Strontium Isotope and Trace Element Analysis of Copper Age Human Skeletal Material from the Great Hungarian Plain

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Abstract

Accelerated mass spectrometry can be used to measure isotope ratios and trace element concentrations in human skeletal material to study diet, demography, and population movement from past cultures. This study utilizes tooth and bone samples from a series of burials excavated in the southeastern portion of the Great Hungarian Plain dating to the Middle Copper Age. Strontium values were compared between tooth and bone samples to identify possible non-local individuals within the burial population. All human samples and two cow samples were tested for trace elements to evaluate levels of contamination, look for correlations between trace elements and strontium isotopes, and identify dietary indicators. Preliminary $^{87}Sr/^{86}Sr$ isotope values suggest that there was insignificant movement within the sample population. Trace element concentrations indicate differential preservation between the bones and teeth. Distinct ranges for the trace elements barium, strontium and zinc between the human and faunal teeth support previous research that these elements are associated with trophic levels.

Introduction to isotope and trace element analysis in anthropology

The analysis of isotopes and trace elements in skeletal material has greatly advanced our understanding of how environment and lifestyle affects the tissues of the human body and how this information can be utilized in the archaeological record to learn about past human behavior. Advances over the last 30 years in the application of biochemical research to anthropology have developed in conjunction with the disciplines of ecology, geology, biology, and chemistry (Katzenberg 2000). It's application to several scientific disciplines has created a dynamic field where the interaction and exchange of information have allowed for an understanding of the how isotopes and trace elements can be accurately interpreted in a relatively short amount of time.

The stable isotopes carbon (C), nitrogen (N), oxygen (O), sulfur (S), strontium (Sr), and hydrogen (H) can be measured in the archaeological context to study diet, demography, residence and migration patterns, infant feeding and weaning, climate, and disease (Katzenberg 2000). Calcium (Ca), Sr, and barium (Ba) are the most widely applied trace elements in anthropology and are used to reconstruct prehistoric diet. Other trace elements are useful for assessing levels of diagenetic alteration in bone and teeth. Chemical analysis is becoming increasingly essential to understanding and interpreting many aspects of prehistory that are insufficient or lacking in the archaeological record. Isotopes and trace elements preserved in the tissues of the human body can supplement evidence of human behavior by providing more holistic information about individual and community practices.

Strontium as an indicator of prehistoric diet and movement

The concept of stable isotope analysis is based on the principle that isotopes are atoms of an element with the same number of protons but a different number of neutrons; therefore they have a different mass that we can measure (Katzenberg 2000). This study measures abundances of the element Sr that substitutes for Ca in the mineral matrix of skeletal tissue. Levels of Sr isotopes in human and faunal skeletal material can be used to deduct movement throughout an individual's lifetime, residence and migration patterns based on the local geology, and diet characteristics (Price 2003; Price 1994b).

There are four naturally occurring stable Sr isotopes: ^{88}Sr, ^{87}Sr, ^{86}Sr, and ^{84}Sr (Grupe 1997, Price 1994b). All of these isotopes are nonradiogenic except for ^{87}Sr, which decays from the radioactive element rubidium (^{87}Rb, $t^{1/2}$~4.7×10^{10}). Because ^{87}Rb is decaying at a constant rate to ^{87}Sr, ^{87}Sr can be used to characterize the age and source of rock formations and these variations in the geological landscape have been characterized. Sr content varies in natural material however. In order to normalize variations in Sr content so that we can measure the absolute ^{87}Sr abundance, the isotope value is expressed in a ratio. ^{88}Sr is the most abundant of total Sr (approximately 82.53%) and ^{84}Sr is the least abundant (approximately 0.56%). ^{87}Sr and ^{86}Sr are the closest in approximate abundances (7.04% and 9.87%) therefore the ratio is expressed as ^{87}Sr/^{86}Sr.

The ^{87}Sr/^{86}Sr isotope ratio is useful in the archaeological context for several reasons. As stated previously, Sr substitutes for Ca in the hydroxyapatite crystals of tooth

and bone mineral. As rocks weather, the local Sr values are contained in the soil and groundwater. As the ecosystem uses these resources the ^{87}Sr signature from the local geology is passed through the food chain and preserved in the bones and teeth of the community, therefore recording the Sr in an individuals diet as well as the local geology. Because $^{87}Sr/^{86}Sr$ is a heavy isotope it is not affected by biological fractionation as it is passed on to higher trophic levels and retains its original isotopic composition (Price 1994a).

Strontium levels in skeletal tissue can be used in some cases as an indicator of paleodiet (Aberg 1998, Sealy 1991). In a study conducted in the southwestern Cape of South Africa, human skeletal material from several sites were analyzed to see how prehistoric people utilized coastal and inland resources for food and land. $^{87}Sr/^{86}Sr$ values were characterized for coastal and inland environments by testing faunal samples from each area. The human material was then compared to the marine and coastal (^{87}Sr depleted) verses terrestrial (^{87}Sr enriched) ranges. Results showed that sites closer to the coast had Sr ranges that matched marine signatures and sites closer to the mountain belt had values that matched a terrestrial diet.

This study focuses on the use of Sr isotopes to study population movement based on the comparison of Sr levels in teeth to an established "local" geological Sr value. The enamel in teeth forms in the first couple years of an individual's life and the mineral structure is not replaced after formation (Grupe1997). Once the enamel has formed it records the level of Sr in the individual's diet and geological habitat as a child. Sr levels in bone, which are continually being replaced, are thought to reflect the individuals average Sr intake over the last seven to ten years of life. Comparisons of Sr isotope ratios between the two tissues reflect whether there has been a significant change in diet and possible geographical area within a lifetime.

Although Sr studies in the past have used compact bone as the local value from which tooth values are compared to identify non-local individuals (Grupe 1997, Price 1994a, Price 1994b), recent studies have discussed the problems with this method (Bentley 2003, Horn 1999). The practice in previous publications defines the 'local' tooth value as within 2 standard deviations of the average human bone value. This is considered to be a conservative estimation but it is not based on isotopic boundaries in the landscape and is therefore subjective. It is important to discuss the complexities that this might cause during analysis and interpretation. First, if tooth values fall outside the analytically defined 'local' range than we know that early in life the individual ate foods mainly from a non-local source. We cannot assume this means the individual migrated from one specific geological area to another. Sr signatures can be an average of multiple diet catchment areas depending on the movement of the person and the location where food was pasteurized or grown. A 'non-local' value can also indicate someone whose subsistent activities included a broader range of territory than other more stationary individuals.

Contamination of bone by post-depositional Sr is also an issue. Tooth enamel is not susceptible to digenesis because it's larger mineral crystals are resistant to biochemical contamination, whereas the porous structure of bone can be highly susceptible depending on the bone structure and the burial environment. Studies have shown that diagenetic Sr can be removed with acid unless the bone's mineral structure has been completely replaced. It is argued that bone contaminated by groundwater and

soil from the local environment can still be used as a valid indicator of the local geology because contamination should reflect the local geology (Grupe 1997). However Bentley et al. (2003) argues that diagenetic Sr could narrow possible variation of the individual's original Sr value. To account for this possibility samples from several species of faunal teeth should be used to establish a more complete understanding of the 'local' geological Sr range.

Trace element application to preservation issues and paleodiet

Trace elements in the skeletal tissue of humans and animals have been studied in the archaeological setting since the 1970s (Katzenberg 2000). The number and function of trace elements considered useful to reconstructing human behavior and diagenetic change have significantly decreased since its inception due to the complex nature of the way trace elements are taken into the skeletal system before and after death.

Bone structure is approximately 77% organic and 23% inorganic (Recker 1992). It is the inorganic portion that is composed of mostly calciumphosphate (hydroxyapatite) that contains a majority of the trace elements. The major and trace elements Ca, phosphorus (P), zinc (Zn), copper (Cu) and manganese (Mn) are fundamental for growth, modeling and remodeling of skeletal tissue. Other trace elements are introduced into the bone structure due to ion exchange with the normal chemical components in hydroxyapatite (Ca, P, H). Trace element concentration in the hydroxyapatite mostly represent proportions of those elements in the diet and certain elements can be used to indicate major dietary components (such as marine vs terrestrial sources or distinctions between trophic levels) however there are several issues in this discipline that are not yet resolved. data.

The replacement with trace elements in bone is influenced by a variety of factors during life and in the burial environment (Katzenberg 2000). Age, sex, health, stress, and environment can affect the manner, location, and degree of trace element absorption. Certain elements can alter the uptake of other elements in a positive or negative way. For example, Ca, Cu, iron (Fe), cadmium (Cd), and chromium(Cr) are antagonistic to Zn, and high P and Ca intake in the diet deters Sr absorption. For this reason it is important to establish the chemical composition of the dietary components a site would be utilizing in order to make valuable interpretations of trace element

It is also very important to take in to account post-depositional alteration of original trace element concentrations. The structure of the hydroxyapatite continues to allow for ion exchange after death. Age of the bone, groundwater, temperature, precipitation, and microorganisms can effect the trace element concentrations, as well as the size, porosity, structure and biochemical composition of the bone itself. In order for trace elements to be used for dietary reconstructions a distinction between in vivo and post depositional concentrations must be made.

Despite the complexity of using trace elements in archaeology there are still applications if used with caution. The trace elements Ca, Sr, Zn and Ba, which can be used to reconstruct diet, are less susceptible to diagenetic change (Katzenberg 2000). The mineral structure of certain areas of the skeleton such as tooth enamel and dense cortical bone are more resistant to replacement. Multielement analysis can be useful to assess diagenetic change in a sample population. For example, the trace element

Uranium(U), a common element found in ground water is not normally be found in the skeletal system and was used in this study contamination between bone and tooth samples.

For this study the elements Sr, Ba, and Zn were used as indicators of dietary differences between human and faunal samples. Previous research (Katzenberg 2000) has shown that Sr concentrations in the diet relate to the trophic position of that organism. Higher Sr values indicate lower trophic levels, so herbivores should have higher values than omnivores and so on up the food chain. Ba has been used in conjunction with Sr in recent dietary studies (Burton 1990) because it behaves in a similar way (reduced at higher trophic levels). Zn has also been used as a dietary discriminator in the case of animal resources verses mainly agricultural resources (Gilbert 1985). Higher Zn levels indicated the use of an animal rich diet.

The archaeology site: Gyula 114

The human skeletal material used for Sr and trace element analysis was excavated near the town of Gyula in southeastern Hungary only a couple kilometers from the Romanian border (Figure 1). A total of 18 burials were removed due to the threat of construction in the area. The excavation took place during the summer of 1999 and was conducted by Attila Gyucha from the Múnkacsy Mihály Múzeum.

Figure 1. Map of Hungary.

The bodies were interred in pits with the head pointing east. Individuals were orientated on their left or right side, which according to the mortuary practice of the Middle Copper Age (5500 B.C.) Tiszapolgár culture corresponds to gender (males buried on their right side and females on their left). Most of the burials included grave goods such as pots, beads, faunal material, lithics, and metal.

Due to the poor preservation of some of the burials only 10 of the 18 had enough material to analyze.

Two cow molars from Vésztõ-Mágor, a nearby archaeological site from the Copper Age, were also analyzed as a preliminary faunal comparison.

The geological setting: Hungary and Romania

Gyula 114 is located on the eastern side of the Pannonian Basin that makes up the Great Hungarian Plain (Figure 2). The plain is mostly composed of quaternary deposits of alluvial silts and clays and is surrounded by the Carpathian Mountains that start near Vienna, Austria, border the northern portion of Hungary and end near Bucharest, Romania. The Carpathians are composed of a series of parallel mountain ranges formed at different time periods. The rock formations most relevant to this study are the young Tertiary volcanic rocks that make up the innermost range. This range makes up the arc that surrounds Hungary to the north and east and was formed less than 50 million years ago.

It is necessary to establish the geological setting to get an idea of the Sr isotope ranges within the scope of possible population movement. The Plain should have a fairly high Sr isotope composition because the average of sedimentary silts and clays that make up the soil are very old. Geological units greater than 100 million years that contain high ratios of Rb/Sr have the highest $^{87}Sr/^{86}Sr$ at present. This includes clay rich and igneous rocks. Younger geological units (less than 1-10 million years) such as basalts have lower Sr isotope ratios.

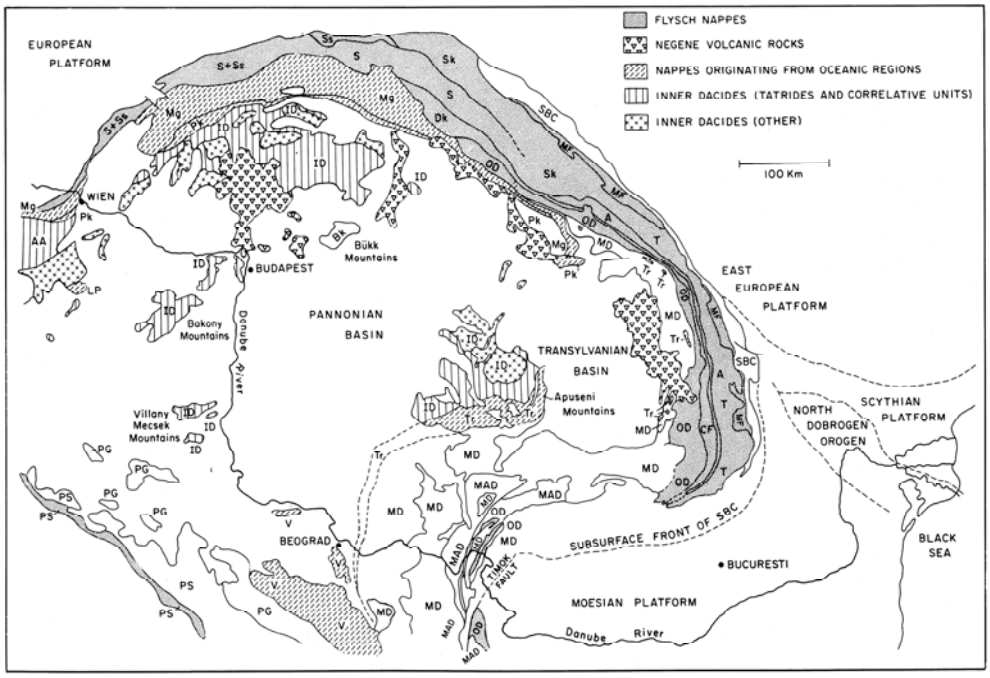

Figure 2: Simplified geological map of Eastern Europe

Samples and procedure

Strontium samples were taken from the first molar (M1) whenever possible in teeth (the second molar was used if M1 was unavailable) and mid shaft from the compact bone in the femur (See figure 3). Enamel and bone fragments were mechanically cleaned and isolated using a steel blade and then ground into a powder with an agate mortar and pestle. In total, 15 tooth samples and 4 bone samples were processed from 10 burials and two cow teeth (duplicates were taken from 3 burials to test reproducibility). All samples were processed in the Geochemistry lab at the National High Magnetic Field Laboratory. Chemical treatments were done in the Class 100 clean lab.

Figure 3. Example of a first molar and femur shaft processed for Sr analysis.

Removal of organics and post-depositional carbonate

Samples were reacted with acid according to the procedures described by Chiaradia et al. (2003) to remove the organic and post depositional portion of the material. Approximately 1 ml of quartz deionized (QD) water plus ½ ml of hydrogen peroxide was added to samples contained in acid treated Teflon beakers and placed in an ultrasonic bath for 30 minutes. This process was repeated twice. The samples were then rinsed with QD water and put in the sonic bath for 15 minutes. One ml of acetone was added to each sample four consecutive times. They were then put on a hot plate with no heat to dry down.

Separation for trace element analysis

All samples were weighed and five mg was taken from each sample for trace element analysis. First, 1 ml of 2.5 N HCl was added and the samples were placed in an ultra sonic bath for 30 minutes to dissolve. Depending on the quantity of each sample, a proportion of solution was removed with a pipette equivalent to 5 mg and placed into a separate Teflon beaker. The trace element samples were dried down on a hot plate and then dried down again with 3 drops of concentrated HNO_3.

A sample solution of 2% HNO_3 was made by adding QD water and concentrated HNO_3 to each sample. The concentrated acid and approximately 3 ml of QD water was added to each beaker and then put on a hot plate to dissolve. The trace element solution was then poured into a larger beaker and the appropriate amount of QD water was added

to make a 2% HNO_3 solution. This solution was introduced into the sector ICP-MS Finnegan ELEMENT for analysis.

Separation for strontium

The remaining portion of each sample was dissolved in 0.5 ml 2.5 N HCl and put in a sonic bath for 15 minutes. The samples were loaded into tubes and placed in a centrifuge for 15 minutes at 10,000 rpm(?). 0.25 ml of sample solution was loaded onto a cation exchange chromatography column to separate out the Sr from the other elements. The Sr solution was collected in a clean Teflon beaker, three drops of $HclO_4$ were added and they were put on a hotplate overnight to dry. Once the Sr dried down to a tiny spot on the bottom of the beaker, 3 drops of concentrated HNO_3 were added and the samples were dried down again.

Tungsten wire was used as a loading platform to ionize the samples once in the mass spectrometer. Once assembled the tungsten filaments were degassed. The samples were dissolved in 0.25 N HNO_3 and then mixed with a TAPHF solution. This mixture was placed into the filament, dried down at 1.1 amps and flashed at 3.2 amps. The samples were placed in a magazine and analyzed using a Finnigen/ MAT 262 Thermal Ionization Mass Spectrometer (TIMS).

Results

Sr isotope data was acquired for 12 out of the 18 samples processed (cow sample Vésztõ -Magor 299 was analyzed for trace elements but has not been separated for Sr at this time) (Table 1).

Table 1. Sr values for human bone and teeth from Gyula 114 and cow teeth from Vésztõ-Mágor.

Site	Burial	Sample	Material	$^{87}Sr/^{86}Sr$	Standard error
Gyula 114	4	4.1	Tooth	x	x
Gyula 114	6	6.1	Tooth	x	x
Gyula 114	8	8.1	Tooth	0.709364	0.000009
Gyula 114	8	8.2	Tooth	x	x
Gyula 114	10	10.1	Tooth	0.709149	0.000007
Gyula 114	11	11.1	Tooth	0.709585	0.000007
Gyula 114	12	12.1	Tooth	0.709659	0.000007
Gyula 114	13	13.1	Tooth	0.709184	0.000008
Gyula 114	13	13.2	Tooth	0.709160	0.000007
Gyula 114	14	14.1	Tooth	0.709526	0.000023
Gyula 114	16	16.1	Tooth	x	x
Gyula 114	17	17.1	Bone	0.709276	0.000007
Gyula 114	17	17.2	Bone	0.709238	0.000006
Gyula 114	17	17.3	Bone	x	0.000007
Gyula 114	17	17.4	Bone	0.709255	0.000007
Gyula 114	17	17.5	Tooth	0.709232	0.000007
Gyula 114	17	17.6	Tooth	0.709273	0.000007
Vésztõ-Mágor		283	Cow tooth	x	x
Vésztõ -Mágor		299	Cow tooth	Not analyzed	Not analyzed

Samples 4.1, 6.1, 8.2, 16.1, 17.3, and 283 did not have sufficient Sr levels to run in the mass spectrometer and had increased amounts of Ca. This was probably due to an error in the chemistry while separating the Sr in the cation chromatography columns. These samples will be Sr separated and run again at a later date.

Trace element data was acquired for all samples except 17.3 due to non-dissolvable solids in the sample (Table 2). Table 2 shows trace element values that had significant concentrations in the bones, teeth or both.

Table 2. Trace element concentrations for all samples. Concentrations are in ppm.

Sample	Strontium	Barium	Titanium	Chromium	Manganese	Nickel	Copper	Zinc
17.1	553.84	1,510.49	52.29	2.82	16,745.49	38.07	14.21	60.07
17.2	616.843	94.961	13.536	1.389	1667.190	7.616	11.772	70.616
17.4	631.096	139.185	28.395	2.232	2758.647	9.980	10.810	75.823
17.5	117.808	10.381	2.570	0.064	50.065	-0.006	0.018	67.400
17.6	123.595	9.619	1.314	0.043	83.422	0.178	-0.134	60.631
4.1	78.827	1.566	0.811	0.040	8.191	-0.274	-0.188	111.813
6.1	66.023	5.574	0.411	0.042	9.752	0.605	0.125	91.164
8.1	97.922	5.825	0.355	0.092	11.695	-0.184	-0.113	72.029
8.2	96.578	5.463	0.272	0.072	9.661	0.068	-0.192	68.459
10.1	129.972	3.914	316.065	0.815	6.253	2.607	-0.004	62.424
11.1	67.694	3.732	303.770	0.021	6.992	-0.230	-0.220	71.440
12.1	90.744	4.924	331.999	0.143	8.728	-0.008	-0.215	86.309
13.1	105.409	3.783	306.691	0.007	13.036	0.295	-0.158	62.353
13.2	100.916	3.723	264.160	0.058	12.740	-0.173	-0.172	65.167
14.1	95.289	4.033	216.194	0.378	15.636	0.282	-0.175	71.057
16.1	99.553	4.438	201.822	0.052	27.339	-0.131	-0.014	107.500
283	167.683	64.015	170.673	0.186	8.595	0.151	-0.113	22.379
299	248.489	117.256	174.333	0.087	6.597	-0.024	-0.238	21.841

Discussion

$^{87}Sr/^{86}Sr$ isotope values

The preliminary 'local' range for Gyula 114 was established based on the bone values from burial 17. With further research, bones from all of the individuals sampled will be analyzed as well as a variety of faunal and soil samples from the area in order to develop our understanding of the local geological range. For the present discussion the 'local' $^{87}Sr/^{86}Sr$ value is estimated to be approximately 0.07092.

$^{87}Sr/^{86}Sr$ values from the rest of the human tooth samples do not show a high degree of variation from this 'local' value (Figure 4). Samples 11.1, 12.1, and 16.1 are slightly higher than the rest of the samples but not by a significant amount. Elevated Sr values might indicate that these individuals subsisted on a larger dietary catchment area than the other individuals that had narrower Sr isotope ranges. There was no apparent distinction between the orientation, sex, and grave good assemblage of these individuals compared to the rest of the burial population.

The lack of variation in the samples might indicate a few things about the burial population. First, with relatively high $^{87}Sr/^{86}Sr$ values it is unlikely that any of the individuals moved from or utilized soils rich in volcanic material in the nearby Carpathian Mountains. This does not necessarily rule out population movement however because the Great Hungarian Plain is a large area with a very homogenous soil composition. If the individuals moved within the plain it would be hard to determine based on variation in their Sr composition. There are also large areas of land to the east of the Carpathian Arc in Romania with a similar soil composition to the Great Hungarian Plain. If there was any movement from this area, evidence in the Sr isotope composition would be small.

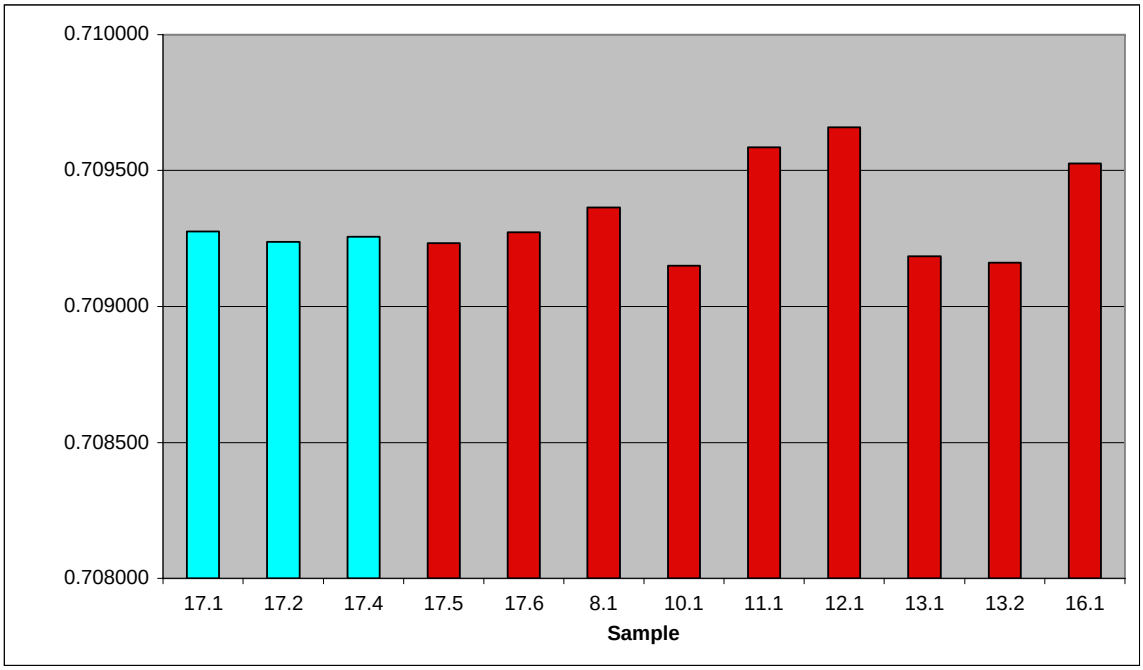

Figure 4. Sr isotope values for human bone (aqua) and teeth (red).

Trace elements

An analysis of multiple trace elements in all of the samples showed distinctions between the composition of human bone and teeth as well as between human teeth and cow teeth.

Bone

Based on the concentrations of trace elements, the bone samples (17.1, 17.2, 17.4) had much higher levels of Mn, Ba, and Sr than the tooth samples (Figure 5 and 6). The bones also contained levels of rare earth elements and metals such as Uranium (U), Copper (Cu), Nickel (Ni) and Chromium (Cr) that were not significant in the teeth. The presence of U in the samples suggests that the bone was affected by groundwater contamination in the post depositional environment (Figure 6). Its absence in the teeth is a good indicator that the enamel was not susceptible to groundwater contamination.

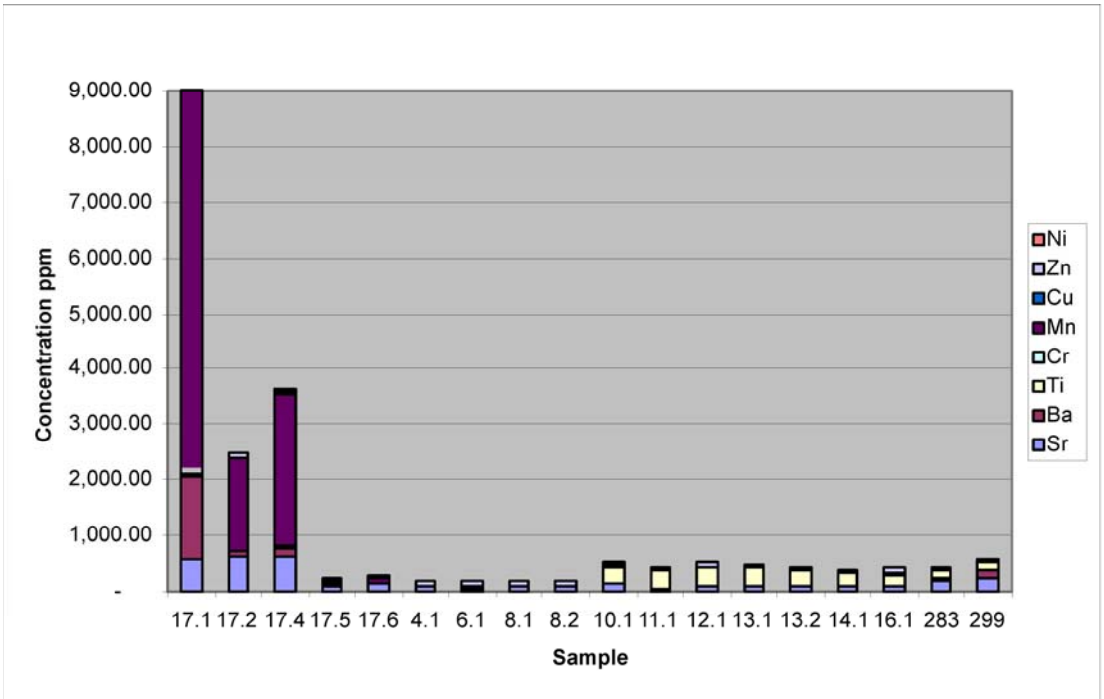

Figure 5: Trace element concentrations for bone and teeth samples

Teeth

The tooth trace element concentrations show much lower levels of Mn, Ba, and Sr in relation to the bone and almost negligible levels of rare earth elements and metals (Figure 5 and 6). The human teeth show variation in composition in titanium (Ti) levels. Samples 4.1 through 8.2 contain less than 1 ppm of Ti whereas 10.1 through 16.1 including the cow teeth range from 170 to 331 ppm Ti. At this time it is uncertain what increased quantities of Ti in some teeth indicate. Contamination is a possibility but the Ti

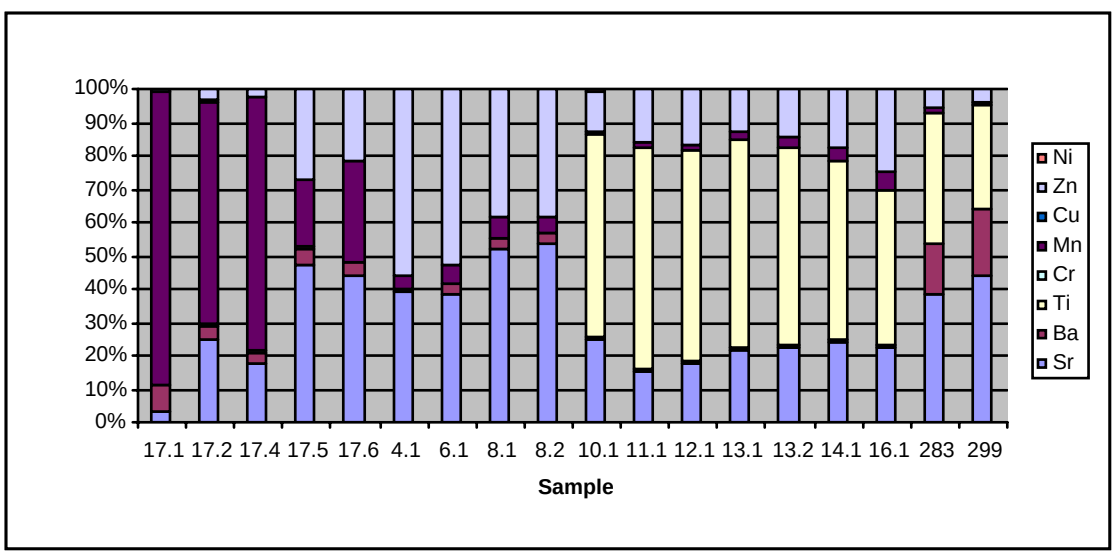

Figure 6. Significant trace element concentration percentages for all samples.

concentrations did not affect the Sr isotope ratio. The cow teeth have high levels of Ba and Sr and lower levels of Zinc in relation to the human teeth.

Dietary indicators

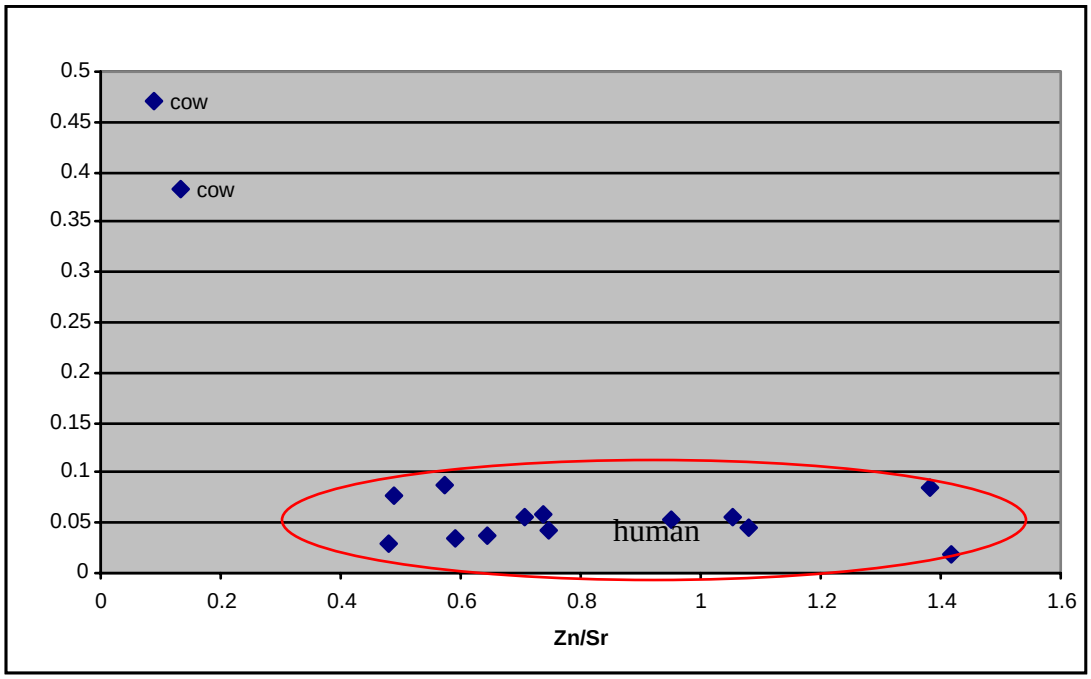

Figure 7. Trophic level distinction between cow teeth (herbivores) and human teeth (omnivores) based on Sr, Ba, and Zn concentrations.

Correlations between the trace elements Sr, Ba, and Zn with diet seem to have been demonstrated in the values from the human and cow teeth (Figure 7). The cow samples express higher levels of Ba/Sr and lower levels of Zn/Sr (both ratios indicate lower trophic levels) than all of the human samples. There is a clear distinction between where the species plot which supports known differences in food resources according to trophic level.

Connections between Sr isotopes and trace elements

Several combinations of trace element concentrations were plotted against the $^{87}Sr/^{86}Sr$ isotope ratios to see if there was any correlation and possibly contamination of the samples. Uranium was used because it is can indicate contamination by groundwater. Figure 8 confirms differential preservation between bone and tooth samples but does not show a correlation with the U concentrations and $^{87}Sr/^{86}Sr$ isotope ratios. Figure 9 shows a positive connection between increased Zn concentrations and increased $^{87}Sr/^{86}Sr$ ratios in bone and tooth samples. Although Zn is correlated with Sr uptake in the skeletal system, it is uncertain why this would affect the isotope ratio.

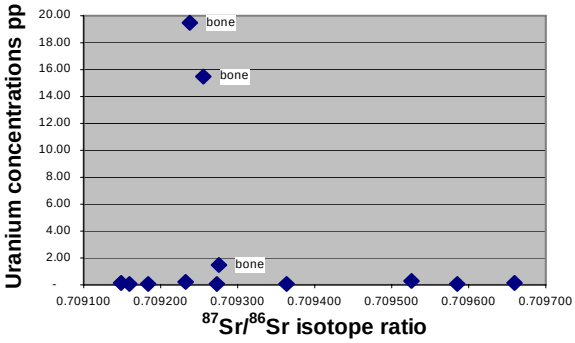

Figure 8. Uranium trace element concentration plotted with Sr isotope ratios for bone and teeth.

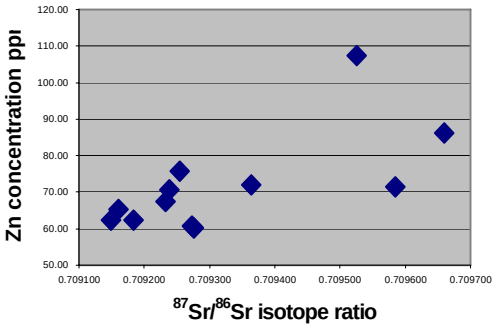

Figure 9. Zinc trace element concentration plotted with Sr isotope ratios for bone and teeth.

Trace element concentrations of Sr were plotted against the isotope ratio in bone and teeth with no strong correlation (Figure 10). When the bone samples were taken out of the case study due to such a large difference in Sr concentrations there seemed to be a negative connection between the concentration and the isotope ratio (Figure 11).

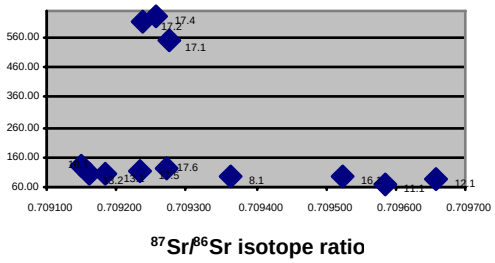

Figure 10. Strontium trace element concentration plotted with Sr isotope ratios for bone and teeth.

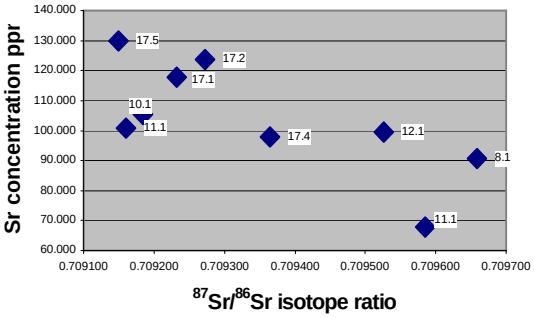

Figure 11. Strontium trace element concentration plotted with Sr isotope ratios for teeth.

Conclusions

The preliminary $^{87}Sr/^{86}Sr$ isotope values do not suggest that the burial population utilized resources from volcanic deposits in the Carpathian Mountains. This data does not rule out population movement within the Great Hungarian Plain or Romania due to the large areas of homogenous sedimentary soil composition and the limited available Sr signatures for comparison. With an expanded database of Sr isotope values for Eastern Europe it might be possible to categorize narrower ranges of Sr isotope values for the human samples within the geological setting.

The results of the trace element analysis provided lots of valuable information to the study. The presence of rare earth elements and metals were useful to identify the

samples that had been affected by the post depositional environment and those that maintained in vivo element concentrations. This evidence supported previous research about differences in the susceptibility of bone and tooth tissues. Trace elements also supported the use of Ba, Sr, and Zn concentrations to distinguish diet between species. Correlations between $^{87}Sr/^{86}Sr$ isotope values and concentrations of Sr and Zn are not fully understood at this point. It will be interesting to see if this trend continues with further research.

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