BONES SPEAK: EVALUATING TRACE ELEMENTS IN TWO MILLENNIA OF TUSCAN HUMAN HISTORY

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Abstract

This study delves into the critical role of essential elements (Ca, P, Zn, Cu, Mn) in growth and bone modeling, emphasizing their incorporation into bone structure through ion exchange with the chemical components of hydroxyapatite (Ca, P, H) (Giblin, 2004). The intricate process of ion exchange is fundamental for mineral homeostasis, as the skeleton serves as a reservoir, releasing and depositing ions in response to the body's requirements. This study explores the significance of trace element analysis in human bone, particularly focusing on its potential for reconstructing diet through the assessment of contaminants and elemental distributions within bones.

The homeostasis of essential elements is paramount for growth and bone health, with certain elements aying a crucial role in bone modeling. This dynamic process ensures that the skeleton functions as a reservoir, facilitating the release and deposition of ions based on the body's mineral needs. In addition to the physiological aspects, exposure to contaminants can be evaluated through trace element analysis, providing valuable insights into diet patterns.

The research highlights the utility of studying the accumulation of various elements in bones, which occurs in proportions determined by dietary choices. As different food sources have distinct elemental distributions, this variation offers a unique opportunity to assess and reconstruct diets through palaeonutritional research. By understanding how trace elements accumulate in bones, researchers can unravel dietary patterns, providing a glimpse into ancient lifestyles and exposure to contaminants.

This paper underscores the increasing importance of trace element analysis in palaeonutritional research, offering a powerful tool for reconstructing dietary habits and assessing environmental exposures. The findings contribute to a broader understanding of human history, shedding light on the intricate relationship between essential elements, bone health, and dietary choices.

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1 Introduction

Certain elements (Ca, P, Zn, Cu, Mn) are essential for growth and bone modelling, whereas others become part of bone structure through ion exchange with normal chemical components of hydroxyapatite (Ca, P, H) (Giblin, 2004). This process is essential for homeostasis of minerals, since the skeleton acts as a reserve from which ions are released and deposited according to the body's needs. Exposure to contaminants can be evaluated and diet studied due to the fact that various elements with different distributions in various food sources accumulate in different proportions that depend on diet. Palaeonutritional research is increasingly demonstrating the potential of trace element analysis in human bone for reconstructing diet.

For correct interpretation of the results of such analyses the following criteria should be observed (Beck, 1985):

- Existence of a direct relation between dietary levels of an element and bone levels;
- Concentrations sufficient to enable accurate and consistent measurements;
- Element concentrations not affected by disease, or evidence of the pathology affecting concentrations in the remains;
- Constant element concentrations in adulthood; - Exclusion of diagenetic phenomena.

The latter point is particularly important for ancient skeletal remains, in which accumulation of trace elements due to physiological processes (biogenesis) may not be the only factor involved in accumulation. Indeed, after death, the bone matrix is still in a dynamic state with its environment in a biogenetic-diagenetic continuum (Sandford, 1992; Von Endt, 1984). Diagenesis includes all physical, chemical and biological processes that alter archaeological materials where they are buried (Wilson and Pollard, 2002).

Continuous stoichiometric variation in composition is caused by absorption or release of ions, altering the molecular structure of bone, often making it difficult to study palaeodiet and contaminants. Not all elements vary to the same extent or in the same way (enrichment or depletion). In evaluating the influence of any diagenetic contaminants, many authors consider it advisable to express trace elements in terms of calcium (Price and Kavanagh, 1982; Sillen and Kavanagh, 1982; Schoeninger, 1982; Ericson et al., 1991). However, element:calcium ratios imply that loss or enrichment of calcium involves similar loss or enrichment of other elements. Since calcium is subject to frequent alterations post mortem (Lambert et al, 1982; Price et al., 1985), ratios with elements less subject to diagenesis may be problematical. Considering the particular diagenetic behaviour of calcium, Bacci et al. (2008) limited the application of correction methods to few, well-defined cases:

1. elements that are normally non diagenetic, such as strontium and zinc, may only be standardized to calcium when calcium is not subject to diagenetic alterations;

2. elements that are normally diagenetic, such as copper and magnesium, may only be expressed in relation to calcium when they and calcium have undergone diagenetic variations of the same nature (enrichment or depletion);

3. "site correction", consisting in correlating Sr:Ca and Zn:Ca ratios of human samples with those of herbivores and carnivores coeval with humans in the same site, is only possible when human calcium and that measured in the bones of animal remains on the site have undergone diagenetic variations of the same nature (enrichment or depletion).

In the present study we investigated concentrations of Cd, Pb, Zn, Ca, Sr, Al, Fe, Ti, Fe and Mn in bone samples from human skeletons dating back to the Copper Age from the Lunigiana region (municipality of Cassola, Massa Carrara, Italy) and to the Late Medieval Period from Pianosa Island (Grosseto, Italy). The objective was to obtain insights into diet, heavy metal contamination and differences in accumulation between humerus and femur. Interestingly, femurs of the Medieval specimens contained soil, allowing for comparison of trace element concentrations in the bones, on the one hand, and in the burial earth on the other. To evaluate diagenetic alterations

we used a statistical approach based on principal component analysis and correlation matrices, comparing trace element concentrations in bones and in the soil within the bones. To evaluate diagenetic effects, the data could not be standardized in relation to calcium because the individuals were of different ages. Since accumulation of certain elements depends on exposure time, high concentrations due to accumulation over a lifetime could be corrected erroneously. Likewise, "site correction" was not possible because no animal remains were found.

We used element:calcium ratios to evaluate and interpret information about palaeodiet only after determining the influence of diagenesis on calcium. We also calculated Sr:Ca and Zn:Ca ratios to use these elements as food markers. Sr and Zn are generally relatively unaffected by diagenesis (Lambert et al., 1983; Price et al., 1985; Sillen, 1981) and are representative of prevalently vegetarian and prevalently carnivorous diets (Lambert, 1984; Sillen and Kavanagh, 1982; Hatch and Geidel, 1985; Busetto et al., 2008).

2 Materials and Methods

2.1 Archaeological evidence

2.1.1 The Copper Age specimens

The human remains from which the samples were collected, have been found in a small cave called *Tana della Volpe* (Fox Den). This is located near the better known Tecchia which is a part of the imposing karst system of the *Grotte di Equi Terme* in the municipality of Casola (Massa-Carrara, Tuscany) (Fig. 1). Tana della Volpe was excavated in the 1960s and is a typical Eneolithic "natural sepulchral cave". Skeletal remains of about 30 individuals were recovered (Formicola 1980) together with grave goods consisting of arrowheads, ornaments and pottery, whose typological characteristics suggest an attribution of the whole complex to the Middle Copper Age. The Copper Age corresponds to the maximum spread of the phenomenon of Megalithism in Western Europe and, in Lunigiana, at the same period, many Stele-Statues were erected. The study of the anthropological features and customs of the individuals buried at Tana della Volpe is therefore of particular interest because this group is a representative sample of the people who conceived and built the intriguing and still mysterious Stele-Statues of Lunigiana.

1.2 The Medieval specimens

Samples for analysis were collected from several human skeletons recovered from the necropolis of Cala San Giovanni on Pianosa Island (Tuscany) (Fig.1), which was excavated between 2007 and 2010 by the Archaeological Office of Tuscany in collaboration with the University of Siena.

The necropolis (which is close to some findings belonging to a Roman villa) can be referred to the late Medieval Period on the basis of both burial typology and rare materials but especially on the basis of ^{14}C dating, carried out on a bone sample from individual no. 1157, which yielded the following date:

Beta-297581 conventional radiocarbon age 930±30 BP, 2 sigma calibrated result Cal AD 1030 to 1170 (Cal BP 920 to 780). The necropolis was possibly used over a long period. The tombs were pits lined with stone slabs and the corpses were covered with earth and sealed with other slabs. Late imperial coins found in the covering earth of the burials can be considered as a *terminus post quem* for the excavation of the graves themselves and confirm the above ^{14}C chronology.
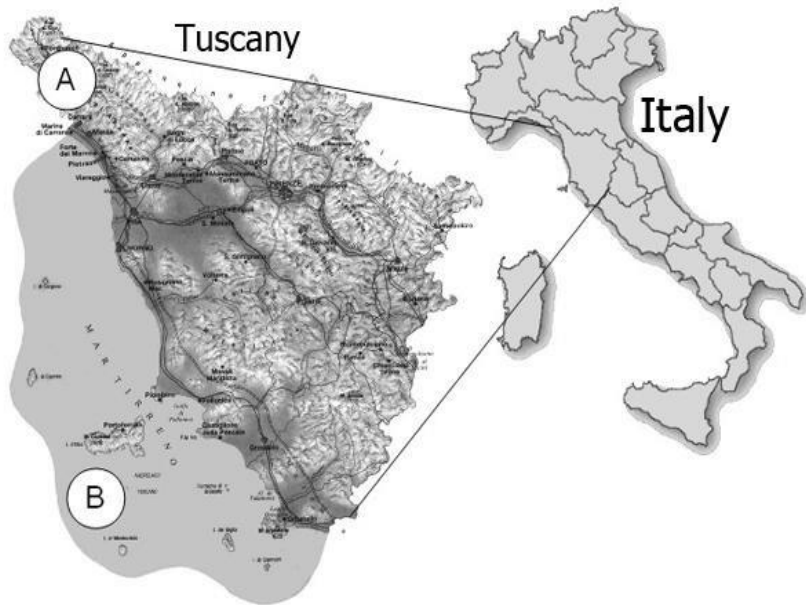
Figure 1 - Location of archaeological sites: A) Tana della Volpe; B) Pianosa Island.

2.2 Sampling of Bone and Soil for Spectro photometric Analysis

Samples from the cortex of the tibia or femur is generally recommended for palaeonutritional analysis because this bone shows more homogeneous individual analytical values than trabecular bone, which is more subject to soil contamination (Bartoli, 2005). Cortical bone is remodelled much more slowly and is therefore suitable for studying long-term accumulation (Sillen and Kavanagh 1982). Medieval samples were obtained from femurs and humeral bones to determine differences in accumulation in a given individual. Copper age bone samples were obtained from five different individuals (Table 1).

Samples were obtained by mechanical abrasion of a portion of cortical bone with an acid-cleaned burr, previously tested on animal bone. Sampling was conducted in three steps:

1.   The sampling area of bone was chosen and an area measuring 0.5 x 1 cm was defined, from which 2-3 mm of bone, where diagenesis is strongest, was removed by abrasion and discarded. Trace element enrichment by diagenesis decreases from the bone surface inwards (Mannino, 2009; Takata, 2005). Removal of the outer surface increases the probability of measuring trace elements due to bioaccumulation in life.

2.   The predefined area was abraded and the powder collected in a 50 mg Eppendorf flask.

3.   Where present, soil samples were obtained from inside femurs and humerus bones. The soil samples were dried completely at 35°C. Coarser material was removed and the soil was homogenized in an agate mortar.

The number of bone and soil specimens sampled is shown in Table 1.

Table 1 - Number of individuals, bone and soil specimens sampled for Copper Age and Late Medieval Period.

Age	no. individuals	Bone type	no. bone samples	no. soil inside bone samples
Late Medieval Period	12	humerus	6	2
		femur	12	6
Copper Age	5	humerus	2	1
		femur	3	0

2.3 Preparation of samples and spectrophotometric analysis

2.3.1 Mineralization of bone samples

Aliquots of about 0.1 g of bone were placed in teflon containers and spiked with 2 ml nitric acid and 0.5 ml hydrogen peroxide analytical grade for acid digestion. The containers were loaded in pressure-sealed steel blocks and held at 160°C for 12 h. The clear solutions obtained were transferred to polyethylene test tubes and made up to 10 ml. The following elements were analysed in these solutions: Pb, Cu, Cd, Ni, Cr, Sr, Ti, Al, Fe, Ca and Mn. A blank was included in each series to verify the purity of reagents, together with standard reference material (Bovine Liver n.1377b from NIST, Gaithersburg, MD - USA) at certified concentrations to determine analytical accuracy. To calculate metal concentrations we used the method of internal additions: to equal aliquots of the same sample we added increasing quantities of a solution containing known concentrations of the metals to analyse.

2.3.2 Mineralization of soil samples

The method is based on dissolving heavy metals in hot nitro-hydrochloric acid attack. Aliquots of about 0.1 g of dried and homogenized soil samples were placed in teflon containers, spiked with 0.3 ml hydrogen peroxide and left to react for 20 min. Then 0.9 ml HCl and 0.3 ml HNO_3 was added for mineralization and the containers were loaded in sealed steel blocks at 160°C for 12 h. All reagents were analytical grade. The resulting solutions were transferred to plastic test tubes, made up to 10 ml and analysed by atomic absorption spectrometry. Each series contained a blank to check reagent purity and six tests of standard reference materials (Montana Soil n.2710 from NIST, Gaithersburg, MD - USA) to test accuracy. Concentrations of metals were calculated by the method of internal additions: to equal aliquots of the same sample, increasing quantities of a solution containing the metals to be analysed, at known concentrations, were added.

2.3.3 Analytical determinations

The instruments used for trace element analysis were:

1.   Analytic Jena Contra 700 atomic absorption spectrophotometer with graphite furnace for Cd, Pb, Ni and Cr;

2.   ICP-Plasma Perkin Elmer Plasma 5100DV emission spectrometer for Zn, Mn, Fe, Cu, Al, Ti, Ca, Cu and Sr.

2.4 Statistical analysis

Graphs and descriptive statistics were obtained using GraphPad Software 1992-2003. The graphs show means and standard deviations on error bars. Significant differences are indicated by an asterisk. The data was processed by PCA, Spearman correlation test, Kruskall Wallis test and Mann Whitney U test using STATISTICA StatSoft. Inc 2007 software.

3 Results And Discussion

3.1 Concentrations of trace elements

Tables 2 and 3 show concentrations of Zn, Fe, Mn, Al, Ti, Ni, Cr, Cu, Pb, Sr and Cd in mg/kg d.w. and Ca as percentage in bone of Late Medieval and Copper Age samples.

Table 2. Concentrations of Zn, Fe, Mn, Al, Ti, Ni, Cr, Cu, Pb, Sr and Cd in mg/kg d.w. and Ca as percentage in bone of Late Medieval samples.

ID	Zn	Fe	Mn	Al	Ti	Ni	Cr	Cu	Pb	Sr	Cd	Ca %
Humerus (n=6)												
Mean	139.25	157.55	19.65	288.69	7.70	3.97	12.90	20.76	42.11	580.33	0.19	38.85

S.D.	46.55	183.74	19.00	275.70	9.89	0.98	3.49	7.66	67.61	281.62	0.21	1.89
Femur (n=12)												
Mean	93.50	171.94	13.97	267.94	6.13	5.19	15.25	22.92	15.25	631.28	0.09	37.45
S.D.	26.81	163.61	9.68	226.05	4.13	3.62	8.82	19.65	10.39	252.06	0.10	2.66

Table 3. Concentrations of Zn, Fe, Mn, Al, Ti, Ni, Cr, Cu, Pb, Sr and Cd in mg/kg d.w. and Ca as percentage in bone of Copper Age samples.

Bones	Zn	Fe	Mn	Al	Ti	Ni	Cr	Cu	Pb	Sr	Cd	Ca%
Humerus (n=3)												
Mean	224.28	174.44	227.01	301.52	2.99	14.59	10.04	506.93	1.59	305.51	1.97	38.89
S.D.	96.95	84.39	138.16	217.46	4.81	11.59	3.48	747.64	0.51	24.69	2.44	3.52
Femur (n=2)												
Mean	144.48	183	39.03	337.55	3.31	2.77	6.25	33.27	1.32	282.78	1.06	37.02
S.D.	78.98	213.07	39.17	243.3	4.43	0.69	1.66	10.91	0.04	7.57	0.2	2.05

Tables 4 and 5 show concentrations of Zn, Fe, Mn, Al, Ti, Ni, Cr, Cu, Pb, and Cd in mg/kg d.w. and Ca as percentage in soil samples obtained from inside the bones.

Table 4. Concentrations of Zn, Fe, Mn, Al, Ti, Ni, Cr, Cu, Pb, Cd in mg/kg d.w. and Ca as percentage in Late Medieval soil samples (n=8) obtained from inside the bones.

Soil (n=8)	Zn	Fe	Mn	Al	Cu	Ti	Cr	Ni	Pb	Cd	Ca %
Mean	38.30	8727.37	248.37	14097.34	21.15	392.35	74.88	281.43	64.00	0.05	17.19
SD	10.64	3261.51	87.44	6298.46	12.07	150.04	31.62	185.87	38.70	0.01	2.51

Table 5. Concentrations of Zn, Fe, Mn, Al, Ti, Ni, Cr, Cu, Pb, Cd in mg/kg d.w. and Ca as percentage found in Copper Age soil samples (n=1) obtained from inside a bone.

Soil (n=1)	Zn	Fe	Mn	Al	Cu	Ti	Cr	Ni	Pb	Cd	Ca %
Single measurement	252.70	7341.39	878.46	14743.88	63.99	265.45	38.09	105.99	18.06	0.65	19.76

3.2 Diagenesis

Before any further analysis or discussion of the results we assessed the influence of any diagenetic processes. Many authors consider it appropriate to express trace elements in relation to calcium (Price and Kavanagh, 1982; Schoeninger, 1982; Ericson et al., 1991). In the present study we did not standardize the data by element:calcium ratios because the individuals whose bone we studied had different ages and as accumulation of certain elements depends on exposure time (Miculescu et al., 2011), high concentrations due to accumulation could have been

erroneously corrected. Nor did we do site correction as suggested in the literature (Bacci et al., 2008; Bisel et al., 1980) because we had no faunal remains from the same sites.

To evaluate diagenetic alterations we used a statistical approach based on correlation tests and multivariate methods (PCA), comparing trace element concentrations measured in bones and in soil removed from inside bones. Because it was possible to obtain soil from inside femurs and humerus bones of subjects living in the Late Medieval

Period, we were able to check the similarity or otherwise of trace elements in these different matrices. Principal Components Analysis showed that soil and femurs formed two well-defined groups in different quadrants (Fig. 2), indicating that the concentrations present in the two matrices were quite distinct.
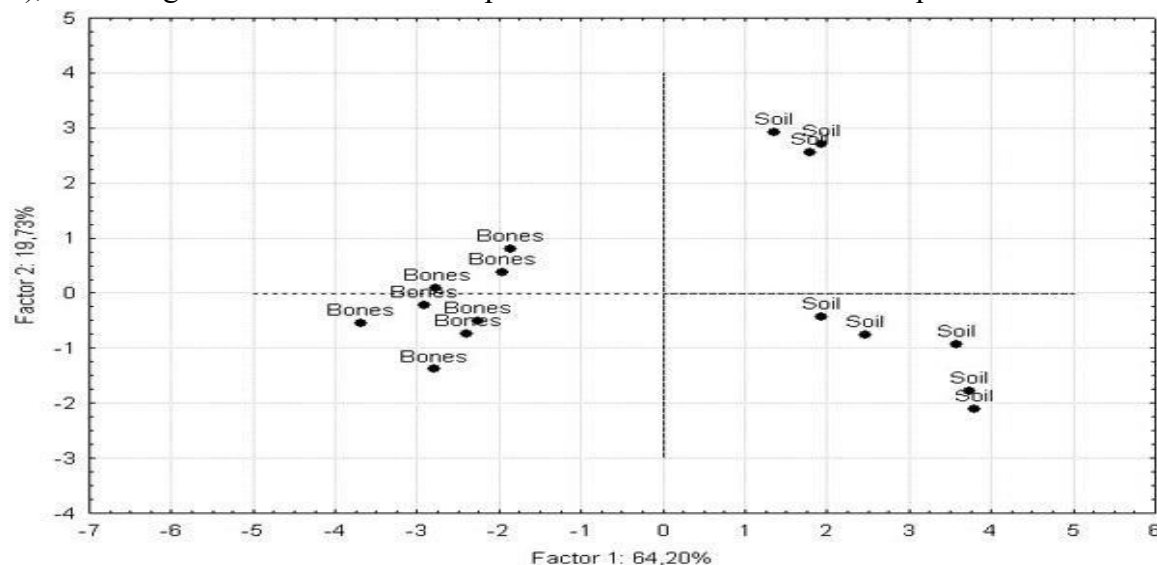

Figure 2 - Projection of cases in the factor plane according to concentrations of Zn, Fe, Mn, Al, Ti, Ni, Cr, Cu, Pb, Sr, Cd and Ca in soil and bones.

Projection of the variables (element concentrations) on the factor plane of the first two factors (Fig. 3), which explained 86% of the variance, showed that the components representing Ca, Sr, Zn, Cd, Ni and Cr were in different quadrants from the components representing lithogenic elements (Al, Fe, Mn, and Ti), which though clustered were not significantly correlated. The components representing Cu and Pb were in the same quadrant as lithogenic elements and irrespective of any statistically significant correlation, this could indicate a modest influence of diagenetic processes.

Figure 3 - Projection of the variables (Zn, Fe, Mn, Al, Ti, Ni, Cr, Cu, Pb, Sr, Cd and Ca concentrations) on the factor plane determined by bones and their respective soil samples.
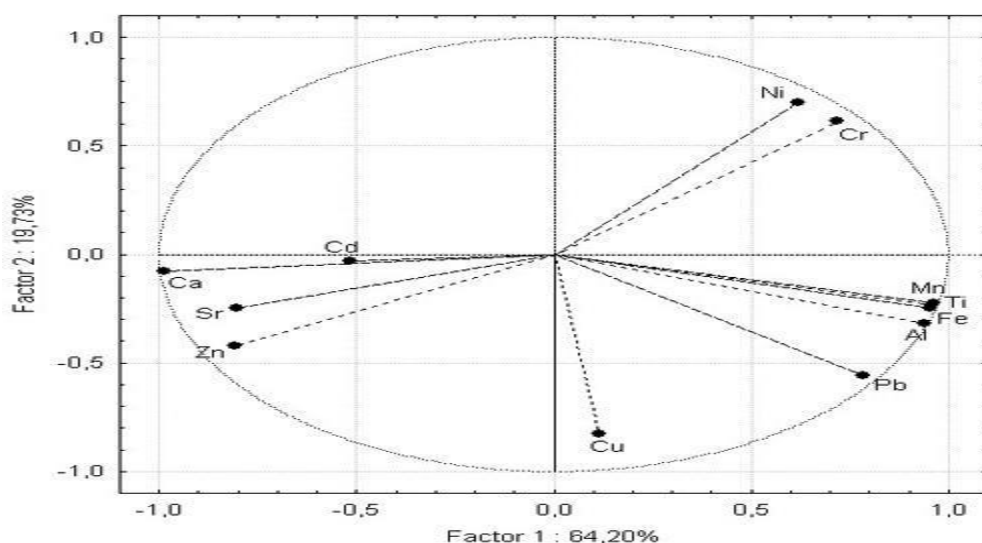
The absence of correlations between Ca, Sr, Zn, Cd, Ni, Cr, Cu and Pb and the group of lithogenic, and typically diagenetic elements, sustained the hypothesis that the influence of diagenetic processes was weak, if not absent. On the other hand, highly significant correlations between Al and Fe ($R=0.88$ $p<0.01$), between Ti and Fe ($R=0.97$ $p<0,001$) and between Ti and Al ($R=0.83$ $p<0.05$) indicated a possible common source (soil) that may have been due to diagenetic enrichment. These results suggest the hypothesis of absence or limited influence of diagenetic processes for elements such as Pb, Zn, Cu, Sr, Ca, Ni, Cr and Cd. Principal components analysis and correlation matrices applied to bones with soil inside them showed which elements underwent depletion (negative correlation) and enrichment (positive correlation). This statistical approach proved to be valid and useful for interpreting the influence of diagenetic processes in ancient skeletal specimens.

Since processes of enrichment or depletion of Ca, Cd, Zn, Sr, Cu, Pb, Cr and Ni were excluded, it was unnecessary to correct the data with calcium ratios (as reported in the literature) to assess the influence of any diagenetic contaminants (Price and Kavanagh, 1982; Sillen and Kavanagh, 1982; Schoeninger, 1982; Ericson et al, 1991). The element:calcium ratio assumes that any loss or enrichment of calcium involves similarly intense losses or enrichments of other elements. However, use of this correction method for the data of certain elements may be erroneous if the data sample does not belong to individuals of the same age and who therefore have not undergone bioaccumulation for the same periods of time. Some individuals could also have been exposed to high inputs of certain elements (smelting of mineral ores and forging of metal artefacts out of Pb, Cu and so forth) that could be mistaken for diagenetic enrichment.

For Copper Age specimens it was not possible to do statistical analysis of femur and soil samples to detect any diagenetic processes because only one soil sample was available. We can nevertheless exclude strong depletion or enrichment because Ca concentrations in femur samples of the two epochs reflected normal concentrations of human bone (Takata et al., 2005; Busetto et al., 2008) (Fig. 4) and were also higher than those measured in soil samples (Tables 2 and 4). After dissolution of soft tissue, bones usually reach a state of chemical equilibrium with the surrounding earth (White and Hannus, 1983). Had there been input from the soil, balancing or enrichment between bone and soil concentrations would have been detected.
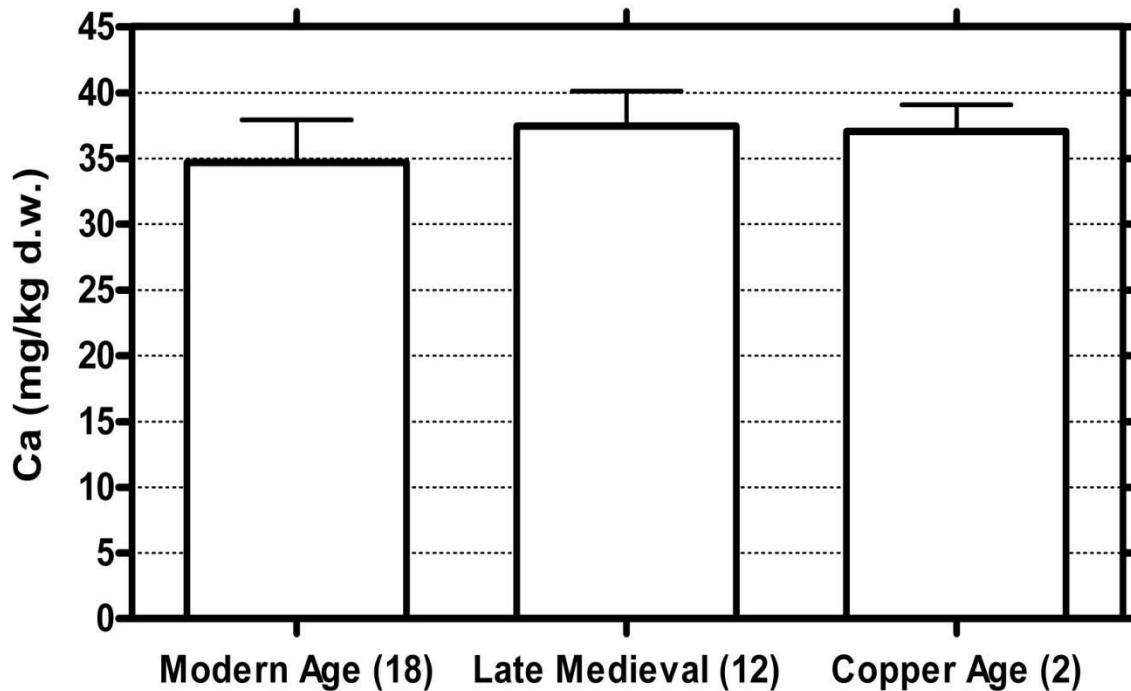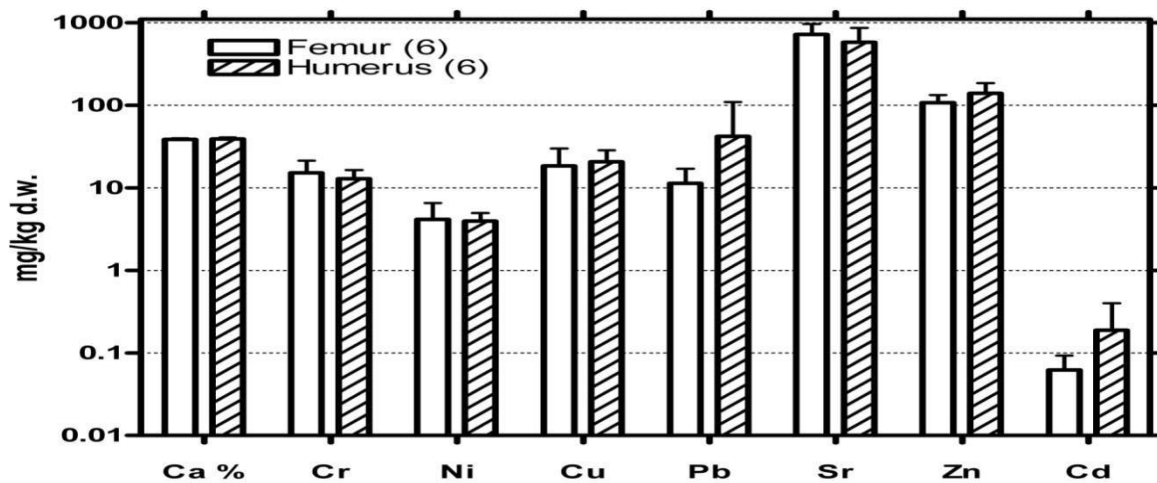
Figure 4 - Calcium concentrations in Copper Age and Late Medieval remains and in modern humans (Takata et al., 2005).

Figure 5 - Concentrations of Zn, Sr, Cu, Pb, Cd, Ni, Ca and Cr (mg/kg d.w.) in femur and humerus samples of the same individuals (n=6) .

The hypothesis of enrichment for the very high copper concentration (1368.64 mg/kg d.w.) measured in one humerus sample was excluded because the other bone samples and the soil sample showed much lower concentrations (25.54 - 121.45 mg/kg d.w. for bone and 63.99 mg/kg d.w. for soil).

3.3 Comparison of humerus and femur

In case physiological accumulation of trace elements differed in different bones of the same individual, we compared concentrations recorded in femurs and humerus bones. Since we did not have both bones of the same individual in Copper Age specimens, we only compared Late Medieval specimens. As expected, calcium concentrations in the different long bones did not differ, since in the absence of diagenetic depletion and

enrichment, the physiological process of hydroxyapatite formation is practically uniform throughout the skeleton (Bratter et al., 1977). The absence of significant differences in concentrations of Zn, Sr, Cu, Pb, Cd, Ni, Ca and Cr between femurs and humerus bones (Fig. 5) suggests that these elements accumulated and were distributed evenly in the two districts. The other elements (Al, Fe, Mn and Ti) were not considered because they are influenced by diagenetic processes.

Although concentrations of Pb in humerus bones were highly variable (42.11 ± 67.61 mg/kg d.w.), they were higher than those in femurs (11.38 ± 5.61 mg/kg d.w.). This slight difference in accumulation could be due to differences in physiological accumulation since metabolic turnover may change in different districts, modifying concentrations of trace elements (Bratter et al., 1977) or to minor diagenetic enrichment. The more robust nature of femurs compared to other bones generally means that the bone is better conserved and resists diagenetic phenomena. The choice of sample is therefore fundamental for obtaining reliable paleonutritional data (Bartoli and Bacci, 2009).

3.4 Comparison of epochs

Concentrations of elements were compared between the two epochs, but for the Copper Age not only data of femur samples was used but also data of humerus samples belonging to different individuals. Figure 6 does not show appreciable differences between concentrations of Ca, Fe, Al, Ti, Ni and Cr, whereas statistically significant differences were found for Mn, Pb, Cd, Cu and Zn.
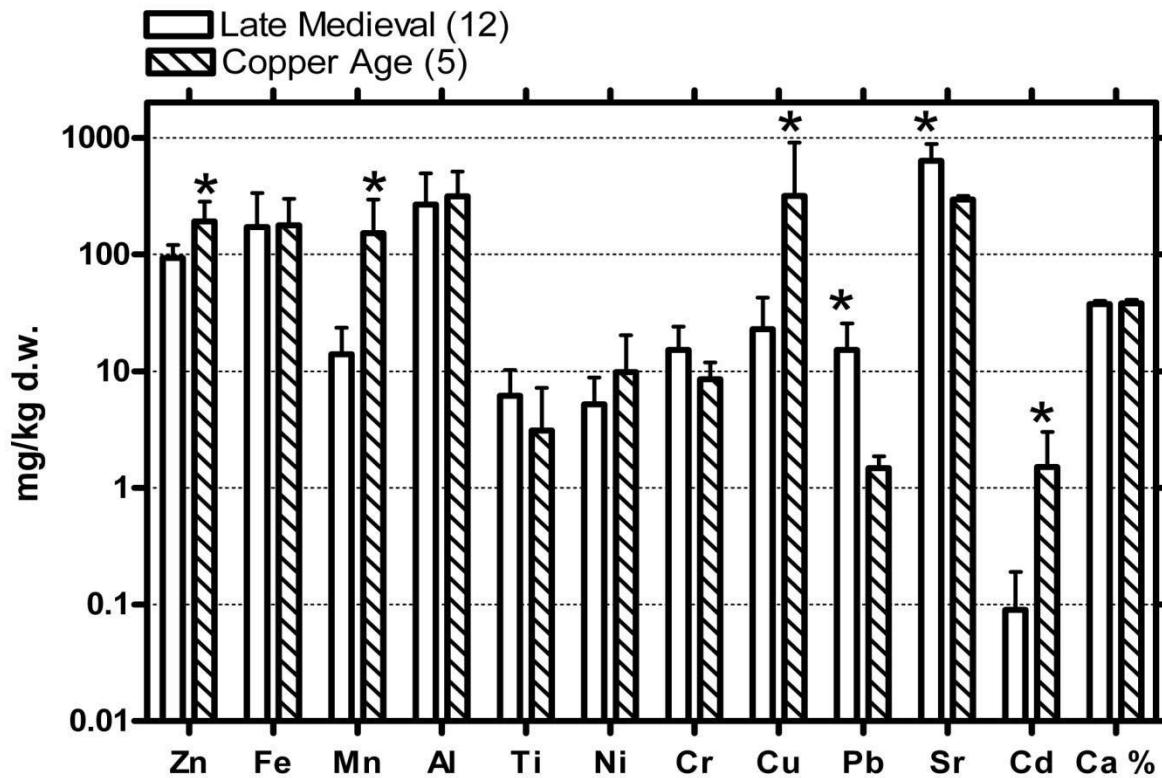

Figure 6 - Concentrations of Zn, Fe, Mn, Al, Ti, Ni, Cr, Cu, Pb, Sr and Cd in mg/kg d.w. and Ca expressed as percentage in femurs and humerus bones from the Copper Age (n=5) and femurs from the Late Medieval Period (n=12).

The significantly higher concentrations of Pb found in femurs of Late Medieval samples (15.25 ± 10.39 mg/kg d.w.) (Mann-Whitney U test; U = 0; p<0.01) than in Copper Age samples (1.48 ± 0.39 mg/kg d.w.) could be due to different social and dietary customs of the two populations. Indeed, the Medieval diet was mixed and balanced

with a predominance of plant foods compared to the diet rich in animal protein of the Copper Age (Bartoli & Bacci 2009). These lead concentrations are, however, lower than those found in Roman samples, a period when exposure to lead was high (Zapata et al., 2006; Aufderheide et al., 1992; Lewis, 1995) and are similar to those determined in the contemporary human population (Becker et al., 1968; Busetto et al., 2008; Miculescu et al., 2011).

An interesting finding was the high copper concentrations found in Copper Age specimens (317.47 ± 588.92 mg/kg d.w.), significantly higher than in Medieval individuals (22.20 ± 16.38 mg/kg d.w.; Mann-Whitney U=6; p<0.05). The greatest contribution to the total was from a humerus sample with a content of 1368.64 mg/kg d.w., which, excluding diagenetic enrichment, was unlikely to come from use of copper utensils but rather from exposure to copper smelting or other source of contamination (Pyatt et al., 2004; Pearce, 2007; Giardino, 2002). The mean copper concentration is much higher than those reported in other studies. Grattan et al., (2005) in femurs from a mining and smelting area in the Jordanian desert, dating back to 1500 BC, reported copper levels in the range of 10-120 ppm d.w.. Koizumi et al., (2009) reported high concentrations of copper of 220 mg/kg d.w. in a Medieval subject exposed to copper fumes during the casting of statues. Recently, Güner et al., (2011) in Central Anatolian Early Bronze Age human bones found copper levels ranging 1.99 to 396.46 mg/kg d.w..

Cadmium levels (Fig. 6) in Medieval (0.09 ± 0.01 mg/kg d.w.) and Copper Age femurs (1.51 ± 1.5 mg/kg d.w.) were in line with reports from humans living prior to the modern age (Martinez-Garcia et al., 2005; Baranowska et al., 1995). In modern man Cd levels are about ten times higher than those found in the present study (Jaworowsky et al., 1985; Miculescu et al., 2011).

The significantly higher levels of manganese in femurs of Copper Age individuals are difficult to interpret, because while not significantly correlated with other elements of lithogenic origin (Al, Fe, Ti) they could be influenced by diagenetic enrichment as shown by multivariate analysis (Fig. 3). As reported in the literature, Mn is very susceptible to diagenetic enrichment (Lambert et al., 1984).

Zinc concentrations, which were significantly higher in Copper Age specimens (Mann-Whitney U=8; p<0.05), usually indicate diets rich in animal proteins. The first evidence of milk processing dates to the Copper Age, accompanied by an increase in animal protein intake (Bartoli and Bacci, 2009; Hatch and Geidel, 1985). In the Late Medieval Period, a diet rich in vegetables was associated with greater accumulation of strontium than of zinc (Sillen and Kavanagh, 1982). Zinc levels and their relation to calcium are discussed in next section.

3.5 Dietary assessment

In the course of human life, trace elements tend to replace calcium in the structure of calcium phosphate. Variations in calcium content are evidence of non physiological accumulation. In the present study, no significant differences in calcium percentage were found between the two epochs (37.92% Late Medieval Period; 38.14% Copper Age) suggesting similar physiological status. These values reflect the normal calcium content of human bones (Takata, 2005) (Figure 4).

After excluding the possibility of diagenesis for calcium, we were able to apply a correction based on Sr:Ca and Zn:Ca ratios, using these elements as dietary markers (Bartoli and Bacci, 2009). Strontium and zinc represent prevalently vegetarian (Sillen and Kavanagh, 1982; Comar and Wasserman 1963; Toots and Voorhies, 1965) and carnivorous (Hatch and Geidel, 1985) diets and do not seem to be subject to diagenesis (Lambert et al., 1983; Price et al.,1985; Sillen, 1981). Thus these ratios were used to standardize the data for comparison of the two epochs (Bacci et al, 2008; Bartoli and Bacci, 2009; Nriagu, 1998, Bisel, 1980) in order to detect any differences in dietary customs. The results showed that Sr:Ca ratios (Fig. 7) were significantly higher (Mann-Whitney U=0; p<0.01) in Late Medieval bone samples, indicating a diet prevalently based on plant foods (Toots and Voorhies

1956; Comar and Wasserman 1963, Bartoli and Bacci 2009). On the other hand, Copper Age bone samples showed a significantly higher Zn:Ca ratio
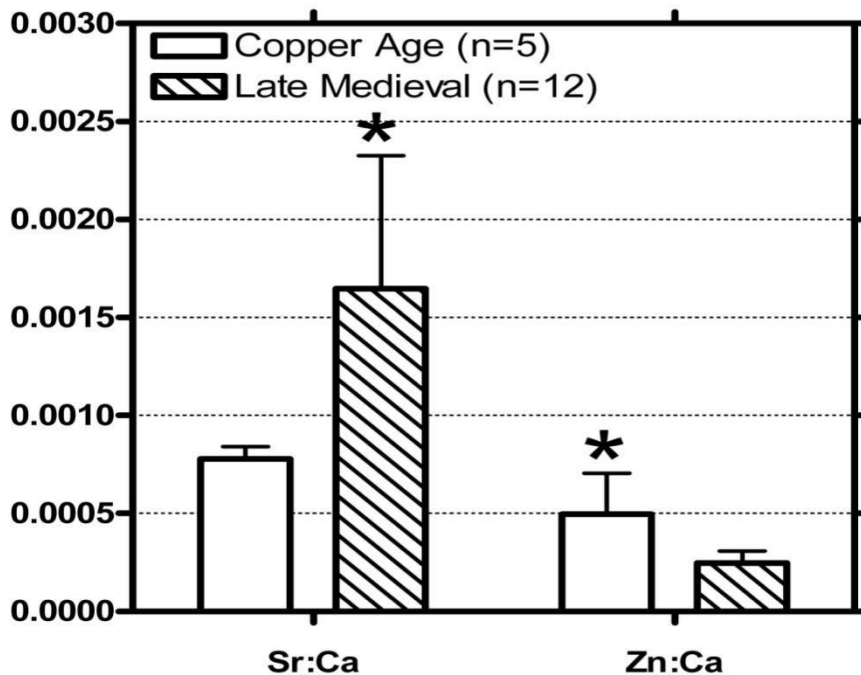
(Mann-Whitney U=0; p<0.01) (Fig. 7) indicating a diet rich in animal proteins (Mirce, 1984).

Figure 7 - Sr:Ca and Zn:Ca ratios in Copper Age and Late Medieval bone samples.

4. Conclusions

The use of small inverter-controlled burrs allowed a non invasive bone sampling, minimizing the amount of material removed from the specimens. Sampling the femur soil enabled direct comparison with trace elements in the two matrices by multivariate analysis.

This provided an assessment of the entity of diagenetic processes, which proved to be minor, if not absent, for Pb, Zn, Cu, Sr, Cd, Cr, Ni and Ca. An interesting result was the high Cu content (1368.64 mg/kg d.w.) found in one humerus of a Copper Age individual, which if not caused by diagenetic enrichment, was presumably due to exposure to the fumes of copper smelting. Thus, these results offer interesting insights into the methodology, since determination of metal levels, in this case copper, in skeletal specimens may in itself be a valid indicator of the presence of smelting in a given site, even in the absence of other archaeological evidence. This method, which requires further study and confirmation, could be applied in the future not only to human bones (generally not easily available) but also to animal bones. The copper content in bones of sedentary domestic species, such as pigs, could, perhaps, be used as a proxy datum for recognizing the occurrence of metallurgical activity in Eneolithic and Bronze Age sites, even if other signs are lacking.

The comparison of trace element levels in femur and humeral bones in the Late Medieval specimens did not show any differences in calcium content. This was expected, as the physiological process of hydroxyapatite formation is practically uniform, in the absence of diagenetic depletion. The absence of significant differences in Pb, Zn, Cu and Sr between femur and humerus indicated that these elements accumulated and were distributed evenly in the two districts. Although lead levels in humeral bones varied widely, they seemed to be higher than in femurs. This difference could be due to soil contamination or, as reported in the literature, to different physiological rates of accumulation, since metabolic turnover may vary in different parts of the body, affecting concentrations of

trace elements. Generally the robustness of the femur with respect to other bones, enables it to better resist diagenesis. The choice of sample is therefore fundamental for obtaining reliable palaeonutritional data.

The study of palaeodiet by analysis of Sr:Ca and Zn:Ca ratios indicated that the Late Medieval subjects had a prevalently vegetarian diet and that Copper Age subjects had a diet based mainly on meat and animal products. As reported in the literature, these results are in line with archaeological evidence about food customs of the two epochs. Indeed, there is clear evidence of an increase in animal proteins (perhaps also from milk and its by-products) since the beginning of the Copper Age, compared to the more vegetarian diet of the previous Neolithic period (Bartoli, 1993; Bartoli et al., 2005; Mallegni, 2001). Moreover, the Eneolithic diet could change depending on sex and/or wealth, revealing the emergence of stratified and socially differentiated communities, as displayed by the analyses on several specimens from the necropolis of Piano Vento in Sicily (Fornaciari and Bartoli, 1995). During the Bronze Age diet characteristics remained essentially unchanged whereas, with the Iron Age, protein intake considerably decreased as reflected by increased accumulation of strontium in bones (Bartoli et al., 2005, Bartoli and Bacci, 2009). This food choice could have been due to the improvement of agricultural techniques and the consequent increased availability of plant products, but more articulated social and economic reasons cannot be excluded.

In the Late Medieval Period, diet was mixed and balanced, but the high values of strontium indicate a prevalence of plant products. These results are consistent with those known for the Late Medieval period in general (Bartoli and Bacci, 2009, Le Goff, 1967; Mallegni et al., 2004). In Medieval society oxen and horses were mainly employed as working power in agriculture and were slaughtered for food only at the very end of their working cycle. This is one of the reasons why noble protein consumption from meat was usually sporadic among most medieval people (Mallegni et al., 2004). Further analyses on human remains (e.g. dental meso- and micro-wear), are in progress in order to confirm this interpretation by means of cutting-edge methods, such as 3D microscopy (Arrighi et al. 2016; Oxilia et al. 2015).

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