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# Enhanced methods for assessment of the trace element composition of Iron Age bone

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ABSTRACT

Modern, ultra-trace, analytical methods, coupled with magnetic sector ICP-MS (HR-ICP-MS), were applied to the determination of a large suite of major and trace elements in Iron Age bones. The high sensitivity and un-paralleled signal-to-noise characteristics of HR-ICP-MS enabled the accurate measurement of Ag, Al, As, Ba, Ca, Cd, Ce, Co, Cr, Cu, Fe, La, Li, Mg, Mn, Ni, P, Pb, Pt, Rb, Sr, U, V, and Zn in small bone sections (<75 mg). Critically, the HR-ICP-MS effectively addressed molecular interferences, which would likely have compromised data generated with quadrupole-based ICP-MS instruments.

Contamination and diagenetic alteration of ancient bone are grave concerns, which if not properly addressed, may result in serious misinterpretation of data from bone archives. Analytical procedures and several chemical and statistical methods (Principal Components Analysis — PCA) were studied to assess their utility in identifying and correcting bone contamination and diagenetic alteration. Uncertainties in bone (femur) sampling were characterized for each element and longitudinal variation was found to be the dominant source of sampling variability. However the longitudinal variation in most trace elements levels was relatively modest, ranging between 9 and 17% RSD.

Bone surface contamination was evaluated using sequential acid leaching. Calcium-normalized metal levels in brief, timed, dilute nitric acid leaches were compared with similarly normalized interior core metal levels to assess the degree of surface enrichment. A select group of metals (Mn, Co, Ni, Ag, Cd, and Pt) were observed to be enriched by up to a factor of 10 in the bone surface, indicating that that these elements may have a higher contamination component. However, the results of sequential acid leaching experiments indicated that the single acid leaching step was effective in removing most surface-enriched contaminants. While the leaching protocol was effective in removing contaminants associated with the bone surface, there remained potentially significant residual levels of soil-sourced contaminant tracers within the leached bone. To address this issue a mathematical procedure, based on metal/aluminum ratios, was developed to correct—for the soil—contaminant metal pools. Soil correction fractions for the primary anthropogenically mobilized metals evaluated were greatest for Pb (13.6%) followed by As

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(4.4%), Ag (3.9%), and Cd (0.94%). Although median soil corrections were typically low, many samples did require a much larger correction, thus both bone cleaning and soil corrections may be necessary to realize accurate endogenous bone elemental data. The results of the PCA analysis were remarkably consistent with outcomes from the chemical and elemental ratio protocols evaluated in the study, and suggest that loadings on certain factors will be helpful in screening for soil-biased samples and in identifying diagenetically altered bone. Application of these contamination evaluation and correction tools was made possible by the high-quality, multi-element, datasets produced by HR-ICP-MS. Large variations in bone core concentrations between the 80 Iron Age specimens examined were observed for all the primary trace elements and in many of the supporting elements, even after correction for major contaminant components.



## 1. Introduction

### 1.1. *Background*

Bone and other calcified tissues can be important archives of environmental and dietary trace element exposures (Martinez-Garcia et al., 2005; Trueman and Tuross, 2002; Farnum et al., 1995). The chemistry of the bone reflects both the integrated exposure and the biogeochemistry and metabolism of the individual elements. In archaeological investigations of humans buried in mass cemeteries of Western Europe, analyses of bone specimens have provided information about potential metallotoxicity (Grandjean 1988) and on paleodietary habits (Van Klinken et al., 2000; Jay and Richards, 2006). Studies of contemporary humans demonstrate that anthropogenic exposures to metals are reflected in bone (Jurkiewicz et al., 2004) and teeth (Budd et al., 1998), and therefore validate, in part, past and future use of ancient bones and teeth as a tool to assess trace element exposure profiles of early societies.

Exogenous contamination and diagenetic alteration of ancient bone are critical concerns potentially limiting the value of information, particularly from trace elements, obtained from bone archives (Stuart-Williams et al., 1996; Trueman et al., 2004; Pike and Richards, 2002; Elliott and Grime, 1993; Radosevich, 1993). The long contact with soil, metal-containing grave goods, and the post-excavation exposures has led to special challenges and controversies. For instance, a popular hypothesis is that the Roman Empire declined largely because of Pb toxicity (Nriagu, 1983, 1998), but diagenetic elevation of bone Pb might also explain some of the findings (Wittmers et al., 2002). Because of the challenge of burial diagenesis, much paleoepidemiologic research on prehistoric populations abundantly available from inhumation burials, such as the Celtic people of the Iron Age, who were famous as metal-workers (Cunliffe, 1997) and were likely exposed to metallotoxicity, has had to be deferred.

Burial conditions, principally the geological environment of burial, are the overarching factor driving the potential biases. Mechanistically, burial biases result from two primary processes; (a) occlusion and incorporation of micro-grains of burial soils into the porous bone matrix, and (b) diffusion/repartitioning of elements from soil-solution into the bone (adsorption or ion substitution). However the degree of environmental contamination and/or diagenetic alteration is often quite variable, and therefore contamination isolation and correction approaches must be sufficiently robust to effectively deal with the wide range of perturbations. Contamination artifacts may also be introduced during handling (e.g. inappropriate "cleaning" or preservation techniques (e.g. the use of arsenic as a preservative) and processing (e.g. coring)).

Few published papers have systematically examined analytical and statistical tools for identification and correction of exogenous contamination or diagenetic alteration. Some researchers have relied on bone cleaning techniques, many of which incorporate an acid rinse to isolate inorganic surface contamination (Chiaradia et al., 2003; Budd et al., 1998; Ericson et al., 1991), however the efficacy of some of these methods is poorly validated. In a large number of studies, pre-cleaning of the bone is much less comprehensive (González-Reimers et al., 2003; Aberg et al., 1998; Baranowska et al., 1995). The relatively limited number of elements quantified in many of these studies also limits the application of post-cleaning correction tools such as contaminant tracer, element ratio, partitioning models, and statistical fingerprinting techniques. Stable isotope ratio methods of lead and strontium may be used to assess anthropogenic contamination and diagenesis/provenance respectively (Webb et al., 2005; Chiaradia et al., 2003; Aberg et al., 1998), however they are of limited use in quantitatively identifying and correcting the contaminant component of the larger suite of trace elements of interest. Direct-solids micro-sampling techniques have provided valuable insight into bone integrity and diagenetic status of major bone elements (Jankuhn et al., 1998; Greenlee, 1996), however, extrapolation to trace elements remains problematic. Correction strategies based on burial soil trace element relationships is an active and promising area of research (Trueman and Tuross, 2002; Pike and Richards, 2002; Shinomiya et al., 1998; Vuorinen et al., 1996).

Analytically, bone is a very challenging matrix for many instrumental techniques because of the relatively low concentrations of most trace elements and challenging hydroxyapatite (calcium phosphate) matrix. As the need grows to quantify ever larger trace element suites at ever lower concentrations, these problems are exacerbated. Application of many solution-based analytical techniques is limited either by sensitivity (ICP-OES: Kuo et al., 2000; Shinomiya et al., 1998; Helliwell et al., 1996) or poor productivity of non-multi-element methods (GFAA: Ericson et al., 1991; Wittmers et al., 2002; González-Reimers et al., 2003). The relatively recent application of plasma mass spectrometry (ICP-MS) to archaeological investigations (Trueman et al., 2004; Lee et al., 1999)