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Characterizing arsenic in preserved hair for assessing exposure potential and discriminating poisoning

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Advanced analytical techniques have been used to characterize arsenic in taxidermy specimens. Arsenic was examined to aid in discriminating its use as a preservative from that incorporated by ingestion and hence indicate poisoning (in the case of historical figures). The results are relevant to museum curators, occupational and environmental exposure concerns, toxicological and anthropological investigations. Hair samples were obtained from six taxidermy specimens preserved with arsenic in the late 1800s and early 1900s to investigate the arsenic incorporation. The presence of arsenic poses a potential hazard in museum and private collections. For one sample, arsenic was confirmed to be present on the hair with time-of-flight secondary ion mass spectrometry and then measured with neutron activation analysis to comprise 176 μg g^{-1}. The hair cross section was analysed with synchrotron micro-X-ray fluorescence to investigate the transverse distribution of topically applied arsenic. It was found that the arsenic had significantly penetrated all hair samples. Association with melanin clusters and the medulla was observed. Lead and mercury were also identified in one sample. X-ray absorption near-edge spectroscopy of the As *K*-edge indicated that an arsenate species predominantly existed in all samples; however, analysis was hindered by very rapid photoreduction of the arsenic. It would be difficult to discriminate arsenic consumption from topically applied arsenic based on the physical transverse distribution. Longitudinal distributions and chemical speciation may still allow differentiation.

Keywords: ToF-SIMS; synchrotron X-ray fluorescence; X-ray absorption near-edge spectroscopy; photoreduction; occupational exposure; toxicology.

1. Introduction

There are many circumstances in which individuals can be exposed to occupational hazards, not least of which can include dealing with materials that have previously been in common use but have since been demonstrated to be much more of a hazard than originally accepted. Arsenic, in particular, has been used for an almost bizarre range of applications, providing a range of useful functions including the treatment of leukemia, but on the other hand has been attributed to numerous deaths, murders and other health implications of millions of people (Bentley & Chasteen, 2002). One scenario involves taxidermy specimens preserved with arsenic-based treatments. Traditionally, arsenic treatments were the optimal choice for preserving specimens to aid in longevity and detract from insect or microbial degradation, for which arsenic has performed exceptionally well with excellent persistence. Unfortunately the arsenic treatments generate a toxic hazard to humans. Such treatments were still common in professional and amateur contexts well into the 20th century, and, as such, has generated an immense quantity of arsenic 'contaminated' material within museum collections and homes. For instance, in the natural history collections from several museums, 530 out of 656 specimens analysed contained arsenic (Sirios, 2001).

This work was primarily motivated by the interest in discriminating arsenic in hair used for preservation from arsenic that may exist owing to poisoning (such as in the case of historical figures). This component of the research has investigated the prevalence, origins and ultimately the physical distribution and chemical speciation of arsenic in hair from preservation practices. The data acquired, however, are of relevance to health considerations. Museum collection environments can present significant toxicological risk to

curators and other people involved in handling specimens (Krake *et al.*, 1999; Schieweck *et al.*, 2005). The objective of this work was to study the occurrence, distribution and chemistry of arsenic in the hair of taxidermy specimens. Unfortunately it was rare for details of preservation methods to be recorded in many museum collections (Goldberg, 1996). However, the most common preservative treatment in the late 1800s to early 1900s included application of arsenical soap, typically comprised of white soap (2 lb), powdered 'white arsenic' (arsenic trioxide) (2 lb), camphor (5 oz), subcarbonate of potash (6 oz) and alcohol (8 oz) (Hornaday, 1916; Davie, 1900). The soap would be diluted with water and applied with a brush directly onto the specimen. Lead arsenate was also used from 1892 (Peryea, 1998) to preserve specimens against insect degradation, although does not appear to be frequently reported, as with mercury-based applications (Goldberg, 1996; Sirios, 2001).

In the context of deducing occupational hazards and considerations in handling, cleaning or disposing of such treated specimens, it is of interest to know where the arsenic resides to be aware of the potential threat it poses to individuals or the environment. Simultaneously, in cultural heritage studies, specimens can be of immense value based on the rarity of the specimen and/or the monetary cost. In either case, analysis should be carried out by the least destructive means. Often, it is not possible to perform measurements *in situ*, hence a small sample may be acquired. While inexpensive and rapid means are available to detect arsenic in a variety of media (Agrawal *et al.*, 1999), it was of interest here to look at the micro-distribution and chemistry of the arsenic. A variety of modern techniques, such as time-of-flight secondary ion mass spectrometry (ToF-SIMS) (Kempson *et al.*, 2003), synchrotron micro-X-ray fluorescence (micro-XRF) (Kempson *et al.*, 2006), micro-X-ray absorption near-edge structure (micro-XANES) (Proost *et al.*, 2004) and neutron activation analysis (NAA) (Meloni *et al.*, 2000), provide a combination of highly sensitive, accurate, chemically informative and spatially resolved methods that enable the acquisition of results utilizing a minimal amount of material.

To characterize the arsenic in taxidermy specimens, the spatial distribution was resolved with ToF-SIMS and synchrotron micro-XRF, and the chemical state with micro-XANES. Sirios (2001) emphasized the relevance of the arsenic distribution. Obviously arsenic on external surfaces of exhibits is readily accessible to becoming airborne or transferred during physical contact. Spatial analysis investigated the extent to which the arsenic treatment penetrated the hair, or if it existed in a purely superficial extent on the outer surface. ToF-SIMS is sensitive to the first one to two monolayers of a sample giving an accurate indication of the surface composition. High-resolution XRF maps were sought to identify the localization of arsenic in a hair cross section. Arsenic on the outside of the hair is a more serious threat for exposure than if it resides internally. In addition, if there is cause to restore specimens to a non-toxic state, by washing for example, then it is of interest to know how involved this could be. In a toxicology perspective, if arsenic has penetrated the hair, it adds complexity in interpreting the occurrence of arsenic as being from biogenic incorporation (*i.e. via* consumption) as opposed to the exogenous application.

Oxidation state information acquired through XANES analysis is valuable in this case since the bioavailability and toxicity of arsenic is highly dependant on its chemical form. Original application of the arsenic treatments was most likely to have involved arsenic trioxide, or white arsenic, in an arsenical soap. In this form, arsenic has been solubilized and is in a highly toxic trivalent form which can be readily absorbed by individuals. However, after interacting with the hair structure and persisting for periods up to over 100 years, it was of interest to investigate the arsenics' current chemical form. XANES is a very sensitive technique for characterizing arsenic chemistry in any media including biological samples (Smith *et al.*, 2005, 2009). Knowledge of this has importance in assessing risks (occupationally or domestically) in exposure to the arsenic and is valuable for determining the optimal way to handle, clean or dispose of specimens. Assessing the chemical form also offers an additional means to discriminate arsenic that has been used for taxidermy from arsenic that is in hair owing to consumption.

2. Materials and methods

2.1. Taxidermy hair specimens

A variety of taxidermed hairs were collected for examination of their arsenic content. Hair from six museum animal exhibits were collected comprising a yak, musk ox, two horses, a mandrill and a zebra (from which white and black hairs were obtained), providing seven different hair samples. All exhibits were suspected of being preserved with arsenic in the late 19th and early 20th centuries.

Two hairs from the mandrill (*Mandrillus sphinx*), which had been taxidermed in October 1895, were used for a highly detailed analysis of the arsenic composition and distribution. An individual strand of hair had been analysed with NAA to provide a bulk quantitative value for the amount of arsenic of 176 μg g^{-1} in a 50 μg sample. A segment of the other hair was studied with ToF-SIMS to investigate the arsenic residing on the surface of the sample. Another segment was embedded in an epoxy resin and transverse sections of approximately 1 μm thickness were obtained using a microtome. These were subsequently mounted onto adhesive Kapton tape and used for elemental mapping by micro-XRF. Another whole segment was used for the micro-XANES analysis.

The other six hair samples were used for studying the transverse arsenic distribution by scanning across whole hairs with micro-XRF. Comparisons with a mathematical model were used to infer the transverse profiles (Martin *et al.*, 2005). The chemical state in each of these samples was also measured.

2.2. ToF-SIMS

ToF-SIMS is a surface analytical technique providing monolayer sensitivity, sub-p.p.m. detection limits and is an

excellent analytical method for studying trace surface compositions (Denman *et al.*, 2008). An ION-TOF (GmbH) reflectron TOFSIMS IV with a Cs^+ primary ion beam was utilized at Surface Science Western, University of Western Ontario, Canada.

2.3. Micro-XRF

Micro-XRF offers an analytical probe with excellent limits of detection and spatial resolution for biological analysis (Martin *et al.*, 2004; Smith *et al.*, 2009). In addition, the tunability of the incident energy allows for optimization for acquisition of data. For instance, in this case, to map arsenic, an energy was selected for which the arsenic *K*-edge was excited without inducing spectral interference from the lead L_{III}-edge. Detection limits have been published in the attogram domain in a 1 s acquisition (Lai *et al.*, 2004), and sub-200 nm beam sizes have been published for hard X-rays since 1999 (Yun *et al.*, 1999).

X-ray fluorescence mapping of the mandrill hair section was performed with a synchrotron X-ray source at the 2-ID-E beamline at the Advanced Photon Source, Argonne National Laboratory, USA. An incident energy of 12 keV generated by an undulator insertion device was used to excite the As *K*-shell electrons. A region 12 µm wide by 42 µm high was scanned with 0.5 µm and 0.3 µm steps in the horizontal and vertical directions, respectively, utilizing a 400 nm beam. Data for each pixel were acquired over a total live time of 2 s. Data were analysed with the *Maps* software produced by Stefan Vogt (Advanced Photon Source, Argonne National Laboratory, USA) to generate chemical images of the sample. The other six samples were mapped at the PNC-CAT 20-ID beamline at the Advanced Photon Source with an undulator source and monochromatization utilizing a Si(111) crystal. A beam focused to 4 × 20 µm was used to analyse the arsenic in whole hair samples. Fluorescent X-rays were detected with a Canberra seven-element Ge detector and analysed with *2-D Scan Plot* (PNC-CAT, in-house software).

2.4. XANES

As *K*-edge XANES analysis was performed at the PNC-CAT 20-ID beamline, described above. Owing to the occurrence of photoreduction of the arsenic, rapid acquisitions were performed across the white line energies prior to more extended scans. The rapid scans spanned an energy range from 11.866 keV to 11.877 keV in 0.25 eV increments. Data were acquired for 1 s per point; however, with 'overheads', each scan required 45 s. The beam was impinging upon the sample for the entire scan but the shutter was opened immediately before the scans. The longer scans spanned from 11.717 keV to 12.110 keV. The pre-edge region was incremented in 10 eV steps up to an energy of 11.850 keV. Increments of 0.5 eV were used to span 50 eV over the edge before returning to increments of 5 eV post-edge. The longer scans required 326 s per scan. Energy calibration was performed by two means. For the short scans, calibration was performed by introducing a grid into the incident X-ray beam to induce scatter through a standard dimethylarsenic acid sample and onto a photodiode to simultaneously record a transmission spectrum for every scan. The longer energy scans allowed the placement of a gold foil succeeding the sample. Ionization chambers were used for the simultaneous acquisition of the Au L_{III}-edge (11.9197 keV). The error in the energies quoted is ∼0.25 eV.

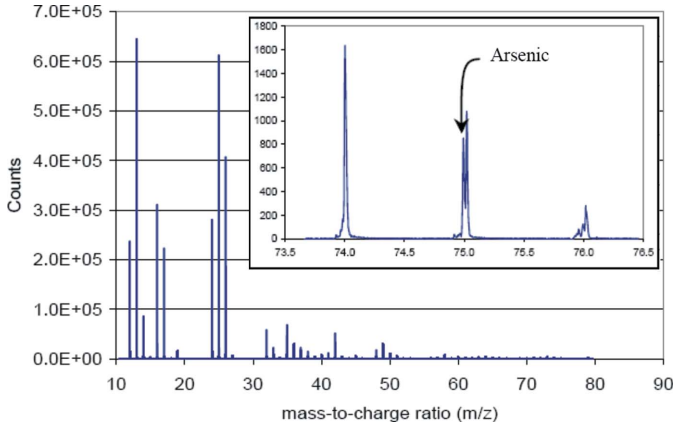

Figure 1
A ToF-SIMS positive ion spectrum confirming the presence of arsenic on the outer surface of the mandrill hair sample. The small peak intensity suggests a low concentration.

3. Results and discussion

3.1. ToF-SIMS

The presence of arsenic on the outer surface of the mandrill sample was confirmed with ToF-SIMS (Fig. 1). The As^+ ion intensity was quite weak suggesting minimal arsenic was present on the outer surface of the hair. While ToF-SIMS peak intensities do not give direct quantitative information, the amplitude of the As^+ peak appeared considerably smaller than anticipated based on the 176 p.p.m. bulk concentration from topical application.

3.2. Micro-XRF

An optical micrograph has been reproduced of the mandrill hair cross section in Fig. 2. The area analysed by XRF is indicated by the boxed region. The central medulla is clearly visible, as are the clusters of melanin granules embedded within the cortex. It was assumed that arsenic was evenly distributed longitudinally and this section was representative of the length of the hair. X-ray fluorescent maps of the mandrill hair cross section are provided in Fig. 3. Sulfur is indicative of the proteins comprising the keratinous structure. Distinct correlations are observed between the elements examined and the clusters of melanin granules and the medulla. Iron exhibits slightly less accumulation within the medulla than the other elements. The bulk of the hair, the cortex, does not contain appreciable quantities of any trace element detected here. A portion of the elements in the hair arise through endogenous incorporation from when the animal was alive; however, as demonstrated by the arsenic distribution, contamination can penetrate into the hair. It is

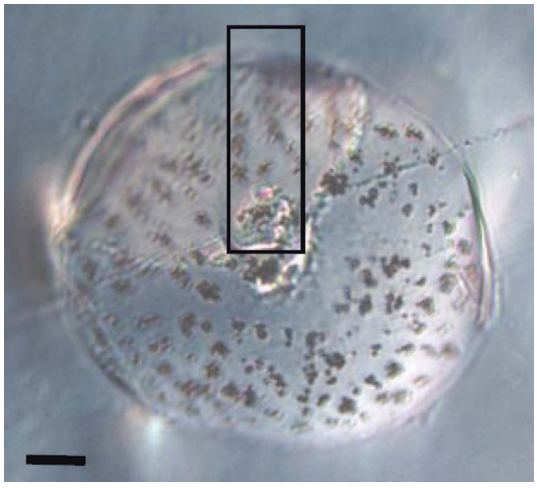

Figure 2
A microscope image of the mandrill hair cross section. Dark clusters of melanin can be seen within the cortex surrounding the denatured central medulla. The boxed region represents the area mapped by XRF. Scale bar = 10 μm.

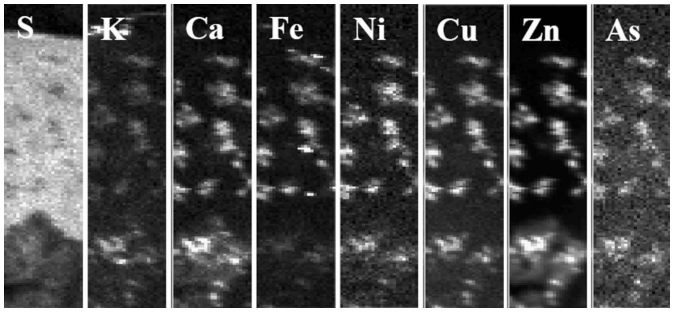

Figure 3
XRF elemental maps of the mandrill hair cross section. Sulfur highlights the hair proteins, particularly in the cortex. Clusters of melanin polymers exhibit accumulations of the other elements presented, as does the medulla (bottom of image). Images are 12 × 42 μm.

therefore difficult to comment on whether the other metals are from endogenous or exogenous incorporation mechanisms. It is most likely that they are a conglomeration of both. It is interesting to note that there was no significant amount of arsenic detected at or near the surface of the hair. In addition, the arsenic has not uniformly penetrated and distributed through the hair. It has, rather, penetrated the hair and preferentially associated with the clusters of melanin granules and in the central medullary canal. This demonstrates several issues in attempting to apply hair mineral analysis to bio-monitoring applications in general. These results have shown that trace elements can penetrate hair and preferentially associate with discrete features of the hair structure. There are diverse opportunities for chemical incorporation in hair from endogenous and exogenous sources (Kempson & Skinner, 2005; Kempson *et al.*, 2006). A spectrum obtained from the hair at higher excitation energy (15.4 keV) indicated that both lead and mercury were also present in the hair sample (Fig. 4). The mercury content could be an additional toxic ingredient to contribute to the preservative properties of the treatment. It

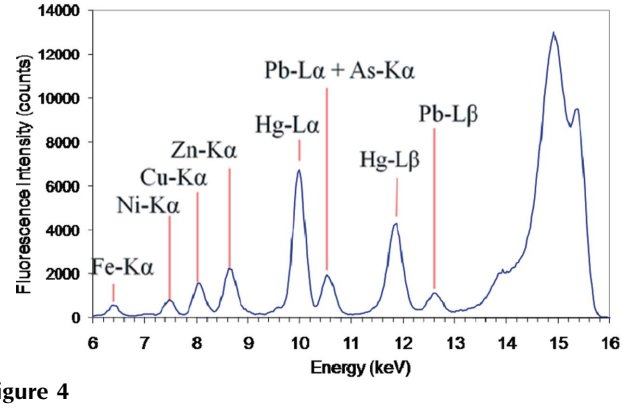

Figure 4
XRF spectrum of the mandrill hair demonstrating the presence of an array of toxic elements: arsenic, lead and mercury.

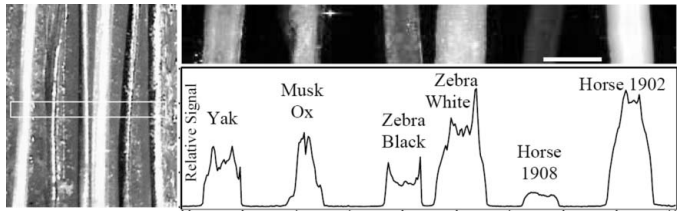

Figure 5
Analysis of arsenic in the hair of a variety of taxidermy specimens. The optical micrograph (left) shows the arrangement of six hairs that were mapped with micro-XRF for arsenic (top, scale bar = 200 μm). The line-scan (bottom) across these samples reveals a surface component as well as significant penetration into the hairs.

has been assumed that the arsenic is from taxidermy processes rather than consumption by the animals. It is unlikely that these animals would have been fed arsenic, and if they consumed a fatal dose it would not appear in the distal regions analysed here.

Fig. 5 presents an optical micrograph of the other hair samples analysed with XRF. Arsenic was clearly present in all samples and the line-scan reveals the distribution of arsenic across the samples. The line-scan profiles can be studied in terms of the internal transverse distributions by fitting with a simple model. By comparing the line profiles here with those presented by Martin *et al.* (2005), it is clear that arsenic in all of these samples has penetrated the hair. In all samples other than the zebra hairs, it appears that the far majority of arsenic occurs within the hair structure. It could be a reasonable assumption to presume that topically applied hair treatments would exist, at least, primarily superficially. These data demonstrate that this is not accurate. Arsenic applied in the preservation of hair penetrates the fibre and is not restricted to the outer surface.

3.3. XANES

Toxicity of metals is dependant on their chemical form and can greatly vary the risk associated with exposure. As *K*-edge XANES was obtained from the musk ox hair (Fig. 6), and was performed to investigate the chemical state in which the arsenic was present. Initially it had been believed the arsenic

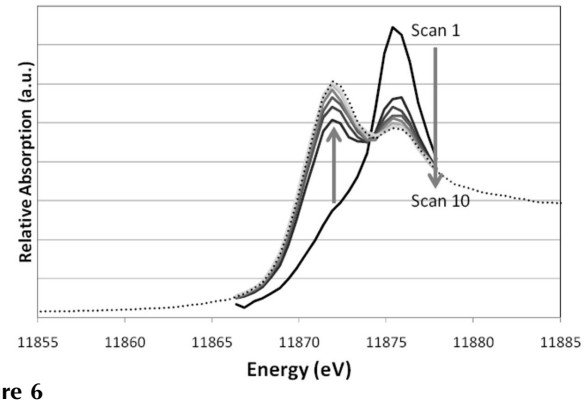

Figure 6
As *K*-edge XANES spectra from the musk ox sample exhibiting very rapid photoreduction of As(V) to As(III). The duration of each of the first nine scans was 45 s each.

existed as As(III), before it was realised that photoreduction was occurring. This process was so rapid that almost complete reduction could occur in a single scan and provided misleading results. As such, rapid XANES scans were performed over the white line energies in an attempt to deduce the peak position(s) before any photodamage. The first scan from all samples exhibited a dominant peak at higher energy (roughly 11875 eV). By the second scan, the lower-energy peak was significantly enhanced at the expense of the reduced higher-energy peak. The initial position of the higher-energy peak is consistent with what is expected for a higher oxidation state and suggestive of an arsenate species, as opposed to arsenic trioxide (commonly used for preservation). All hair samples exhibited an almost identical trend, but with differing initial ratios in the two peaks' intensities from the first scan. The higher-energy peak was always dominant, however. The initial arsenate state was somewhat surprising since arsenic was typically applied in a trioxide form as an arsenite. It is possible that in all of these samples an arsenate had been applied. The presence of lead in the madrill sample could then suggest a lead arsenate. Alternatively, it could indicate that, after application, any arsenite is progressively oxidized into the arsenate state. This latter scenario is quite conceivable since the exhibits have been exposed to air for of the order of 100 years. Sulfur is abundant in hair and is quite possibly involved in the reduction of the arsenic in the presence of the analysis beam. This photoreduced form is similar to the XANES spectroscopy presented by Gault *et al.* (2008) who analysed arsenic in hair from Cambodian individuals exposed to arsenic-contaminated water used for cooking and washing. Toxicity of the arsenic is also dependent upon the solubility of a compound. The arsenic was most likely to have been applied in solution form, and hence existed in a soluble form of greater bioavailability. However, aging of the arsenic and interactions with the hair composition and other metal ions could reduce the bioavailability.

4. Conclusions

While it is not feasible to apply the suite of techniques utilized here for routinely scanning large numbers of samples, this research demonstrates factors that should be taken into account when contemplating the potential hazards of taxidermy specimens treated with arsenic-based preservatives. For instance, the total quantity of arsenic can be quite substantial (the concentration was 176 $\mu g\ g^{-1}$ in the instance of the mandrill). Contrary to what may be expected, the distribution of arsenic is predominantly inside the hair, despite being topically applied. This adds some comfort in reducing physical exposure while handling such specimens, and reduces the threat of the arsenic becoming airborne. The form of the arsenic is difficult to predict and may exist in multiple states. The toxicity is therefore also unpredictable. Other toxic metals, lead and mercury, can pose additional threats. Handling, cleaning or disposal of such specimens should be done so with consideration of the risks involved for individuals and the environment.

In the context of toxicology, the penetration of arsenic into the hair is problematic. It adds significant complexity in identifying arsenic reflecting ingestion as opposed to being due to preservation (in the case of preserved hair from historical figures). If arsenic is detected in a hairs' internal structure, it is not a sufficient observation to conclude the arsenic originated by being consumed by the individual.

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