**Synchrotron X-ray Fluorescence Imaging Evidence of Biogenic Mercury Identified in a
Burial in Colonial Antigua**

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Abstract

A mass spectroscopic analysis of bone samples from 17 individuals once buried in a Royal Naval Hospital cemetery (1793-1822) in Antigua revealed a high level of mercury (Hg) in one individual. While the toxic properties of Hg are now recognized, this metal was used for centuries to treat ailments such as syphilis and yellow fever. Synchrotron radiation X-ray fluorescence imaging (XFI) was employed to determine whether the Hg was present in the bone as a result of environmental contamination or due to biogenic uptake. The XFI study revealed that only a localized subset of the osteons within a 2.5 mm by 1.5 mm scan contained Hg – a finding consistent with biogenic uptake. The near-edge portion of the X-ray absorption spectrum was used to determine that the Hg was present in the bone tissue as an inorganic mercuric sulfide.

1. Introduction

Mercury (Hg) has been recognized as an element of human interest for millennia (Aufderheide and Rodriguez-Martin, 1998). The first evidence of Hg in the archaeological record was identified in 18th dynasty Egyptian tombs circa 3500 years BP (Abramowitz, 1934). This element has been used for decoration (Yamada, et al., 1995, Yamada, et al., 1997) and as a component in medicine, especially in recent centuries when it was used to treat syphilis (Lopes, et al., 2010, Tucker, 2007, Kepa, et al., 2012). As part of a larger study of identifying the biogenic uptake of trace elements in archaeological bone through the use of synchrotron techniques (Swanston, et al., 2012), inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the concentrations of trace elements in 17 bone samples from individuals recovered from a colonial Royal Naval Hospital Cemetery (1793-1822). This cemetery, which is located in Antigua, West Indies, was partially excavated due to modern construction. Our primary research goals included using ICP-MS to identify bone samples with high lead concentrations; however, this analysis revealed that a sample from one individual contained a high concentration of Hg.

Hg is a heavy metal that exists in three broad chemical species: elemental Hg, inorganic Hg, and organic Hg (Guzzi and La Porta, 2008), all of which show some level of toxicity. Hg can be identified using standard X-ray fluorescence instrumentation (Toribara and Jackson, 1982) but it is difficult to determine whether the element was introduced in the burial environment or associated with a biological event during life. In the 17th century, topical mercuric treatments for syphilis became popular and calomel (inorganic mercurous chloride) was used as a purgative in medicine. By the late 18th century, it was common to take elemental Hg orally (Sartin and Perry, 1995). Interestingly, five years after the final burial at the Antigua Royal Naval Hospital

cemetery (1793-1822), Dr. Anthony Musgrave, the Surgeon-General on the Colonial Staff of Antigua, wrote an article on the use of Hg in the early 19th century (Musgrave, 1827). He described how patients were treated with a course of quicksilver pills (elemental Hg) when they had symptoms of syphilis or yellow fever.

As a way of testing the hypothesis that Hg was deposited in the bone sample from the Royal Naval Cemetery in Antigua via biogenic uptake rather than post-mortem diagenetic modification we employed synchrotron radiation X-ray fluorescence imaging (XFI) to examine the spatial distribution of the element and X-ray absorption spectroscopy (XAS) to obtain chemical speciation information. Specifically, we sought to determine 1) if the spatial elemental distribution was correlated with microstructural features in the bone and 2) if the nature of the chemical species was consistent with biogenic deposition.

2. Material and Methods

The colonial Royal Naval Hospital cemetery (1793-1822) in English Harbour, Antigua was excavated between the years of 1998 and 2001, and bone samples are currently curated in co-author Varney's laboratory at Lakehead University, Thunder Bay, Canada. Ethics approval was obtained from the University of Saskatchewan Biomedical Research Ethics Board. Prior to this Hg study, ICP-MS was completed to identify bulk element concentrations using standard methodologies at the ICP-MS Laboratory in the Department of Geological Sciences, University of Saskatchewan (Jenner, et al., 1990, Stefanova, et al., 2003).

The bone sample of interest had a Hg level of 94.6 ppm in comparison to the other sixteen samples that ranged between 0 and 2 ppm. The sample was a tibial cortical fragment that belonged to a male individual who was approximately 25 to 29 years of age at death and was

likely of European ancestry based on the cranial features present. A second sample from the same tibia was tested with similar results. A gross osteological analysis of the complete set of remains identified a healed fracture on the right fourth metacarpal and Schmorl's nodes on thoracic vertebrae eight through twelve. In addition, the maxillary and mandibular alveolar sockets were noted to be abnormally porous.

2.1 Histological Analysis

Bone samples were sectioned with a Buehler® IsoMet® Low Speed Saw with a diamond wheel (Norton Company, TX). A 300 µm bone fragment was ground to a thickness of approximately 150 µm with 2000 Grit 3M sandpaper. Residual material was removed by soaking the bone sample for 10 min in an ultrasonicator. The sample was placed between Thermanox® metal-free plastic coverslips (Cedarlane, Burlington, ON) and examined by light microscopy. The digital histological image was created using a PAXcam® high-resolution digital microscope camera (PAXcam, Villa, IL) at 10x magnification.

2.2 Synchrotron Radiation X-ray Fluorescence Imaging

A two dimensional spatial map of Hg associated with the bone sample was initially generated using XFI on the VESPERS (Very powerful Elemental and Structural Probe Employing Radiation from a Synchrotron) hard X-ray microprobe bending magnet beamline located at the third generation Canadian Light Source (CLS) in Saskatoon, Saskatchewan, Canada (Feng, et al., 2007). The incident X-ray energy was set to 15 keV (flux of approximately 2×10^{11} photons/sec) and the double multilayer monochromator had a bandwidth of 1.6%. Micro-focussing of the incident X-rays was achieved by using Kirkpatrick-Baez (KB) mirrors

and the resulting beam spot size was approximately 5 μm in diameter. Data were collected by rastering the sample in steps of 10 μm in length while capturing the signals from the Hg $L\alpha_{1,2}$ (9980 eV) and Hg $L\beta_1$ (11822 eV) lines. A Vortex® Silicon Drift Detector, calibrated with standards containing multiple elements, was used to measure the X-ray fluorescence.

A larger map of the sample in question was created using the CLS-administered beamline 20ID at the Advanced Photon Source (APS), Argonne National Laboratory (Heald, et al., 2007) using a liquid-nitrogen-cooled silicon (111) double-crystal monochromator set to an incident X-ray energy of 16.5 keV. The APS was operating in top-up mode with 102 mA of current in the ring. The incident flux was approximately 2×10^{11} photons per second within the $5 \times 5 \mu\text{m}^2$ micro-focus beam spot obtained from the KB-mirrors.

Open source (ImageJ: <http://rsbweb.nih.gov/ij/>) and custom software were used to convert the raw data into images. Since a correlation between biological structures and the spatial distributions of trace elements is indicative of biogenic uptake, the XFI data were compared with the bone histology.

2.3 Synchrotron X-ray Absorption Spectroscopy

XAS measurements were conducted at the Stanford Synchrotron Radiation Lightsource (SSRL) with the SPEAR storage ring containing 450 mA at 3.0 GeV. Hg L_{III} -edge data were collected on the structural molecular biology XAS beamline 7-3, employing a Si(220) double-crystal monochromator and an upstream rhodium-coated collimating mirror was used to reject photons above 15 keV. Beam size was approximately 1 mm by 3 mm (VxH). X-ray absorption of the bone sample was collected in fluorescence mode using the Hg $L\alpha_{12}$ X-ray fluorescence excitation spectrum with a Canberra (Meriden CT, USA) high-purity germanium array detector

(Cramer, et al., 1988), with incident and transmitted X-ray intensities being measured using nitrogen filled gas ionization detectors with a sweeping voltage of 1800 V. The larger beam size was chosen to reduce flux density on the sample and lessen the likelihood of beam damage. Multiple sweeps of the spectra were recorded to improve the signal to noise ratio but to also ensure that photoreduction was not taking place. During data acquisition all samples were maintained at 10K using an Oxford Instruments (Abingdon, Oxfordshire, UK) CF1204 liquid helium flow cryostat.

In addition to the bone sample, spectra of standard Hg containing species were collected for comparison and for use in data analysis (George, et al., 2009). These included Hg in the elemental state as well as a wide range of organometallic and inorganic Hg species. XAS data were processed using standard techniques (George, et al., 2008) using the EXAFSPAK suite of software (<http://ssrl.slac.stanford.edu/exafspak.html>). The near-edge portion of the Hg L_{III} XAS was used to provide speciation information on the Hg present in the bone.

3. Results

In the XFI study, a portion of the scan indicated higher levels of Hg in the bone sample and the pattern was clearly distinct from surrounding structures (Fig. 1). The spatial localization of the Hg corresponds with compact bone features (osteons) that are present as a result of bone remodelling. Interestingly, the Hg is constrained within a small number of osteons.

A second sample from the same bone was used to determine the species of Hg through the use of near-edge analysis. The chemical sensitivity of the Hg L_{III} near-edge spectra are much less than those of K-edge spectra in the same energy range (e.g. As and Se). Because of this, quite subtle variations in the spectra must be used to obtain speciation information, and as

147 previously discussed (George, et al., 2008) spectra must have good signal to noise for a valid
148 analysis. In general, and as expected (George, et al., 2008, Korbass, et al., 2010), near-edge
149 spectra from similar structural sites resemble each other. For example, spectra from two-
150 coordinate linear oxygen-coordinated species (such as $\text{Hg}_3(\text{PO}_4)_2$) (Aurivillius and Nilsson,
151 1975) resemble one another, with a distinctive low-energy peak at 12285.8 eV, and are distinct
152 from two-coordinate sulfur bound species such as $\alpha\text{-HgS}$ and these in turn from three and four
153 coordinate sulfur bound species. We note that some species such as HgCl_2 do show a peak in
154 their Hg L_{III} near-edge spectra, but that this is at a higher energy around 12286.7 eV. Automatic
155 software (the EXAFSPAK program MATCHIT) was used to compare the experimental spectrum
156 with spectra from a library of 41 standards of Hg L_{III} near-edge spectra. Figure 2 shows a
157 comparison of the bone spectrum with those of a small number of selected standard compounds.
158 The MATCHIT program uses non-linear optimization of a polynomial background function and
159 linear scaling to find the closest match of experimental data from our library of 41 standard
160 compound spectra. For our bone samples, the closest match was found to be the beta form of
161 inorganic mercuric sulfide ($\beta\text{-HgS}$) which has four-coordinate Hg(II) with sulfides. The next
162 nearest matches were all four-coordinate sulfur-bound species, and when two components were
163 allowed the best fit was found with essentially 100% $\beta\text{-HgS}$, with minor components being
164 incorporated below the threshold of detection which we estimate as about 4%. Very similar
165 results were obtained with larger scale multi-component fits using the EXAFSPAK program
166 DATFIT. Even empirically, the spectrum of the bone appears quite distinct from that of
167 $\text{Hg}_3(\text{PO}_4)_2$ and lacks the characteristic low-energy peak at ~12285.8 eV. The spectrum from the
168 bone sample is a close match to the spectrum of the $\beta\text{-HgS}$, mineralogically known as
169 metacinnabar, indicating that the structure is four-coordinate with sulfur, and strongly suggesting

the substantial presence of this compound in the sample. The close correspondence between the bone spectrum and β -HgS is evident upon examination of Fig. 2.

4. Discussion and Conclusions

This study began after a standard ICP-MS analysis of bone samples from 17 individuals recovered during salvage operations on the island of Antigua in the West Indies identified that one bone sample contained an elevated level of Hg. Our initial goal was to discern whether the Hg was present due to biogenic uptake or as a result of post-mortem contamination. The soil at the site was slightly alkaline and consisted of a hard mix of volcanic ash and gravel deposits. The burial in question was the last in a row of 5 graves, 120 cm below the surface and similar to the surrounding burials. The well-preserved remains resided within a six-sided wooden coffin with ferrous metal nails. Associated artifacts were limited to eight bone buttons alongside the lumbar and lower thoracic vertebrae. Bone buttons were common in the burials at the site and not a likely source of Hg since this is the only skeleton in which the element was found at anything but negligible levels.

For many centuries, the use of Hg was in fact controversial due to the limited knowledge surrounding the impact of the element on healthy tissue. Abramowitz (1934) commented that research in the 19th century revealed Hg had no effect on the bone of humans or animals. In the recent past, with technological advances, there have been new opportunities to identify metals associated with bone (Lindh, et al., 1980).

A biogenic source of Hg associated with archaeological bone has been noted in previous studies, including research by Yamada and colleagues (1997) who analyzed a number of burials from the 3rd to 6th century in Japan when inorganic mercuric oxide was used in red cosmetics.

The authors used the method of Inductively Coupled Plasma Atomic Emission Spectroscopy and while their results suggested a connection between the presence of Hg and the use of red cosmetics, they also commented that environmental contamination may have occurred.

A study by Rasmussen and colleagues (2008) revealed through atomic absorption the presence of Hg in Danish medieval bones associated with individuals who likely had leprosy and syphilis due to the apparent bone lesions. They noted that Hg-containing medicine was used in the majority of leprosy cases and almost half of syphilis cases during that time period (Rasmussen, et al., 2008). As a way of identifying possible environmental contamination, the authors tested the soil at regular intervals both vertically and horizontally away from where the remains were excavated. They also noted that while they were able to achieve results indicating various levels of Hg in the bones, the levels were too low for “any feasible mapping technique to reveal even the location of Hg.”

XFI, a technique that can localize specific chemical elements by monitoring their characteristic X-ray fluorescence, has been used to study Hg in a variety of systems including modern bones of skua and penguin, which are seabirds from the Antarctic (Zhouqing, et al., 2005). This seabird study found that the Hg levels were higher in the periosteal and endosteal surfaces. It is intriguing to use this same imaging technique on archaeological bone because of the advantages of using synchrotron light that are not found in conventional techniques such as faster scan speeds, higher spatial resolution and sensitivity to low trace element concentrations.

Hg can be isolated in the natural environment in areas with volcanic soil (Martin, et al., 2012). The most common ore of environmental Hg is inorganic alpha mercuric sulfide (cinnabar), but mercuric sulfide can also exist in the beta mercuric sulfide form (Barnett, et al., 1997). Under normal environmental conditions, both forms are stable and highly insoluble in

water (Dickson and Tunell, 1959). Recent work by Avila and colleagues suggests that cinnabar does not interact with hydroxyapatite associated with post mortem remains (Avila, et al., 2014).

In this study, the data that were acquired from the XFI scan revealed the presence of Hg in some of the osteons but not in all of the osteons within the field of view. This evidence rules out environmental contamination and is consistent with the biogenic uptake of Hg though it is currently not possible to determine a time sequence.

The question how long mercury remains in the system, is one of practical importance. The antimercurialists speak in support of their favourite views, of its remaining many years in it; but there is no proof of this.

(MacPherson, 1874)

The level of Hg toxicity varies dramatically with the chemical form in which it is presented. The speciation of the Hg in the bone sample identified a form of inorganic Hg β -HgS, which can form *in vivo* and is known to accumulate in the kidneys; high levels will result in the termination of kidney function (Clarkson and Magos, 2006). In colonial times, the toxicity of Hg was acknowledged. One historical incident in 1810 involved the Royal ship H.M.S. *Triumph* while in harbour at Cadiz, Spain. The ship received approximately 130 tons of quicksilver (elemental Hg) from a damaged Spanish ship (Earles, 1964). Dr. William Burnett documented the accidental contamination of the *Triumph* with Hg (Burnett, 1823, Doherty, 2004). He commented that many of the crew were affected with ptyalism (excessive salivation), and some individuals suffered from partial paralysis and ulcerations of the mouth. It has been suggested that due to the mouth ulcerations, there is a greater potential for periodontitis, and when this condition is severe, the alveolar bone is impacted (Coventry, et al., 2000). It is interesting to speculate that since the individual in this current study had porous alveolar sockets, this individual may have been poisoned with Hg.

Improvements in synchrotron technology and the dissemination of its applicability have resulted in not only modern bone studies (Pemmer, et al., 2013) but also in studies of relevance to bioarchaeology and paleopathology (Bertrand, et al., 2012). In this study, the technique of XFI provided evidence supporting the interpretation that the Hg was present due to biogenic uptake with the additional structural information gained through XAS revealing that it was an inorganic form of Hg.

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Figure Legends

Fig. 1. XFI Spatial Map

Region of interest with the corresponding XFI spatial map (scale bar = 1 mm): (A) bone histology observed through light microscopy; and (B) a composite of the mercury XFI map (data collected at the APS) at 40% opacity with the histological image. The color gradient (value range from 0.001 to 0.078) indicates the normalized XFI intensity but does not reflect the true concentration.

381 Fig. 2. Speciation using XAS

382 Comparison of Hg L_{III} X-ray absorption near-edge spectra of bone and selected standards. The
383 very close correspondence of the β -HgS standard and bone sample spectra indicates that the
384 majority of Hg present in the bone is present as β -HgS.

385

Figure 1

Figure 2