced X-ray emission (PIXE). The samples were placed on a X,Y,Z-motorized stage and held in a plastic envelope, with a hole punched over the fluorescent area circled for analysis (Fig.2).

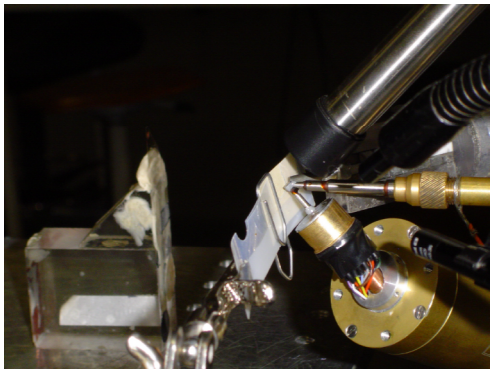

FIGURE 2. Sample of herbarium backing sheet, showing the punched plastic envelope holder.

PIXE Analysis

PIXE analysis was carried out using a NEC 2 MV tandem pelletron accelerator at the C2RMF, Centre de Recherche et de Restauration des Musées de France.

The proton beam (3 MeV) was aligned parallel to the surface normal, and two Si(Li) detectors were placed at 45° – one with an ultra thin polymer window optimized for the lower energy X-ray (1–10 keV), the other with a 50 μm Al filter for higher energies. The utilized beam size was about 30 μm in diameter. A positioning system with CCD camera and laser was

[1] The specimens were previously removed to allow the original backing sheets to be destructively analysed

used to place the zone of interest in the beam, at a 3 mm distance from the exit window. The proton dose was monitored by measuring the Si X-ray signal emitted from the 0.1 µm silicon nitride exit window with a peltier-cooled silicon drift detector. Quantification was achieved using the GUPIX software[10], calibrated against Micromatter thin-film standards. Samples were treated as thin samples, and hence no correction was made for X-ray absorption by the matrix [11]. The accuracy of this quantification is expected to be around 15–20% Rsd, mainly due to the faint precision dose monitoring (±10%) and the accuracy of the standard material (±5%).

Multielemental spot analyses (100x100 µm scan using the controlled stage) were carried out on all fluorescent areas, whilst elemental area and line mapping (500 µm^2, 1000 µm apart, over a distance of 4 cm) was used to determine variation in the Hg and As contamination across the sample, incorporating both fluorescent and non-fluorescent areas, and areas with visible staining. In order to eliminate elements inherent in the paper, control samples were taken from the edges of all specimen sheets.

RESULTS AND DISCUSSION

Eight distinctive colours were identified subjectively under UV light, using the Munsell colour system as a reference: white, cream, yellow, peach, orange, brown, grey and black. Black was visible without UV; grey and brown could often only be observed under UV; all other colours were fluorescent.

The majority of the samples were found to contain significant amounts of mercury, but only a few contained significant amounts of arsenic.

Elemental line mapping, for both arsenic and mercury, showed that elevated concentrations of these toxic metals were correlated to the position of the fluorescent areas (Fig.3), thus indicating a link between the observed areas under UV light and biocide application.

Six replicate analyses of each coloured area were carried out to determine whether the biocide residues were evenly distributed. From the results (Table 1) it was apparent that, even over these small areas (2–3 mm), the variability of both mercury and arsenic was high (≤ ±78% for Hg; ≤ ± 64% for As).

Any correlation between concentration and observed colour under UV was difficult to determine due to the inhomogeneity. However, the black areas observed were significantly higher in mercury than all other samples (Fig.4), and no arsenic was observed (<dl).

To determine whether any of the other observed colours could be distinguished from each other, backing sheets with several different coloured areas were used for comparison.

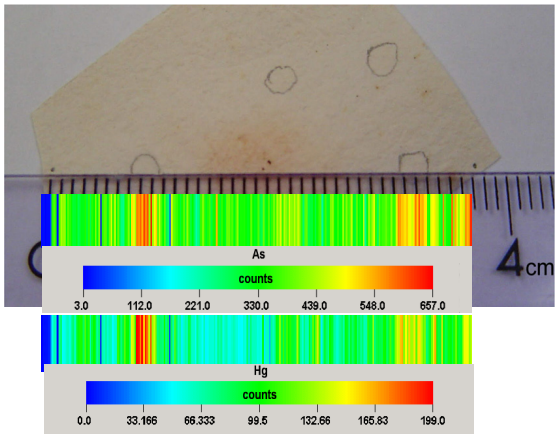

FIGURE 3. Distribution of arsenic and mercury across a backing sheet from the Bute collection (18th century). Element line mapping was carried out over the 4 cm distance between the two pencil dots, along the line indicated by the ruler. Two fluorescent areas (one circled in pencil) correlated exactly with the observed elevated concentrations.

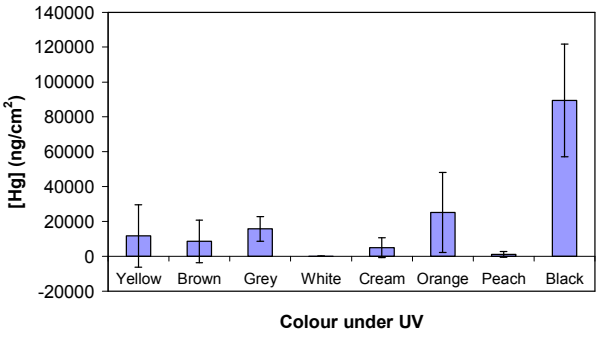

FIGURE 4. Mean mercury concentrations (± σ) for the eight identified coloured areas under UV light.

TABLE 1. Replicate analyses (n=6) for the eight distinctive colours under UV, showing the general inhomogeneous distribution of mercury and arsenic

Colour under UV	[Hg] (ng/cm^2)	Rsd (%)	[As] (ng/cm^2)	Rsd (%)
Yellow	15835	78	75	44
Brown	4444	54	304	14
Grey	18165	18	183	64
Black	89460	36	70	16
Cream	3488	59	312	17
Orange	76	63	67	40
Peach	30	9	50	29
White	27	53	159	11

Figure 5 compares the yellow, orange and peach areas for four different backing sheets (samples 11, 21, 52 and 76). Although the yellow and peach areas show no significant difference in their mercury content, the orange areas are consistently higher, and significant differences are observed.

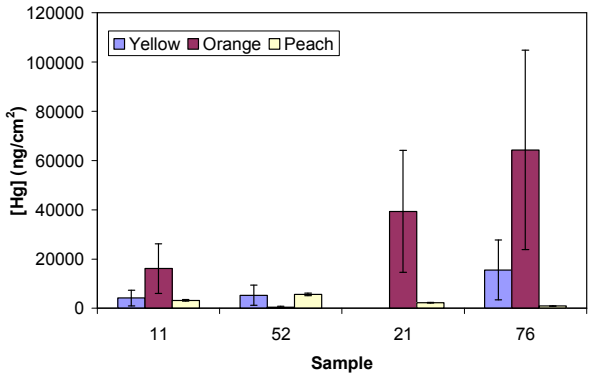

FIGURE 5. Comparison of the mean mercury content (± σ) of the yellow, orange and peach UV areas on four different backing sheets.

Other backing sheets with brown, grey and cream areas present indicated that, although the brown and cream showed no significant difference in mercury content, the grey areas could indeed be differentiated (Fig.6).

No correlations were identified between colour and arsenic concentration, as only a few of the backing sheets analysed contained arsenic. In order to determine a relationship, a larger sample base would have to be analysed.

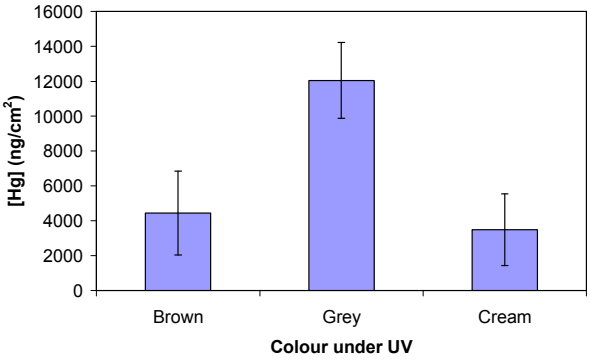

FIGURE 6. Mean mercury concentrations (± σ) for backing sheets with brown, grey and cream UV areas all present.

CONCLUSIONS

The majority of the backing sheets analysed showed evidence of historic mercuric chloride treatment. Clearly, the application was not evenly applied, leading to an inhomogeneous distribution of mercury across the backing sheet. Although the results have indicated that high concentrations of mercury occur in the coloured areas visible under UV light, it is unclear what mechanism has led to this phenomenon. Mercuric chloride does not itself fluoresce. Reaction with degradation products from the paper, which readily biodegrades over time, could, however, provide an explanation. Certainly, preliminary investigation by X-ray photoelectron spectroscopy has indicated differences in mercury oxidation state in some of the colours observed.

From the results obtained to date, it seems likely that a hand-held UV lamp may provide a rapid and effective method of identifying those samples within the collection that have been highly contaminated with mercuric chloride, and provide a means to prioritise which collections require immediate re-mounting. Furthermore, this will inform the implementation of standard procedures to protect personnel and visitors handling the collections, and enable the removal of a large amount of hazardous chemical from the herbarium environment.

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