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Full Length Article

The poisoned samples from the *Herbarium Centrale Italicum*: A study of the mercury contamination by ion beam analysis techniques

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ABSTRACT

Museum herbaria are collections of preserved, dried plants, bearing an exceptional value as archives of botanical history and biodiversity, an invaluable resource for modern research in several fields. The Museum of Natural History of the University of Florence (Italy) houses the Florentine Botanical Collections (FI), which include some of the most ancient and largest herbaria in the world, such as the Herbarium Centrale Italicum (FI-HCI). Like many herbaria worldwide, these collections are affected by Hg contamination resulting from the historical use of corrosive sublimate ($HgCl_2$) as a pesticide for specimen preservation. Although this practice was discontinued over a century ago, elemental Hg degassing from the samples and residual Hg compounds on them prevent the full accessibility and valorisation of these collections.

This work aims at characterizing the concentrations and in-depth distributions of Hg and other possibly related elements (Cl, and S) within contaminated samples from the Florentine collections using external beam ion beam analysis (IBA) techniques. Nine representative specimens – eight from the 19th century and a recent, untreated one (blank) – were selected for PIXE and EBS measurements, with a 3 MeV proton beam, at the INFN LABEC 3 MV Tandem accelerator.

The study revealed that contamination variably affects both the plant matrix and the support paper: Hg concentrations measured on dark stains on paper, which were identified as superficial accumulations of Hg, were significantly higher than those measured on clear paper and on the plant matrix, where Hg penetrates deeply instead. It was also possible to make some hypotheses about the speciation of Hg in the samples.

1. Introduction

Museum herbaria, i.e., collections of dried plants, represent a unique historical record and a key resource for modern research on climate change and biodiversity [1].

The susceptibility of these collections to infestations has meant that, since their establishment, herbaria have been treated with a large number of pesticides in order to overcome them [2,3]. During the 19th century, and up to the beginning of the 20th century, one of the most used pesticides worldwide for botanical collections was "corrosive sublimate", i.e., an alcoholic solution of mercury dichloride, $HgCl_2$ [2,4,5]. The decomposition of $HgCl_2$ causes the release of gaseous elemental mercury (GEM, Hg^0), as attested in many herbaria worldwide [5], with clear consequences for the health of both workers and visitors to the herbarium collections [6].

The Botanical Collections of the Museum of Natural History of the University of Florence (in the following referred to as FI, its code in the international Index Herbariorum), Italy, host some of the most ancient and important herbaria in the world [4]. The main nucleus of these botanical collections is represented by the Herbarium Centrale Italicum

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(FI-HCI), founded in 1842, implementing the few pre-existing botanical collections of the Imperial and Royal Museum of Physics and Natural History, established in Florence in 1775. The Sicilian botanist Filippo Parlatore, first director of FI-HCI, was able to polarize the attention of many national and international botanists and collectors, who decided to donate their personal herbaria for the growth of the Botanical Collections, which from that moment onward continued to expand, reaching today more than two million samples, including rare and precious specimens from all around the world [4]. Nowadays, collections of dried plants of FI are divided into an "open herbarium", i.e., the collection continuously updated as new material arrives, and "closed herbaria", i.e., historical collections not incremented by the arrival of new material and maintained in their original structure, such as the herbaria Cesalpino (16th cent.), Micheli-Targioni Tozzetti (17-19th cent.), Webb (18-19th cent.), Beccari (19th cent.) and Pichi-Sermolli (20th cent.).

Although the practice of treating samples with $HgCl_2$ was discontinued over a century ago at the FI collections [2], they still suffer from GEM [1,5] and particulate-bound mercury (PBM) [5,7] contamination, which prevents the full accessibility and valorisation of the Botanical Collections, complicating their management [5].

Since 2013, several investigations and analyses have been conducted in the FI collections halls [1,5,7] with the aim of determining GEM concentrations in air, assessing their seasonal and diurnal trends, and developing strategies to mitigate airborne Hg levels [4,5]. Notably, GEM concentrations were high, although Hg was applied as chloride ($HgCl_2$) to the plant specimens. The reduction of Hg^{2+} to Hg^0 was observed in the environments of many natural history museums and has been attributed to different pathways, including enzymatic reduction driven by bacteria [8], reaction between Hg^{2+} and the carbonyl groups of the cellulose of the support paper on which plant samples are mounted [9], temperature increases [10], and photolytic reactions [11]. PBM pollution is also present at FI collections: a recent study indicated that the museum particulate is fine (PM2.5) and rich in Hg (up to 530 mg kg^{-1} Hg) [7]. Regarding particulate chemistry, the study indicated that Hg is surprisingly rarely bound to Cl (the original anion in $HgCl_2$), whilst it mostly occurs bound to S. The presence of high amounts of S in the FI collections indoor atmosphere is compatible with the application of fumigation treatments with carbon disulfide (CS_2), which were practised for many years [7,12]. As detailed by scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) microanalyses [7], S was often in a molar ratio of approximately 1:1 with Hg, while O was not detected, suggesting the presence of a Hg sulfide (HgS) in the FI dust.

Until now, no targeted analyses have been carried out directly on the FI botanical samples, at least not in recent days. In 1927 Passerini and Pampanini [2], suggested the presence of Hg-based organometallic compounds (i.e., chemical compounds formed by the binding of Hg and organic matter, such as the plant matrix) on the treated samples and metallic Hg in the dark stains often present on their support paper, as a result of a 5-year long survey based on visual observations and classic analytical chemistry analyses. However, no other identifications had been done to date by modern spectroscopic or mineralogic techniques, and the speciation study of the Hg-based compounds on herbaria plants remains a challenging task, which can be accomplished with non-routinary techniques, such as synchrotron radiation [13], and by destructive techniques, such as thermal desorption [14]. Mercury speciation is fundamental to determine its bioavailability to humans [5,6], and in the risk assessment scenario [6,7]. As an example, the solubility and bioavailability of Hg sulfides is very low if compared to other Hg compounds: the absorption of cinnabar (HgS) by the gastrointestinal tract is very scarce (0.05%) whilst $HgCl_2$ (7–15%) and Hg-based organometallic compounds are almost totally absorbed [15], and finally Hg sulphate ($HgSO_4$) is considered to be highly toxic causing acute poisoning [16]. A full understanding of the Hg speciation in the herbaria is therefore of utmost importance, considering also different exposure pathways: accidental ingestion, inhalation or skin and eye contact [7].

This work aims at evaluating the contamination extent on the herbaria plant samples and their support paper sheets by non-invasive, multi-elemental, quantitative and highly sensitive ion beam analysis (IBA) techniques, focusing on the determination of Hg concentration and its in-depth distribution. Furthermore, IBA techniques were employed for the simultaneous determination of the concentrations of other elements in the samples, and especially of the potential Hg ligands, such as Cl and S.

2. Materials and methods

2.1. Samples

Nine herbarium samples were selected for this study (Fig. 1, Table 1): eight are dated to the 19th century and were most likely treated with corrosive sublimate; a recent one, dated 1984, was certainly not treated with $HgCl_2$, and was used as a blank (CAMP1).

Each sample is made of a dried plant specimen, secured to a ~ 30x50 cm^2 support paper using paper strips and metal pins. An exception is the 19th-century sample named *Plantago*, stored in the deposit and never formally accessioned in the collections, which is kept between two newspaper sheets. Samples FI071379, FI090808 and FI090807 belong to Filippo Parlatore's collection, also known as *Herbarium Parlatoreanum,* part of the FI botanical collections*,* and are dated between 1842 and 1869. They bear the stamp «*avvelenata col sublimato*» (poisoned with sublimate), which testifies that they have certainly been treated with $HgCl_2$. Samples FI064120 and FI063946 are part of Odoardo Beccari's collection (*Herbarium Beccarii,* FI-HB), while samples FI090823 and FI090824 belong to Philip Barker Webb's collection (*Herbarium Webbianum,* FI-W). Both Beccari's and Webb's herbaria are stored in the so-called "Webb Hall", which hosts important historical collections and was identified as the most Hg-contaminated room in previous studies [1]. Even though they do not show the stamp attesting the poisoning treatment, they were likely treated as well, as they date from between the 1820s and 1870s, a period during which the use of corrosive sublimate is well documented. In addition to this group, sample *Plantago*, dating back to 1896, was chosen to represent specimens that never left the deposit area, in order to evaluate the extension of the Hg contamination to other building's rooms.

2.2. Experimental setup and data analysis

External beam IBA measurements, namely particle induced X-ray emission (PIXE) and elastic backscattering spectrometry (EBS), were carried out at INFN-LABEC laboratory in Florence [17] which hosts a 3 MV Tandetron accelerator, using the external beamline set-up dedicated to the analysis of cultural heritage samples [18]. An in-vacuum 3.00 MeV proton beam, collimated at 0.5 mm in diameter, was extracted into ambient pressure at the end of the line through a 200 nm Si_3N_4 membrane. The proton energy at the sample has been estimated to be 2.90 MeV, taking into account the energy loss in the Si_3N_4 extraction window and in an 8 mm atmospheric path from the exit nozzle to the sample. It can be assumed that the 2.90 MeV proton beam does not penetrate through the supporting paper (a heavy paper) nor through the plant sample, both being reasonably hundreds of micrometers thick at least, while 2 MeV protons have a range of about 100 μm in cellulose. Each measurement lasted for about 300 s and the beam current was kept between 200 and 500 pA. The beam current is measured via an indirect method: normalization to the Ni characteristic X-ray yield from a graphite vane with a Ni evaporation, rotating in front of the beam with a frequency of 1 Hz.

The experimental setup included two X-rays detectors for PIXE measurements, each 450 μm thick: a small-area Ketek Silicon Drift Detector (SDD) for light elements analysis (Na-Ca), with an active area of 10 mm^2 (collimated to ~ 4 mm^2), a 10 μm Be window and He flow to reduce X-ray absorption, placed at a 45 mm distance from the sample,

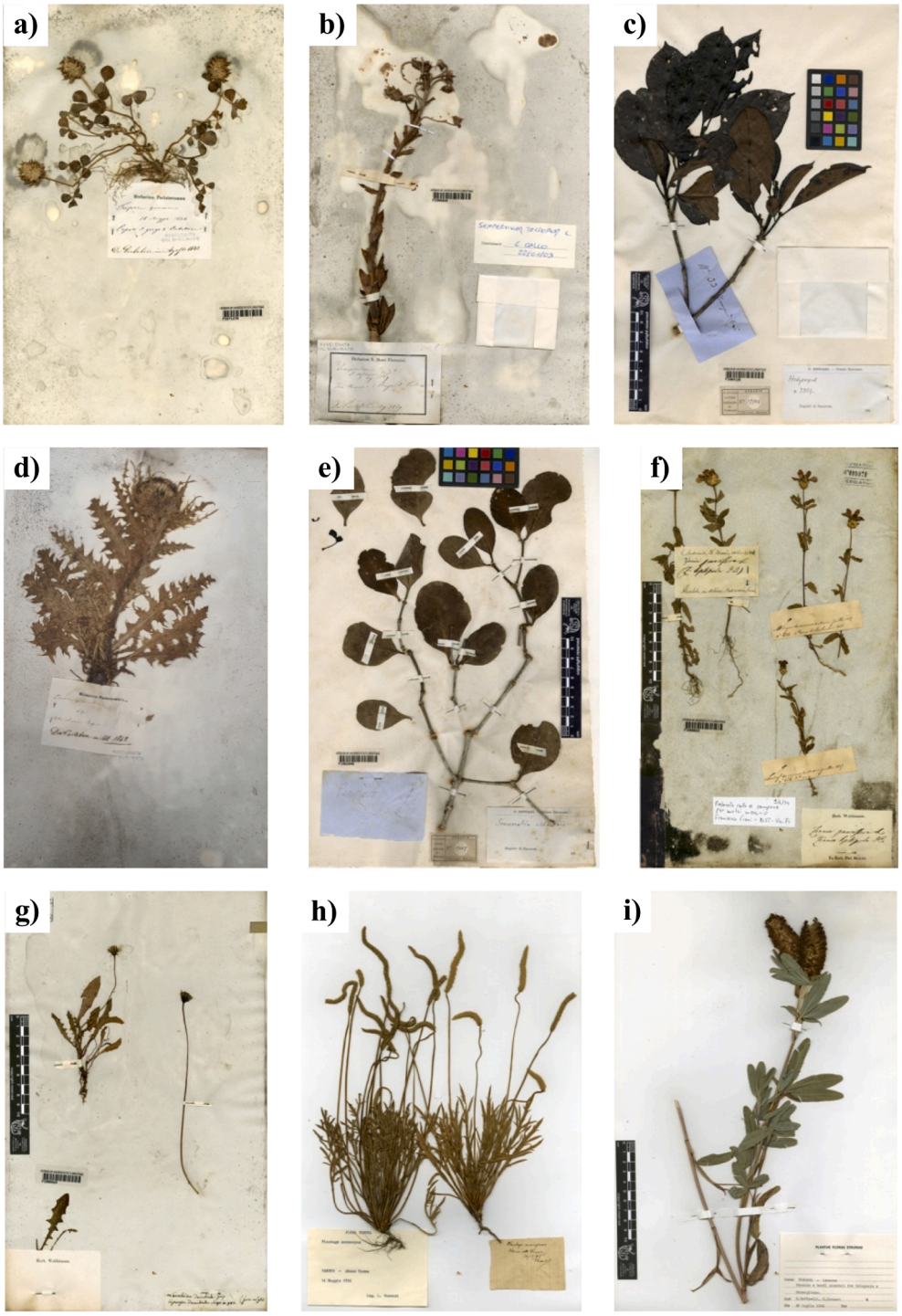

Fig. 1. The herbarium samples selected for the study. a) FI071379; b) FI090808; c) FI064120; d) FI090807; e) FI063946; f) FI090823; g) FI090824; h) Plantago; i) CAMP1.

and a large-area Ketek SDD for medium–high elements analysis (>Ca), with an active area of 150 mm^2, a 25 μm Be window and a 450 μm Mylar filter to cut low-energy X-rays, placed at a 20 mm distance from the sample. The detector used for EBS analysis was a Hamamatsu Si-PIN diode with an active area of 10x10 mm^2 (collimated to 2.5x5mm^2), 300 μm of thickness, placed at a 135° scattering angle at 25 mm distance from the sample in an aluminium case, closed by a 2 μm Mylar foil with a thin Al layer on top, in order to place it under a pressure of 10^{-1} mbar.

The quantitative analysis of PIXE spectra acquired by measuring the samples was performed with the GUPIXWin software [19], after a calibration process that involved measurements on different reference materials including thick high-purity metal foils, thick SiO_2 standard, NIST 610, 620 and 1412 multielemental glass standards, NIST 1573a, 1515 and 1547 dried leaves standards (tomato, apple and peach leaves, respectively), and MicroMatter HgTe and CsBr thin standards, in order to calculate a proper instrumental correction factor (H factor). PIXE analysis provided the concentrations of all the detectable elements (>Na) that could be found in the samples.

The calibration process for EBS measurements involved the analysis of some of the reference materials previously mentioned, namely the

Table 1
The herbarium samples selected for the study. The (s) symbol indicates the presence of the stamp «*avvelenata col sublimato*» ("poisoned with sublimate").

Sample name	Botanical species	Year	Poisoned	Notes
CAMP1	*Trifolium*	1984	No	Not accessioned, blank
FI090823	*Zinnia pauciflora*	before 1831	Yes	Herbarium Webbianum, Webb Hall
FI090824	*Leontodon danubiale*	before 1854	Yes	Herbarium Webbianum, Webb Hall
FI071379	*Trifolium spumosum*	1856	Yes (s)	Herbarium Parlatoreanum
FI090808	*Semprevivum* sp.	1869	Yes (s)	Herbarium Parlatoreanum
FI090807	*Crisium spinosissimus*	1842	Yes (s)	Herbarium Parlatoreanum
FI064120	*Hedycarpus* sp.	1865–77	Yes	Herbarium Beccarii, Webb Hall
FI063946	*Sonneratia alba*	1865–77	Yes	Herbarium Beccarii, Webb Hall
Plantago	*Plantago coroponus*	1896	?	In the deposit (not mounted, not accessioned)

thick high-purity metal foils, the thick SiO_2 standard and the two MicroMatter thin standards, performing the spectra simulations with the SIMNRA software [20] implementing non-Rutherford elastic cross sections from IBANDL [21] and SigmaCalc [22]. The EBS spectra were mostly useful to gather information about the in-depth distribution of the elements in the samples.

Usually in PIXE data analysis, when the goal is to obtain the concentration of certain elements inside a matrix, the composition of the latter should be known. In this case, the composition of neither the support paper nor the plant bulks is known a priori. So, in order to get any valuable results, the PIXE analyst can choose to analyse the data using the thin target approximation offered by the GUPIXWin software, which does not require a matrix composition to be inserted by the user. However, this approach might lead to an over- or an underestimation of the concentration of the considered elements. In some of the present cases, the thin target approximation was probably not far from reality, for instance for the dark spots on support paper in Section 3.1 (concentrations reported in ng cm^{-2}), where in the EBS spectra a peak due to Hg surface accumulation was visible, but in some others, it might have led to a more significant deviation. However, simultaneous EBS measurements allowed to obtain information about the matrix composition, since both support paper and plant specimens are composed of organic materials. Therefore, it was possible to use such information to refine PIXE data analysis of the spectra acquired on the clear support paper and on the botanical specimens by using the thick target analysis option, and thus obtaining elemental concentrations in ppm. The EBS spectra were analysed with the SIMNRA simulation code, fitting only the major elements, with H as "free" element and He as "parasitic" element in the first layer of the sample, using the instrumental parameters obtained from the analysis of thick and thin reference samples, and implementing evaluated non-Rutherford elastic scattering cross sections for proton elastic scattering on He, C, O, and Ca. Using for the analysis of each PIXE spectrum the matrix obtained by the EBS spectrum collected simultaneously would be indeed the optimal approach, but time consuming. However, since the simple superimposition of the different EBS spectra from plant samples revealed very similar structures, hence very similar compositions in terms of the major elements, it was considered sufficient to have a common matrix composition representative of all the plant samples. The same happened for the clear parts of the support paper, so a common matrix composition was considered also in these cases. The average composition of the matrix in terms of the concentrations of the major elements was 4 wt% of H, 46 wt% of C, 46 wt% of O, and 4 wt% of Ca for the support paper, and 4 wt% of H, 50 wt% of C, 42 wt% of O, and 4 wt% of Ca for the plant specimens. This is obviously an approximation, but it saved analysis time since EBS spectra analysis with the SIMNRA software takes a long time to get to an accurate simulation result. In some cases, there were indeed minor compositional changes that nevertheless, when implemented as (slightly) different matrix in GUPIXWin thick target analysis, did not influenced the PIXE results beyond the uncertainties.

3. Results and discussions

The characteristic Hg X-rays peaks were well visible in all the PIXE spectra obtained from both the plant samples and the support paper sheets, with Hg being easily detectable. The only exception was the blank sample CAMP1, where Hg was not detected, as expected (Fig. 2). This confirmed that all the 19th-century FI samples have been treated with the corrosive sublimate, regardless of their date, the botanist who prepared them, and the presence or not of the «*avvelenata col sublimato*» stamp (Table 1).

In addition to Hg, these other elements were also identified in most spots: Mg, Al, Si, P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, and Br. In some cases, non-negligible concentrations of Ti, Sr, and Pb were also detected, although more rarely.

3.1. Support paper

Macroscopically, the support paper sheets are not homogeneous in colour, often presenting irregular dark spots or stains (Fig. 3), which in

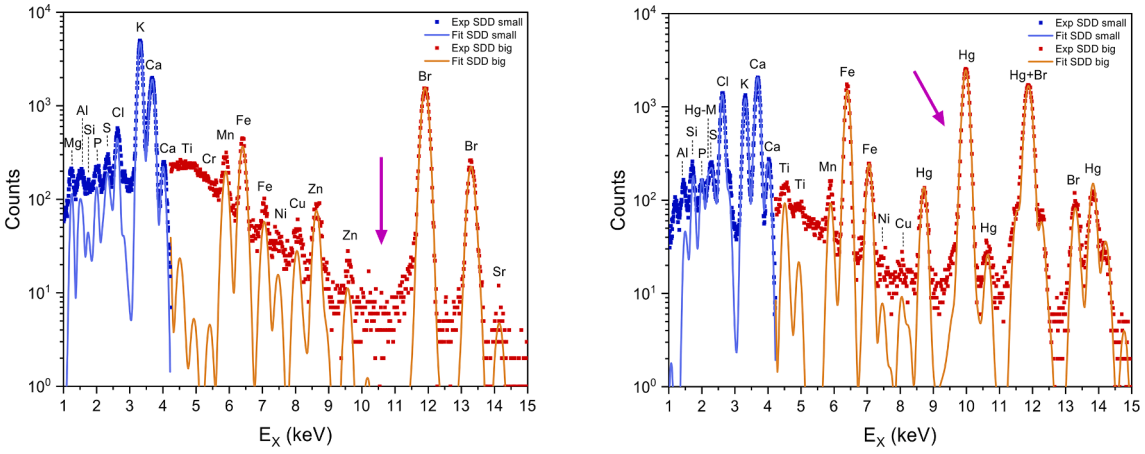

Fig. 2. PIXE spectra obtained with the two SDDs, together with the corresponding GUPIXWIN fit, for the flowers of samples CAMP1 (left) and FI090807 (right). The violet arrow points at the principal Hg X-ray line (Lα at 9.989 keV), which is absent in the spectra of the blank sample (CAMP1).

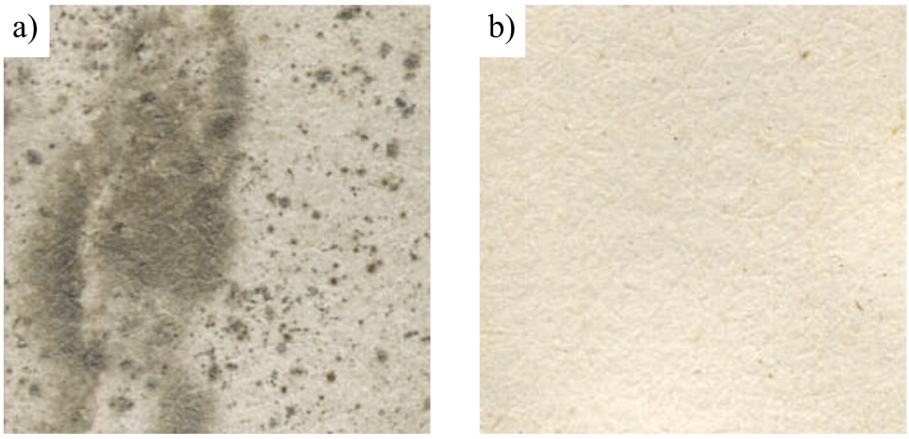

Fig. 3. An example of the irregular dark spots or stains present in the support paper of sample FI090808 (a), in comparison with the clear paper (b) of the same sample.

1927 were reported by Passerini and Pampanini as possible areas where Hg^{2+} is reduced to metallic Hg^0 [2].

The analyses on the support paper were performed with two main goals: first, detect and quantify Hg, and then, define any compositional difference between clear paper and the dark stains visible on some samples.

For what concerns the first point, Hg was detected by PIXE analysis in the support paper of all the samples, with concentrations ranging between 300 and 91,700 ng cm^{-2}. Moreover, a maximum value of 461,690 ± 1110 ng cm^{-2} was measured on a corner of the support paper of sample FI090823 (Fig. 1f), in correspondence of an exceptionally dark stain, not found in any other sample.

The detection of Hg not only in the plants but also in the support paper sheets, suggests that the $HgCl_2$ solution contaminated the paper as well. Considering the methods by which corrosive sublimate could have been applied, listed by Mattirolo in 1900 [12], the application by spray and, possibly, the immersion of whole herbarium folders, seem to be the most compatible ones with the detection of Hg in the paper, while the application by brush and the immersion of single plant specimens are less likely.

The comparison between the Hg concentrations measured on clear and dark spots showed remarkable differences: Hg concentration in the dark stains was at least three times higher than that measured on clear support paper (Fig. 4, Table 2). Moreover, EBS spectra allowed to highlight another important difference regarding the in-depth distribution of Hg: dark stains are the result of surface Hg accumulations, whilst the same element is distributed more homogeneously into the paper in the clear areas (Fig. 5). However, in order to compare directly the very different Hg concentrations between dark and clear spots on the support paper (Table 2), it was chosen – for those cases – to assume the samples always as thin targets, otherwise the Hg concentration would have been expressed in ng cm^{-2} in one case and in ppm in the other.

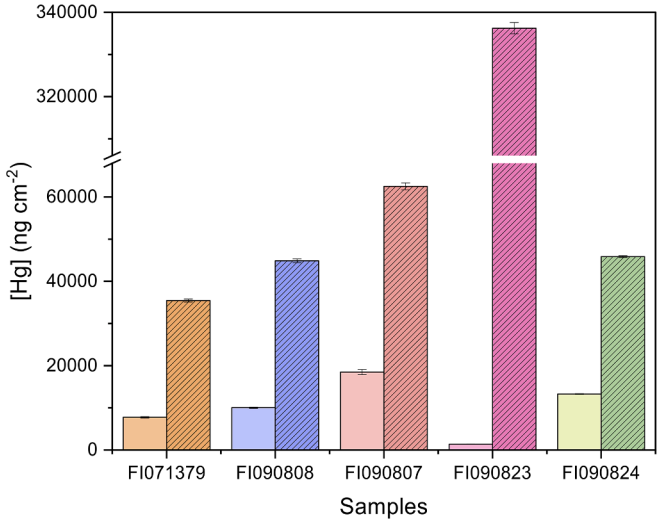

Fig. 4. Comparison between the mean concentrations of Hg measured in the clear spots (plain background) and in the dark spots (striped background) of the support paper of the herbarium samples.

Regarding the other elements, particular attention has been put into the quantification of Cl, the anion originally present in the applied sublimate, and S, already found in association with Hg in previous studies on the PBM [7].

In clear support paper (Fig. 6a), the concentration of these elements was found to be really variable between the samples. Moreover, the Hg concentration was always lower than that of Cl and S, highlighting that the presence of these two elements in the paper is not only linked to Hg compounds. Cl is indeed commonly present in paper materials due to the use of chlorine bleach in paper production [23,24], while the ubiquitous presence of S is probably to be attributed to the CS_2 fumigations performed at the FI collections against pest infestations [3].

In the dark spots (Fig. 6b), Hg was found in concentrations from a minimum of 35,440 ng cm^{-2} to a maximum of 336,200 ng cm^{-2}, ten times higher than the concentration of all the other elements detected in every sample. This is fully consistent with the evidence provided by EBS spectra, which showed that these dark stains are the result of a surface Hg enrichment.

Some interesting hypotheses on the Hg-based compounds that might be present in these samples can be deduced from Hg:X correlation graphs, where X is alternatively Cl or S. For clear support paper, all the graphs showed poor correlation significance, a result that can be

Table 2
Comparison between the mean concentrations of Hg on the clear paper and dark stains on the support paper of the herbarium samples measured by PIXE in the thin target approximation. The quoted uncertainties are the square root sum of the fitting and statistical uncertainties from GUPIWin (5–10%), of the uncertainty on the measured charge (1–2%) and of the uncertainty on the measured H factors (2.5% and 4.5% for small and large detector, respectively).

Sample	Hg [ng cm^{-2}] in clear support paper	Hg [ng cm^{-2}] in dark stains
FI090823	1354 ± 43	336200 ± 1400
FI090824	13260 ± 120	45860 ± 220
FI071379	7750 ± 170	35440 ± 350
FI090808	10030 ± 160	44870 ± 450
FI090807	18470 ± 600	62520 ± 820
FI064120	310 ± 35	–
FI063946	360 ± 20	–

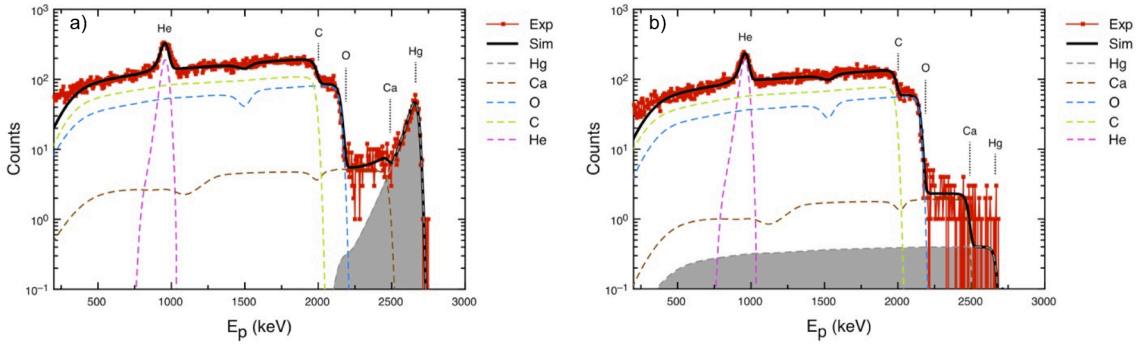

Fig. 5. Comparison between EBS experimental spectra with the SIMNRA simulations for a dark spot (a) and a clear spot (b) on the support paper of sample FI090807. The contribution of the different elements to the simulations is also shown. He is not present in the sample itself, but it is a "parasitic" element, common to EBS spectra when measurements are performed in an external beam set-up under helium flowing.

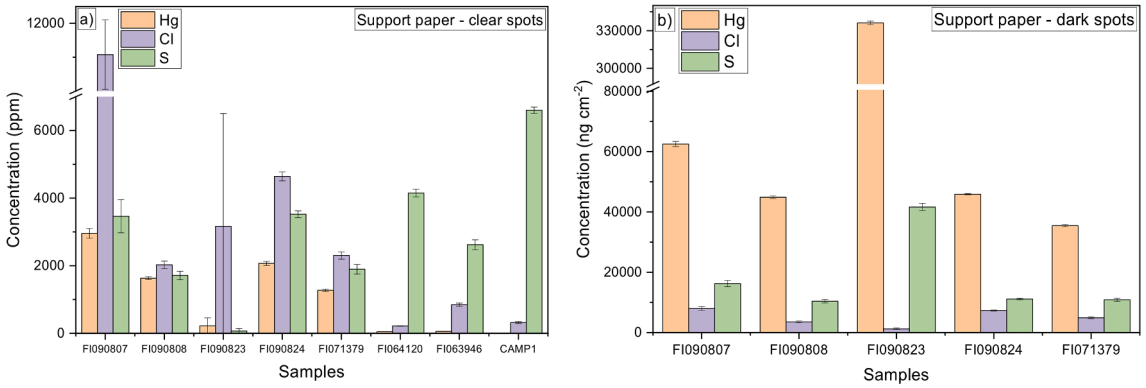

Fig. 6. Concentrations of Hg, Cl and S measured with PIXE on: a) clear support paper, obtained using the thick target solution with a fixed matrix, b) dark spots on paper, obtained using the thin target solution.

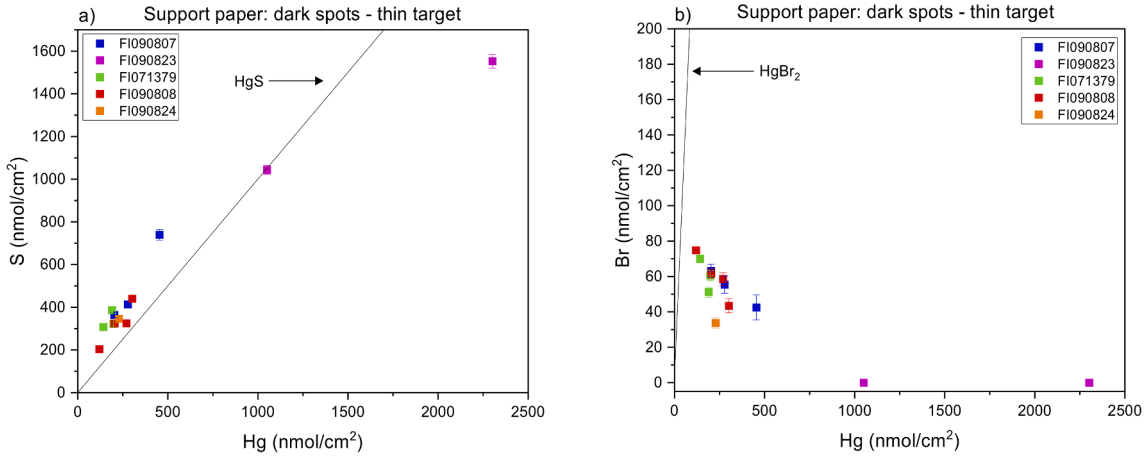

Fig. 7. Correlation plots between Hg and S (a), and Hg and Br (b) for the dark spots on paper, expressed in nmol cm^{-2} by dividing the concentration in ng cm^{-2} for the atomic mass of the single element.

explained also by the fact that it is difficult to separate the contribution of Cl and S coming from the support paper, from the one coming from Hg-X compounds. Indeed, it is nearly impossible to define a common composition of the samples in order to subtract the paper contribution from the total Cl and S concentrations, since these support paper sheets were produced in different years, possibly by different manufacturers and even in different places.

On the contrary, in the case of the dark stains the Hg:X plots showed a good linear correlation between Hg and S, suggesting that these two elements might be bound (Fig. 7a). Moreover, it has already been mentioned that EBS analysis indicated that the stains correspond to surface Hg enrichment. If they were composed solely of metallic Hg, as proposed by Passerini and Pampanini [2], the characteristic S X-rays (Kα line at 2.308 keV) originating only from the underlying material should be attenuated. Therefore, this hypothesis seems unlikely. At the same time, the Hg-S linear correlation does not reflect the 1:1 ratio expected for mercury sulphides (HgS). All these elements suggest that in the dark stains there might be a co-presence of HgS (most likely the black-coloured metacinnabar) and possibly metallic Hg and/or organic Hg compounds, whose presence could not be determined by IBA analysis.

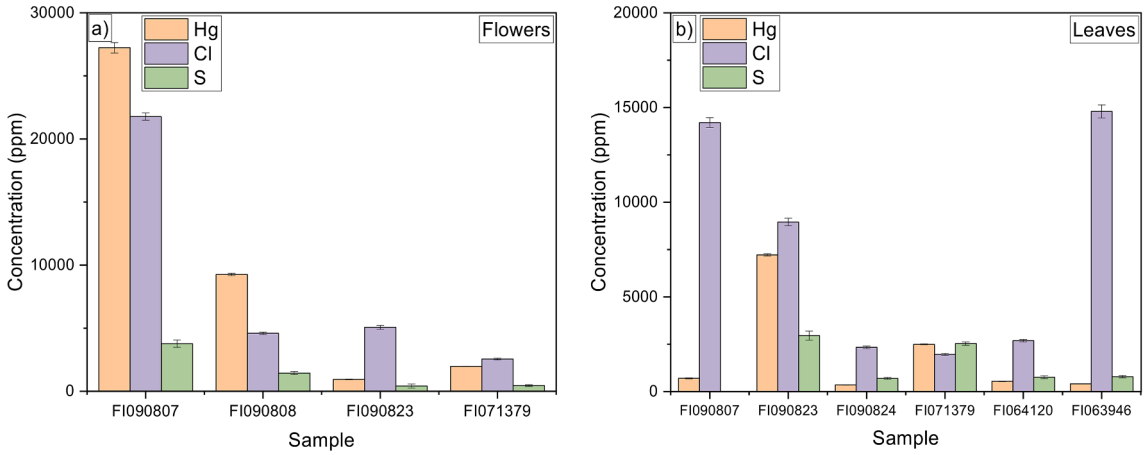

Fig. 8. Concentrations of Hg, Cl and S measured on flowers (a), and leaves (b) of the botanical specimens, obtained from PIXE using the thick target solution with a fixed matrix.

Noteworthy, X-ray absorption near edge structure (XANES) spectroscopy performed on herbarium samples of the University of Manchester in a previous study [13] also identified metacinnabar (β-HgS) as the main Hg compound.

It is interesting to mention that Br was also identified in most cases in the analysed paper spots. Its presence can be explained by the fumigation treatments with CH_3Br [3], used as a pesticide until recent times in the FI collections. This element has been found to be ubiquitous and cannot be directly correlated to Hg, as shown by the Hg:Br correlation graph (Fig. 7b).

Notably, also Purewal et al. studied the support paper of the herbarium samples of the National Museum of Wales using PIXE analysis [25], detecting Hg concentration values similar to the ones resulting from this study for the clear paper spots. It is relevant to point out the fact that they also detected As, an element typically related to pesticide treatments [2,26]. This element, however, was never detected in the FI samples, thereby suggesting that the past use of this compound in the FI collections is unlikely.

3.2. Botanical specimens

The Hg concentrations measured on the dried plant specimens differed from one sample to another, but in general, they were systematically higher than in clear support paper. Moreover, higher Hg amounts were detected on thicker parts (i.e., flowers, Fig. 8a) than in thinner ones (i.e., leaves, Fig. 8b). The same consideration is applicable for the other considered elements (Cl, and S), suggesting that thicker portions might have retained a higher amount of the substances applied over time.

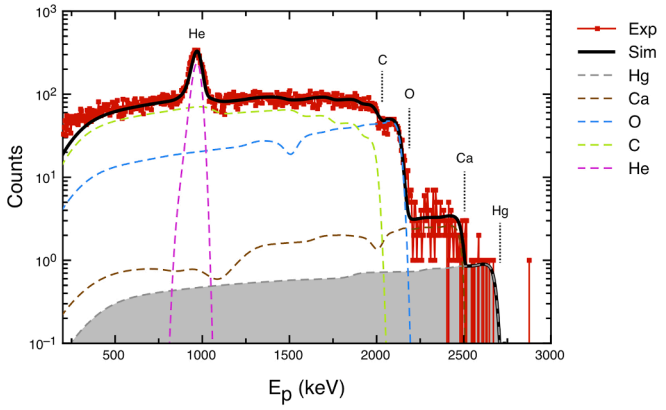

Fig. 9. EBS experimental spectrum with the SIMNRA simulation for the flower of sample FI090807. The contribution of the different elements to the simulations is also shown. He is not present in the sample itself, but it is a "parasitic" element, common to EBS spectra when measurements are performed in an external beam set-up under helium flowing.

In plant specimens, Cl content was much higher than in support paper sheets, being after Hg the second – and even the first in a few cases, overcoming Hg – most abundant detected element (Fig. 8). This might be due to the persistence of *sublimate* residues in the plant specimens or to other Cl-based compounds derived from its decomposition, but also to the presence of Cl in the original plant specimens, being chloride an essential plant micronutrient crucial, for instance, for photosynthesis. Since a more accurate speciation is not possible, these remain hypotheses. However, as for clear support paper, the Hg:X (X = S, Cl) correlation graphs did not highlight significant results, neither for the flowers nor for the leaves.

Furthermore, as regards EBS spectra acquired from plants, a similar behaviour to that of clear support paper was observed: Hg penetrates deeply into the botanical matrix, rather than accumulating on the surface. This is particularly evident when comparing EBS spectra acquired on a dark spot on support paper (Fig. 5a) and on a plant specimen (Fig. 9), in which the different Hg distribution is clearly visible. What is really interesting is that for these same two spots, Hg concentration measured with PIXE was found to be nearly the same.

As regards the extension of the contamination to the deposit area, the sample named *Plantago* was taken as a representative one. Dating back to 1896 but never officially part of the collections, the plant matrix of this sample was also found to be contaminated: a Hg concentration of 89 ± 4 ppm has been measured. Although significantly lower than the ones measured on other samples (ranging between 350 ± 9 and 27,230 ± 410 ppm), this value is not negligible. This might indicate that the treatment with the sublimate solution was extended also to samples in the deposit area, or that it is the result of a cross-contamination with the other samples of the collections, which are stored on the same floor. However, the latter hypothesis appears to be less plausible, since blank sample CAMP1 has also been stored in the deposit for a long time (more than 30 years), but no traces of Hg were detected on it.

4. Conclusions and perspectives

This study revealed that contamination affects both the plant matrix, and the support paper of the FI botanical collections specimens. The results thus confirm the persistent presence of Hg, with a mean concentration of 47,170 ± 460 ng cm^{-2} (but that could reach up to 461,690 ± 1110 ng cm^{-2}) in the dark spots on paper, 1181 ± 74 ppm in the clear paper, and 5902 ± 81 ppm in the plant matrix, where Hg penetrates

deeply. As regards the support paper, a notable difference was observed between the Hg concentrations and distributions measured on clean paper and on dark, irregular stains. In the latter ones, high Hg concentrations are associated with surface accumulations of Hg, or Hg-based compounds, whilst in clear support paper lower concentrations of Hg are distributed in depth in the paper matrix. Interesting associations were found between Hg and S in several samples, suggesting the possible presence of HgS or other Hg- and S-based compounds. In the reference blank sample, collected in the 1980 s, Hg was not detected. Differently, the sample stored in the deposit area from the end of the 19th century showed non-negligible Hg levels (89 ± 4 ppm), albeit significantly lower than those observed in historical collection specimens.

Ion beam analysis techniques, namely PIXE and EBS, allowed the gathering of valuable and complex information regarding the concentration and distribution of Hg, Cl, and S in the different components of the FI samples, in a non-invasive and non-destructive way, with important implications for both the management of herbarium collections and health and safety protocols. They confirmed that 19th-century specimens were likely treated with $HgCl_2$, even when no explicit indication of poisoning is present ("poisoned with sublimate" stamp). The detection of Hg not only on the plant matrix but also on the support paper indicates that the paper itself can act as a source of GEM, coming from $HgCl_2$ decomposition, and that both specimens and their supports should be handled with caution, using appropriate personal protective equipment (PPE), such as gloves. This applies also to samples held in the deposit, which, if collected in the 19th century, are likely to have been treated with $HgCl_2$ as well. Moreover, the data acquired in this study enabled the generation of new and interesting hypotheses on the evolution of the contamination process over time. Hg was applied as a chloride, but its speciation changed over time. The availability of S in the atmosphere, probably due to the CS_2 fumigations, might have favoured the formation of HgS compounds, most likely metacinnabar, which have low bioavailability for humans; however, the presence of Hg^0 and other Hg-based organometallic compounds, not directly identifiable by IBA, is probable. The presence of Hg^0, if confirmed, would be consistent with the observed GEM emissions in the herbaria halls.

Although not exhaustive for a complete understanding of the Hg contamination in herbaria, this study allowed a first investigation on museum herbaria specimens in a non-invasive way, thus guiding the research on this topic to move towards the application of different and targeted analytical methods, in order to get to a full comprehension and accessibility of these precious collections.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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