TECHNICAL NOTE
ANTHROPOLOGY

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Practical Considerations in Trace Element Analysis of Bone by Portable X-ray Fluorescence*

ABSTRACT: Forensic anthropologists are more often turning to nondestructive methods to assist with skeletal analyses, specifically for trace elemental analyses. Portable XRF (pXRF) instruments are versatile and are able to be used in diverse settings or for specimens of a shape and size that cannot be accommodated by laboratory-based instruments. Use of XRF requires knowledge of analysis parameters such as X-ray penetration and exit depth. Analysis depth was determined by examining pure elements through known thicknesses of equine bone slices. Correlation between the element’s X-ray emission energy and the depth of reading was observed. Bone surfaces from a small unknown historic cemetery were analyzed before and after sanding of the periosteal surface to observe possible changes in XRF readings based on potential diagenesis. Results validate the pXRF device as a powerful and convenient instrument for nondestructive analysis, while highlighting limitations and considerations for the analysis of osseous materials.

KEYWORDS: forensic science, forensic anthropology, portable X-ray fluorescence, trace elements, bone chemistry, diagenesis

Energy dispersive X-ray fluorescence spectroscopy (EDXRF) can reveal the trace element concentrations in parts per million (ppm) with nondestructive analysis of the material under investigation. XRF is well established as an analytical method (1) and has been utilized by forensic anthropologists to determine the origin of materials in question (2,3), assistance in identifying contamination in cremains (4), sorting commingled remains (5), as well as determining whether packaging materials lead to contamination of human remains (6). Portable XRF (pXRF) spectrometers have been developed that allow for in situ trace element analysis of samples. The earlier versions of these devices used radioactive materials as excitation sources, but more recently, miniaturized excitation sources have been developed that do not use radioactive material therefore avoiding issues with radioactivity [transportation and disposal] (7,8). These types of units are being utilized in the field in a number of ways: sourcing obsidian (9), ceramic paint analysis (10), environmental sampling (11), dental restorations in a forensic setting (12), and museum artifacts (13). With the dramatic increase in usage of this portable device in forensic sciences, especially forensic anthropology, there is a need for a dialogue on appropriate methods that should be established across the field (For further discussion see [14,15]). To evaluate this device’s utility and limitations to analyze bone, we examined the depth that the device can read into cortical bone, as well as the taphonomy (diagenetic) of archaeologically-derived bone samples.

In XRF, high-energy primary X-ray photons from an energetic source [X-ray beam] strike the sample causing the emission of characteristic X-rays. The resulting energy spectrum shows peaks corresponding to the energy of detected characteristic X-rays, representing an elemental signature of the sample under investigation. In an XRF spectrum, the background is low, allowing detection of very low concentrations of an element. A technical limitation of using pXRF is that lighter elements, such as phosphorus (P), are not detectable due to absorption of low-energy X-rays by air and the detector window. Some units, however, do have vacuum attachments to compensate for this dilemma—although they are expensive and reduce the portability of the instrument. Due to these limitations, some constituents of bone are not within detectable limits of a given type of instrument.

The use of such instruments requires knowledge of analysis parameters, such as X-ray penetration and exit depth. This is especially important if nonuniform whole bone samples are to be analyzed. In this study, two experiments were carried out; the first was to observe the analysis depth of the XRF unit for cortical bone. In the second experiment, we examined the effects of removing surface layers of cortical bone to see whether diagenesis or adhering soil may be having significant effects on the XRF readings. This step has been recommended by other researchers examining forensic and archaeologically-derived bone (2).

Bone is a dynamic tissue, consisting of both organic and inorganic components: collagen and hydroxyapatite (16). The structure of cortical bone is an important factor because the newly deposited bone layers on the periosteal surface may have different readings of a given element than an older deposited layer.

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deep to the surface, as bone undergoes constant remodeling throughout an individual’s life (17). Cortical bone is more conducive to use with this portable XRF device, as trabecular bone has a number of inconsistencies such as an uneven surface (due to porosity) which can inhibit the analytical performance of the procedure. Flat, regular surfaces with no air gaps in the analysis area are ideal for this instrument to report the real concentration of the material being analyzed (18). However, according to Gonzalez-Rodriguez and Fowler (5), in instances where the bone surface being analyzed was irregular in shape, accurate readings were still obtained. Another consideration when analyzing bone is that cortical bone remodels over a much longer period of time than trabecular bone (19). It is not exactly known how long this period is according to more recent research in bone turnover (20,21). Even so, this can help determine which bones should be favored for analysis and, more specifically, what sections of those bones when considering the content of cortical versus trabecular bone.

Bone chemistry can reveal much about an individual’s life history and XRF has been used to study bone chemistry as it has the ability to analyze a number of different elements at once. Certain trace elements give specific and pertinent information about environmental exposure (22), diet (23), and geographical location of residence (24,25). However, caution must be taken as in a forensic setting where the bone sample has been exposed to the environment, the elements being studied may be altered due to diagenetic changes. Depending on a variety of factors such as soil and ground water composition, the observed reading may not fully represent the actual bone chemistry composition of the individual.

Diagnosis from the surrounding soils and ground water in the burial is a real and highly probable hindrance when interpreting results; this adversely affected the accurate re-association of commingled remains as the sample size increased when trace element results from a pXRF were used (5). A study of lead (Pb) diagene
sis of bone in an archaeological context utilizing pXRF showed that the soil can significantly affect the levels read in the associated remains buried in the soil matrix (22). Further, bone is more susceptible to diagenesis (26) than tooth enamel (27) due to its susceptibility to diagenesis (26) than tooth enamel (27) due to its susceptibility to diagenesis (26) than tooth enamel (27) due to its porosity.

Strontium (Sr) has a great affinity for bone (28,29) and is incorporated into it by two mechanisms: surface exchange or ionic substitution (30). Strontium can take the place of calcium in the hydroxyapatite component of bone (31,32). Due to strontium’s natural incorporation into bone and the fact that it is found naturally in bedrock and associated soils, it was chosen as the element of interest for a comparative investigation of potential changes in XRF readings due to diagenetic factors.

We examined the practical applications of pXRF as well as the limitations of this technology for analyzing bone chemistry.

Materials and Methods

The pXRF unit used in this study was the Innov-X Systems Alpha-2 (Woburn, MA), provided by the South Campus Instrument Center from the University at Buffalo, under the direction of P. Bush. This portable device is an EDXRF analyzer, equipped with a tungsten (W) tube run in soil mode at 60 s per beam. This device has a silicon (Si) PiN diode detector, which operated at 40 kV and 10.0 μA from an external power source. The analysis area of the XRF unit is c. 8 mm in diameter. The unit was operated in a vertical position in a laboratory test stand for fixed positioning during analysis, and the device was controlled by a laptop computer to ensure physical stability. Spectra were obtained with an analysis time of ~30 sec. The X-ray beam produced from the instrument runs at a 45° angle from the center of the analyzer’s end—therefore, to maximize the read area based on the beam pathway, the smoothest surface of the sample was positioned to cover the beam angle path to have the greatest amount of X-rays possible bombard the sample.

X-ray counts were processed via the Innov-X software spectra program, where they are converted to parts per million concentrations using the Innov-X calibration program whereby it utilizes the Compton’s scatter derived from silver backscatter. A standard calibration plate provided by Innov-X was used before and after analyses were complete, that was composed of Alloy 316, which was used to empirically calibrate the instrument by comparing expected values with those produced by the instrument. The instrument was set to capture the fluorescence spectra containing strontium (Sr) Kα lines (Kα1 line at 14.166 keV), copper (Cu) Kα lines (Kα1 line at 8.05 keV), lead (Pb) Lα lines (Lα1 line at 10.551 keV), zirconium (Zr) Kα lines (Kα1 line at 15.78 keV), and tin (Sn) Kα lines (Kα1 line at 25.27 keV).

Apparent Analysis Depth

The goal of this part of the study was to determine to what depth in bone might be analyzed for a given element of interest. While the higher energy exciting radiation will reach a certain depth in the bone, the characteristic X-ray for the element of interest will have a different exit depth, due to absorption of the matrix elements in bone and respective lower energy of the characteristic X-ray. Hence, the apparent analysis depth will be different for each element.

In an empirical approach, analysis depth (and hence X-ray exit depth) was determined by analyzing selected pure elements through varying known thicknesses of equine (Equus ferus caballus) cortical bone slices. The slices were prepared by sectioning with a precision slow-speed diamond saw (Buehler, MI). Four bone slices of 0.76 mm thickness and one 0.2 mm slice were prepared, each section large enough to encompass the area of analysis. The thickness of the samples was measured using a digital micrometer.

A sample of a pure element (copper, lead, zirconium, and tin) was placed on top of the slices to observe how many bone slices the device could read through to reach the element of interest.

Sanding Effect

A small human population (i.e., seven individuals) from an unknown historic graveyard from Youngstown, NY, was utilized to examine the effects of sanding of cortical bone. A summary of the demographics of the burials can be found in Table 1 (33). The initial bone surface was analyzed with the pXRF before and after sanding with 600 grit silicon carbide paper. While the amount of material removed by sanding was not quantified, every effort was made to sand the bone samples for the same amount of time at a similar pressure. Bone samples from each individual burial were analyzed before and after sanding by placing the sample on top of the vertically positioned XRF device. The samples were always positioned in a manner to cover as much of the reading window of the XRF as possible. Only skeletal elements that still had cortical bone were analyzed. Some individuals were better preserved than others, leading to differing amounts of data for each individual.

In a burial setting, the positioning of the bones in the grave may affect the readings taken from one side of the bone to the
other (i.e., posterior versus anterior). When possible, bones were analyzed from multiple locations to control for the possibility of diagenetic effects. To statistically analyze the data from this experiment, a paired-samples sign test was performed. There were a total of 33 samples from the seven individuals analyzed.

**Results**

**Analysis Depth**

It was determined that the depth of the reading is dependent upon the emission energy of the characteristic peak for the element under investigation. There was a direct correlation between the element’s X-ray emission energy and the depth of reading by the device into cortical bone (Fig. 1). The higher the emission energy of the element being examined, the greater the depth of the reading. Using the slope of the correlation, analysis depth for other elements can be extrapolated based on the emission energy as the dependent variable. This was done for strontium on the histogram in Fig. 2. These results only apply to cortical bone, which is essentially a matrix of calcium phosphate. The analysis depth would be different for other elemental matrices. The analysis depth for strontium in bone was determined to be 1.9 mm. These results coincide with the exponential attenuation law equation used to determine the penetration into a material (34).

\[
t = -\frac{\ln(I/I_0)}{((\mu/\rho) + \rho)}
\]

(1)

(where \(t\) is the thickness of material attenuating X-rays; \(\mu/\rho\) is the mass attenuation coefficient dependent on material and emission energy of element; \(\rho\) is the density of material that the X-ray is penetrating; \(I\) is the beam of intensity with attenuation; \(I_0\) is the beam intensity without attenuation; and \(I/I_0\) is 0.01 assuming that all but 1.0% of X-rays have been stopped).

**Sanding Effect**

Analysis of cortical bone before and after sanding showed significant differences in levels of strontium. The range of change in Sr level was -81 to +25 ppm when compared to the initial (before sanding) readings (Table 2). This is a large range due to the interindividual variation of strontium, where some were averaging 600–700 ppm and others 200 ppm. Therefore, there is the potential for greater change for these individuals with higher levels than those at the lower levels. Shapiro-Wilk’s normality test results were statistically significant for both the data sets before \(p = 0.000\) and after sanding \(p = 0.001\), indicating that they are both not normally distributed. The paired-samples sign

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**TABLE 1—Summary of Jackson Street burial demographics (33).**

<table>
<thead>
<tr>
<th>Burial</th>
<th>Orientation of Burial</th>
<th>Depth of Grave</th>
<th>Coffin Status</th>
<th>Completeness Code*</th>
<th>Sex</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E/W</td>
<td>2.72 82</td>
<td>Present</td>
<td>Adult</td>
<td>3</td>
<td>Female 25–35</td>
</tr>
<tr>
<td>2</td>
<td>E/W</td>
<td>2.40 72</td>
<td>Absent</td>
<td>Adult</td>
<td>2</td>
<td>Male &gt;40</td>
</tr>
<tr>
<td>3</td>
<td>E/W</td>
<td>3.00 90</td>
<td>Absent</td>
<td>Adult</td>
<td>1</td>
<td>Female 20–35</td>
</tr>
<tr>
<td>4</td>
<td>E/W</td>
<td>2.64 80</td>
<td>Absent</td>
<td>Child</td>
<td>3</td>
<td>Undetermined</td>
</tr>
<tr>
<td>5</td>
<td>E/W</td>
<td>2.53 77</td>
<td>Present</td>
<td>Child</td>
<td>3</td>
<td>Undetermined</td>
</tr>
<tr>
<td>6</td>
<td>E/W</td>
<td>3.00 90</td>
<td>Present</td>
<td>Adult</td>
<td>2</td>
<td>Female 30–35</td>
</tr>
<tr>
<td>7</td>
<td>E/W</td>
<td>3.39 102</td>
<td>Present</td>
<td>Adult</td>
<td>2</td>
<td>Female 20–35</td>
</tr>
</tbody>
</table>

*Completeness Codes: 1 = >75% present; 2 = 25–75% present; 3 = 25% present.

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FIG. 1—Direct relationship between emission energy (keV) of element and cortical bone thickness.

FIG. 2—Maximum read distance of pXRF through cortical bone based on emission energy (keV).
test was used to compare the differences in strontium before and after sanding. A statistically significant median decrease in strontium was found after sanding ($Z = -3.359, p = 0.001$).

## Discussion and Conclusions

Diagenesis and other taphonomic changes in bone are of great interest in forensic anthropology where human remains may be deposited in an environment for a given amount of time. We conclude that it is important to take these factors into consideration when performing XRF bone analysis. When determining what areas of the bone to analyze for a particular element under study (i.e., a cranial fragment versus a long bone fragment), cortical bone thickness and the emission energies of the element of interest should come into consideration. This is important because if the device analyzes the first 1.9 mm into cortical bone, but is not that thick in the area being analyzed, this would affect the output (quantitative) by not being a true representation of the relative concentration in that section of bone. The X-ray depth is dependent on the emission energy of the element of interest as well as the density of the material under investigation—in this case, dry cortical bone. When the density changes, so does the depth of analysis of the X-ray for that particular element. Therefore, to stay consistent with the volume of bone being represented, the thickness of cortical bone should be known to surpass the expected penetration distance of the X-ray for a particular element.

Analyzing uniform samples versus in situ samples with this device should be considered with this information on depth of reading dependent on the element(s) of interest. Although it is desirable to keep samples in situ, it may be of interest to see the absolute differences between in situ bone samples (before) versus homogenized bone samples (after) with the pXRF device. In a review by Zamburlini (35), *in vivo* human bone measurements taken with a XRF device were compared with dual-photon absorptiometry (DPA) when analyzing for strontium. Some conclusions were that if the strontium was not uniformly distributed in the bone, the signal for strontium bone depth would need to be corrected as the device is not reading the absolute bone strontium concentration. Although there are ways to analyze and interpret this uneven distribution of elements in bone as Swanson et al. (36) displayed with SR-XRF and bone histology, this is destructive.

Statistically, sanding the bone surface to be analyzed did affect the trace element analysis in a burial setting. This process removed the initial soil-stained surface and we suggest that this makes the reading more representative of the actual cortical bone strontium concentration in the area being analyzed. Diagenesis though is a potential source of error. Small sample sizes due to the varying degrees of preservation of the remains from this historic graveyard are also problematic. This limits the areas available for analysis. Future studies should examine more taphonomic-related questions on how well the pXRF device detects levels of diagenesis in bone exposed to the environment, as this will be the most encountered type of sample needed for analyses. Using a pXRF versus a laboratory-based XRF was determined to have no significant differences when analyzing obsidian sourcing (9,37) or bone samples (38). However, further research should examine larger sample sizes to confirm this in bone, as the study by Pessanha (38) only examined one bone ash sample and one femur.

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