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Review

Chemical Differentiation of Osseous, Dental, and Non-skeletal Materials in Forensic Anthropology using Elemental Analysis



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ABSTRACT

Forensic anthropologists are generally able to identify skeletal materials (bone and tooth) using gross anatomical features; however, highly fragmented or taphonomically altered materials may be problematic to identify. Several chemical analysis techniques have been shown to be reliable laboratory methods that can be used to determine if questionable fragments are osseous, dental, or non-skeletal in nature. The purpose of this review is to provide a detailed background of chemical analysis techniques focusing on elemental compositions that have been assessed for use in differentiating osseous, dental, and non-skeletal materials. More recently, chemical analysis studies have also focused on using the elemental composition of osseous/dental materials to evaluate species and provide individual discrimination, but have generally been successful only in small, closed groups, limiting their use forensically. Despite significant advances incorporating a variety of instruments, including handheld devices, further research is necessary to address issues in standardization, error rates, and sample size/diversity.

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1. Introduction

While identification of osseous and dental materials by the forensic anthropologist is generally established on gross anatomical features, it may be problematic to identify highly fragmented or taphonomically altered materials as osseous or dental in origin and/or to distinguish between human and non-human. Multiple chemical analysis techniques focusing on the elemental composition of materials have been shown

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to be reliable laboratory methods that can be used to determine if questionable fragments are osseous, dental, or non-skeletal in nature [1–3]. Once the forensic anthropologist determines that questionable material is osseous or dental in nature, the next step in the analysis is to determine if osseous/dental material is human or non-human. Traditionally, methods such as bone histology [4–12], protein analysis [13,14], and DNA [15,16] may be used to determine if questionable materials are human or non-human. Further, in the case of DNA, this method is routinely used for individual determination of commingled remains when sorting through fragmentary material. More recently, studies suggest that elemental analysis combined with multivariate statistical analysis [3,17–19] may be useful in the discrimination, or the initial sorting

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prior to DNA analysis, of multiple individuals when presented with highly fragmentary and taphonomically modified material.

The purpose of this review is to provide a detailed background of the chemical analysis techniques introduced in forensic anthropology to differentiate materials as osseous, dental, or non-skeletal using the elemental composition of these materials. In addition, there are numerous studies evaluating species and individual discrimination focusing on the elemental composition of the samples. These studies use a variety of chemical methods combined with multivariate statistical analyses, and, as such, will be discussed separately. However, before discussing how these methods are used to distinguish osseous and dental tissues from non-skeletal materials it is first important to provide an overview of the anatomy, chemical composition, and variability of bones and teeth.

2. Anatomy, Chemical Composition, and Variability of Bones and Teeth

Dental and osseous tissues consist of both organic and inorganic components (Table 1). The organic component of bone consists of the bone cells (osteoblasts, osteoclasts, and osteocytes) and the osteoid, the organic portion of the bone matrix. The osteoid consists of collagen, proteoglycans, and glycoproteins, all of which are secreted by osteoblasts, and makes up approximately one-third of the bony matrix. The organic component of bone, particularly collagen, provides bone with great tensile strength and flexibility, as well as the ability to resist stretching and twisting [20]. The inorganic component of bone, accounting for the remaining two-thirds of the bony matrix, consists of hydroxyapatites, or mineral calcium phosphates, with the empirical formula, $Ca_5(PO_4)_3OH$. The hydroxyapatite gives bone its incredible strength, allowing it to resist compression [20].

Teeth also have a chemical composition similar to bone (Table 1). An acellular material called enamel composes the crown. Enamel is the hardest substance in the body, with a chemical composition of 96% inorganic material (mostly hydroxyapatite), 1% organic material, and 3% water [20]. The enamel protects the underlying dentin, which is a living, porous, bone-like material that forms the bulk of the tooth. Dentin is harder than bone but softer than enamel, with a chemical composition of 70% inorganic material (mostly hydroxyapatite), and 30% inorganic material and water. A substance called cementum covers the outer surface of the root, attaching the tooth to the periodontal ligament and anchoring the tooth in the bony alveolus. The layer of cementum is thin and composed of 55% organic material and 45% inorganic material (mostly hydroxyapatite) [20].

Once laid down, the trace element component of the inorganic portions of bone can change due to ionic substitution of other elements (Table 2). Different portions of the calcium phosphate matrix can be replaced by various trace elements [23]. Calcium ions are reported to be replaced by: Li, Be, Na, Mg, Al, Si, K, V, Cr, Mn, Fe, Cu, Zn, Ga, Sr, Y, Zr, Nb, Cd, Sn, Ba, Au, Hg, Pb, Bi, Ra, Ac, Th, U, and Pu. The phosphate group (PO₄), may be substituted by citrate, phosphate esters, diphosphonates, pyrophosphates, and amino acids in vitro. Finally, the

Table 2Common Trace Elements found in Bone Tissue and Tooth Enamel.

(Compiled using [21], [22])

hydroxyl group (OH) can be replaced by F or Cl [23]. Bone turnover and external conditions can also cause these elements to filter back out of the bone matrix [24].

As a result of these substitutions and the resulting fluctuations of the calcium-phosphorus ratios within individual bones, presence and quantities of trace elements can vary significantly between individuals. Factors influencing trace elemental disbursement include bone location and type, diet, growth environment, and taphonomic modifications. One of the strongest influential factors related to elemental distribution within the bone matrix is bone type as trace elements are not evenly distributed within individuals or even individual bones [25]. For example, Zn, V, Ni, Cr, Pb, Mn, Co, and Sn are found in higher concentrations in bone epiphyseal regions while Ca, Sr, Na, and K are found more often in the central portion of the diaphysis [21].

Diet and growth environment also have a significant impact on the distribution of trace elements within bone. Research is being conducted to establish past dietary habits by using stable isotope analysis to detect specific trace element ratios associated with particular dietary patterns [26–32]. Environmental contaminants, such as F or Pb in the water supply, will also be incorporated into the bone matrix [33]. Research has also shown increases in Fe, Mn, and Cu is possible due to leaching from the burial environment [34].

Similar conditions impact chemical alterations to dental materials. Dental materials' higher hydroxyapatite concentration results in a more homogenous composition [35] which makes them less susceptible to change [36]. Similar to osseous materials, trace elemental concentration in dental materials is most effected by location. Concentrations of heavy metals increase when progressing from the outer enamel to the inner dentin root, while leeched materials exhibit decreased concentrations towards the inner portion of the tooth [34]. Finally teeth in different portions of the dental arc will reflect different life periods as a result of dental formation patterns. As a result, while trace elements can be deposited in bone during one's lifetime, due to factors such as diet or

Table 1Bone and tooth organic and inorganic components [20].

	Bone	Tooth
Water (H ₂ O)	10-20% in living bone, but <1% in dry bone	~ 3% in living tissue, <1% in dry tissue
Organic Components	~ 33% (dry bone)	~ 1-55% (dry tissue) depending on tooth element
Main Component(s)	Bone Cells (osteoblasts, osteoclasts, osteocytes), Osteoid (collagen, proteoglycans, glycoproteins)	Protein (amelogenins and enamelins in enamel; odontoblasts in dentin)
Function(s)	gives bone its flexibility and great tensile strength; allows bone to resist stretch and twisting	Framework for the development of enamel/dentin
Inorganic Components	~ 66% (dry bone)	~ 45-96% (dry tissue) depending on tooth element
Main Component(s)	Hydroxyapatite (Ca5(PO4)3OH (mineral calcium phosphates))	Hydroxyapatite (Ca ₅ (PO ₄) ₃ OH (mineral calcium phosphates))
Function(s)	give bone it's incredible strength; allowing bone to resist compression	makes teeth strong and resistant to degradation; protects living portion of tooth

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