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PAPER

ANTHROPOLOGY

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Validation of X-Ray Fluorescence Spectrometry for Determining Osseous or Dental Origin of Unknown Material*

ABSTRACT: Forensic anthropological examinations typically involve the analysis of human skeletal remains, but in cases where samples are very small and/or physically compromised, it may first be necessary to determine whether the material is even osseous or dental in origin. X-ray fluorescence spectrometry (XRF) is a technique that reveals the elemental composition of materials and is hypothesized to have utility in such cases. XRF analysis was conducted on a variety of tissues and materials in unaltered and altered (damaged) states. With few exceptions, osseous and dental tissues in unaltered and altered conditions contained characteristic levels of calcium and phosphorus, while other materials did not. Materials could be accurately identified as osseous or dental in origin based on the calcium and phosphorus levels identified by XRF, and we therefore conclude that XRF analysis is a valid and effective means of determining osseous or dental origin of unknown material.

KEYWORDS: forensic science, forensic anthropology, X-ray fluorescence, elemental composition, osseous or dental origin

Forensic anthropological examinations typically involve the analysis of human skeletal remains, but it is sometimes necessary to first determine whether the material in question is even osseous or dental in origin (i.e., whether it is, in fact, a part of a skeleton) versus some other type of material (such as mineral, wood, and plastic). This is especially relevant in cases where the material may be submitted for DNA analysis. When specimens are sufficiently large and in good condition, this can usually be achieved through visual macroscopic, microscopic, and in some cases, radiographic examination by a trained anthropologist. Occasionally, however, specimens are very small and/or compromised by deliberate or natural taphonomic processes, making this determination difficult. Most commonly, these difficult cases involve small fragments of burned/charred material that are suspected to be bone.

X-ray fluorescence spectrometry (XRF) is a technique used for characterizing the major, minor, and trace elemental constituents present in a sample and is hypothesized to have utility in these analyses. It is nondestructive and can be used to analyze a wide variety of materials for elements ranging in atomic number from 9 (fluorine) to 92 (uranium). X-rays passing through matter are subject to three processes: absorption, scatter, and fluorescence (1). X-ray radiography is based on absorption—areas of higher atomic number will attenuate the beam to a greater extent than areas of lower atomic number. X-rays may also be scattered by many solid materials to produce diffraction patterns that can be used to study the crystalline structure of the materials. Fluorescence occurs when sufficiently energetic incident X-ray photons eject an inner shell electron creating vacancies in the specimen. Subsequent filling of these vacancies by electrons from the outer shells results in the emission of fluorescent radiation at specific X-ray wavelengths that are characteristic of the elements making up the specimen. The isolation and measurement of individual characteristic wavelengths following excitation by primary X-radiation is called X-ray fluorescence spectrometry. Because of the speed, accuracy, and versatility of XRF, it has gained major significance as a routine means of elemental analysis (1).

XRF has been used in forensic investigations as a means of identifying particular dental restorative resins (2,3), and portable XRF units have been suggested for the detection of body fluids and gunshot residue at crime scenes (4,5). Chemical analysis of bone and other tissues using XRF has also been widely used in archaeological studies for the determination of diet and medical studies for the detection of lead, zinc, arsenic, and other toxic elements (6).

A study of osseous and dental tissue composition was conducted by Ubelaker et al. (7) using scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDS) in conjunction with a Federal Bureau of Investigation (FBI)-developed spectral database, SLICE (8). The database included X-ray spectra for various materials including bones and teeth from numerous contexts and of various taphonomic conditions. Their results indicated that spectra from unknown materials could be compared with this database to determine consistency with bone or tooth or other material. While they found that the proportions of calcium and phosphorus were particularly important for identifying bone and tooth, they noted that other minor differences in profiles could also be useful for discrimination.

The SEM/EDS process, however, involves sample destruction and considerable sample preparation in addition to significant equipment costs. Therefore, validation of the XRF technique for

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identifying osseous or dental tissue could supply an additional analytical tool for forensic anthropologists to more quickly and effectively assess the potential skeletal origin of unknown material. The following study was undertaken with the goal of determining whether this technique can reliably discriminate osseous and dental tissue from other material.

Materials and Methods

XRF analysis was conducted on a variety of tissues of known osseous and dental origin in good condition including human bones, human teeth, nonhuman bones, and nonhuman teeth. In addition, other biologic hard tissues such as horn, beak, coral, and shell were analyzed, as well as other materials that may appear similar to osseous or dental tissue when in small fragments or altered conditions such as wood, minerals, plastic, metal, and glass (see Table 1). XRF was also conducted on some of these same tissues and materials in thermally, chemically, and taphonomically altered states. These states included various degrees of burning (e.g., charred and calcined), weathering (e.g., bleached and exfoliated), antiquity (up to 9000 years old), and exposure to destructive chemicals. In Ubelaker et al.'s (7) study, bone and other mineral samples were prepared for analysis by transferring dry powdered samples to a double-faced carbon film over the stub and then coating the samples with gold and observing them under the SEM microscope. By comparison, in most cases, no sample preparation was performed for this study, and therefore, no destruction of the samples was required.

Samples were procured in several ways. First, a "control" set of samples was created by the authors specifically for this study. These consisted of pairs of specimens, originating from the same item, one of which was tested in an unaltered condition, and one of which was burned and then tested. These included human bones (humerus, fibula, cranium, and subadult cranium), a human tooth (molar), a pig tarsal, a pig tooth, a mollusk shell, coral, wood (dowel, board, and stick), rocks (quartz and gravel), ceramic, float glass, plastics (hard, semi-hard, and soft), metals, carpet, and fabric. Burning was achieved using a hand-held propane torch (Bernzomatic TS3000™, Medina, NY) in a ventilated hood. In addition, a third sample from the human bone, human tooth, pig bone, and pig tooth were each placed in a pH1 solution of nitric acid (HNO_3) for a period of 1 h and then tested.

Second, the samples used in the Ubelaker et al. (7) study were obtained and analyzed using XRF procedures. These included human bones (cranial, foot phalanx, femur, subadult bones, rib, spongy bone, cortical bone, and ancient bone of 9000 years), human teeth (molars, premolar, and canine), animal bones (rib, vertebra, cortical bone, clavicle, and turtle shell), animal teeth, wood, and a garden hose. Some specimens were in unaltered conditions, but many were altered by burning or weathering. The methods or circumstances of alterations were in most cases unknown. Third, samples of lime and dental deposits (apparent calculus) utilized in a study by Ubelaker and Stothert (9) were obtained and analyzed. Finally, various other specimens available to or procured by the authors were analyzed and included animal teeth, mineral apatite, coral, octocoral, brachiopods, sanddollar, sea turtle beak, and chemically altered (30% solution sodium hydroxide) cow bone. Several samples were analyzed multiple times to verify the reproducibility of results.

Specimens were analyzed using a Kevex Omicron Micro-X-ray Fluorescence Spectrometer (Kevex Instruments, Valencia, CA) and WinXRF software (Kevex Instruments) housed in the Chemistry Unit, Metallurgy Subunit of the FBI Laboratory in Quantico, VA. A 100-watt, Rh anode X-ray tube with a maximum operating voltage of 50 kV was used as the source of the incident radiation. The intensity and energy of the resulting X-ray fluorescence were measured using a standard energy dispersive X-ray detector employing a lithium-drifted silicon wafer. The relative intensities of the X-rays of different energies were determined by counting the number of photons of each type received in a given time frame. These relative intensities were the basis for determining the concentrations of each detected element in the sample. Micro-X-ray fluorescence spectrometry is a modification of standard X-ray fluorescence spectrometry in which the X-ray beam is collimated down to a small spot size (50–1000 μm). On the Omicron, this is accomplished using a mechanical aperture to permit elemental analysis of samples whose size is of the same order as that of the spot size. Because collimation of the X-ray beam reduces the net X-ray intensity reaching the sample, the system requires a relatively high powered, 100 watt X-ray source to produce adequate X-ray intensities. A 300μm diameter aperture was used for all of the analysis carried out in this study.

Samples smaller than 50 μm in diameter can potentially be examined using the instrument if they are mounted appropriately. If fully quantitative results are desired, it is usually necessary for the samples to have a flat, polished surface and to be sufficiently thick. X-rays produced by low atomic number elements (i.e., elements of lower atomic number than vanadium) are subjected to significant absorption by air resulting in significant attenuation of the fluorescent X-rays produced. This effect becomes more acute with decreasing atomic number and will dramatically increase the minimum detectable concentration of the affected elements. Where these lighter elements are of analytic importance, samples are usually analyzed under a vacuum of 500 millitorr or less (standard atmospheric pressure is 760 torr) to eliminate the deleterious effects of the air. In samples where evacuation is not possible, however, such as materials that are vacuum sensitive (including many of our

TABLE 1—*Summary of materials analyzed.*

Material Types	Alterations/Conditions
Human bones and teeth (cranium, humerus, femur, fibula, foot phalanx, molars, premolar, canine, dental plaque, and calculus)	Unaltered, burned, chemically altered (nitric acid), ancient (up to 9000 years), and subadult (including newborn)
Nonhuman bones and teeth (pig, cow, turtle, dog, rodent, and ungulate)	Unaltered, burned, and chemically altered (nitric acid and sodium hydroxide)
Other biologic materials (shell, coral, octocoral, sand dollar, brachiopod shell, and beak)	Unaltered and burned
Nonbiologic materials (various woods, various plastics, various minerals/rocks, ceramic, lime, various metals, glass, garden hose, carpet, and fabric)	Unaltered and burned

severely burned specimens), the chamber can be flushed with helium to displace the air (because of its low atomic number, helium is a poor absorber of X-rays). A helium atmosphere was used on all samples in the study.

With XRF, acquisition parameters can be adjusted to optimize the analysis conditions for a particular range of elements. This may involve changes to the X-ray source filters, voltage, and current to adjust the operating conditions. Specific conditions depend on the analysis objectives and material under analysis. In this case, the tube was operated in an unfiltered condition at 30 kV. The collection live time was held constant at 100 sec. The X-ray tube current was varied, as needed, to produce a detector dead time between 38% and 42%. The need to vary the tube current reflects differences caused by the somewhat irregular sample geometries and the presence of contaminates on the surface. The overall conditions were chosen to permit the detection of a significant portion of the periodic table while simultaneously maintaining sufficient detection limits for calcium and phosphorus.

Determination of the elements detected in the spectra involves careful analysis of the peak shapes, the peak energy positions, the relative heights of adjacent peaks, consideration of the effects of secondary and tertiary fluorescence, and other X-ray/specimen interactions. System peaks (sum and escape), Rayleigh and Compton scattering, and diffraction may also contribute peaks to the spectrum and should be considered when interpreting spectra. In general, the production of fluorescent X-rays follows a Poisson distribution. In consequence, the expected reproducibility (1 sigma) in the number of counts in any peak is equal to $N^{1/2}$ where N is the number of counts in the peak. This yields a relative uncertainty of $(N^{1/2})/N$ or $N^{-1/2}$ in repeated measurements of the emission intensity.

Results

Analysis of the human and nonhuman osseous and dental tissues in good, burned, and weathered conditions revealed characteristic levels of calcium and phosphorus (see Fig. 1 and Table 2). For samples that contained peak levels of calcium and phosphorus, the Ca/P ratio was calculated based on peak volume (vs. maximum height, which was used in the Ubelaker and Ward 2002 study). For unaltered osseous tissue, the average Ca/P ratio was 4.92 (SD = 1.19), and for unaltered dental tissues, the average ratio was 4.02 (SD = 0.83)—even the mildly to severely altered osseous and dental tissue samples showed the characteristic Ca/P ratio (see Fig. 1 and Table 2). Osseous and dental tissue samples also commonly (though not always) contained trace levels of strontium, which can substitute for calcium in the hydroxyapatite component of osseous and dental tissues (6). Coral, shell, and some treated wood samples similarly contained high levels of calcium but no phosphorus. The presence of a calcium peak alone is therefore not sufficient to conclude that the material is osseous or dental in origin—a phosphorus peak must also be present. Horn, plastic, wood, metal, and other materials in either good or compromised conditions did not contain these characteristic levels of calcium, phosphorus, or strontium, in most cases, lacking all of them. Individual samples that were run multiple times demonstrated reproducible spectra.

No sample preparation was initially performed in the analysis, and many specimens contained low levels of various other elements, most likely due to surface contamination that did not substantially affect the results. In cases of ancient osseous and dental samples, however, initial readings indicated much higher Ca/P ratios (because of lowered detection of phosphorus), often with accompanying iron and silicon peaks, which suggested interference from surface contaminants. For these specimens, the outermost layer of the sample was removed by scraping with a scalpel to permit a subsurface reading. These readings revealed Ca/P levels consistent with unaltered samples. Low P levels were also initially observed for unprepared osseous and dental samples that were chemically altered, but again, removal of a thin surface layer with a scalpel resulted in Ca/P levels resembling the unaltered specimens. In some cases (especially when no or few other elemental peaks were present), rhodium peaks were observed because of the presence of rhodium in the X-ray tube. Occasional diffraction peaks were also observed.

Three categories of specimens that were *not* osseous or dental in origin had profiles/ratios that were indistinguishable from osseous and dental tissue: mineral apatite, octocoral, and brachiopod shells. Members of the apatite group are common accessory minerals in many types of rock and are the most abundant phosphorus-bearing minerals (10). Mineral apatite is predominantly green or brown in color, although it may also be blue, violet, or colorless (11). The commonest varieties are fluorapatite, chlorapatite, carbonate apatite, and hydroxyapatite (10). Hydroxyapatite is the inorganic mineral

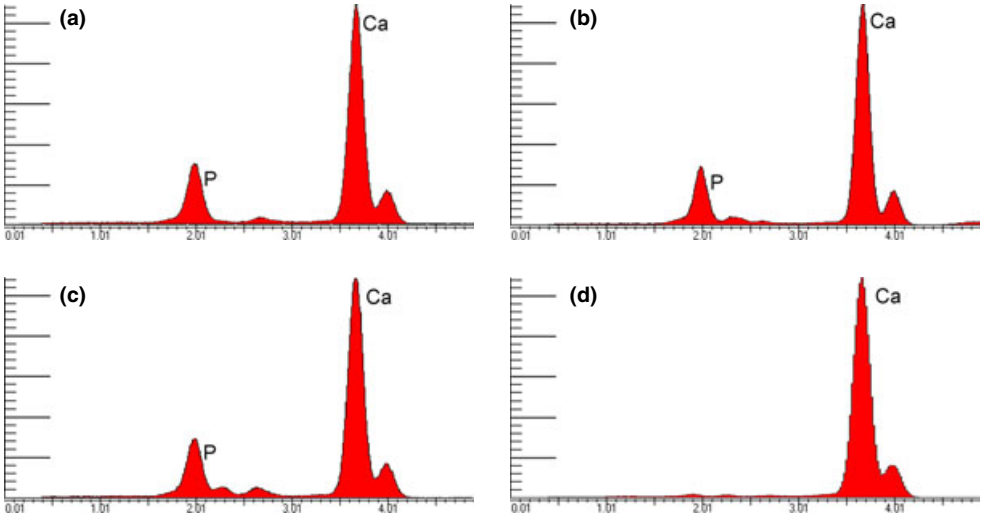

FIG. 1—*XRF spectra from various materials analyzed in this study. (a) Typical spectrum for osseous and dental material in unaltered or altered conditions. (b) Spectrum for mineral apatite. (c) Typical spectrum for octocoral and brachiopods. (d) Typical spectrum for shells.*

TABLE 2—*Ca/P ratios for various materials in this study.*

Material Types	Mean Ca/P	Min/Max	SD	N
Osseous and unaltered	4.92	3.42–8.71	1.19	17
Osseous and burned	4.57	2.64–7.89	1.37	24
Osseous and weathered/ancient	5.00	3.99–7.58	1.14	9
Osseous and chemically altered	4.58	3.80–6.15	1.35	3
Dental and unaltered	4.12	3.22–5.95	0.83	15
Dental and burned	3.76	3.69–3.83	0.10	2
Brachiopod shell	3.68	3.53–3.83	0.21	2
Octocoral	3.03	n/a	n/a	1
Mineral apatite	4.41	n/a	n/a	1
Wood, unaltered, and burned	No P, some sm. Ca peaks	n/a	n/a	7
Plastic, unaltered, and burned	No P, no Ca	n/a	n/a	6
Lime	No P	n/a	n/a	4
Nonapatite minerals	No P, some Ca peaks	n/a	n/a	4

component of bone, comprising about 65% of bone mass (the other 35% being the organic component containing primarily cells and collagen fibers). Hydroxyapatites form tiny crystals and are tightly packed into the extracellular matrix of the collagen fibers (12).

While the majority of skeletal structures of invertebrates are composed of calcium carbonate, a couple of exceptions have been noted to contain calcium phosphate. Octocoral is a modern coelenterate of the family Gorgoniidae, with a skeletal structure that contains carbonate hydroxylapatite (13). Gorgonian octocorals are found primarily in the shallow waters of the Caribbean. Atremate brachiopods of the genus *Lingula* and *Glottidia* also possess shells that contain apatite (14,15). Although at one time quite abundant, they are now rarely seen, living primarily in the very cold waters of polar regions or at great depths (16).

These materials, however, are unlikely to be confused for osseous or dental tissue because of their macro- and microscopic appearances and are also less likely than many of these other materials to be encountered in forensic contexts.

Discussion and Conclusions

XRF is a routine and reliable method of elemental analysis of questioned material in forensic contexts. It is nondestructive and relatively easy to use and straightforward to interpret with the appropriate training. These features make XRF analysis a very appealing possibility in forensic anthropological examinations where it may be necessary to determine the potential skeletal origin of unknown material.

XRF analysis is normally performed in a laboratory setting, but portable XRF units have recently been suggested for use as a possible screening tool at crime scenes (4,5,17). Such portable instruments may have potential for separating osseous and dental material from other materials at forensic scenes (especially fires). Currently available portable instruments, however, may not be able to detect elements of low atomic number (2), and given that the detection of phosphorus plays a key role in the use of XRF for identifying osseous and dental tissue, portable instruments may not yet be useful in these contexts. One possible solution is to create portable instruments capable of excluding air and incorporating an appropriate X-ray source, but more research is needed in this area, and laboratory analysis using instrumentation capable of effectively detecting phosphorus is highly recommended.

Although in most cases no sample preparation is needed for effective XRF analysis, significant surface contamination, especially in ancient samples, affected the detection of phosphorus in this study. At least one other study (17) found similar interference with previously buried cemetery samples. It is therefore recommended that, where possible, the surface should be cleaned of possible contaminants or the outermost layer removed prior to analysis, especially for potentially ancient, heavily soiled, or chemically altered samples.

Materials in this study were accurately identified as osseous or dental in origin based on the calcium and phosphorus levels detected by XRF using the analytical parameters of this study, with few exceptions. Mineral apatite, octocoral, and brachiopod shells were the only materials identified to have Ca/P profiles similar to osseous and dental tissue, but are structurally distinct from bone and tooth and are unlikely to be encountered in forensic contexts; therefore, nonbone and nontooth materials are unlikely to be misclassified as osseous or dental tissue. We conclude that XRF analysis is a valid and effective means of determining osseous or dental origin of unknown material.

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