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Original Scientific Article

Elemental Status in Individuals from Naklice Burial Site (Southern Croatia): Mediaeval Diet Reconstruction

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Abstract. Elemental status of mediaeval individuals from Naklice burial site (Southern Croatia) was analyzed and their diet was reconstructed. Samples from different human bones were taken from 16 individuals who died in the 9th century and were recently excavated from Naklice burial site. The metal content of iron, lead, cadmium, magnum, zinc, copper, strontium, and calcium were determined by atomic absorption spectrometry (AAS) in flame mode. Mercury concentration was determined by three direct consecutive measures taken with a mercury analyzer. When comparing our results to the modern bone heavy metal concentrations, it is obvious that Cu, Fe, Ca and Sr greatly exceeded the values while concentration of Zn and Pb were lower. Concentration of Sr was about ten times higher than in modern bone samples. Due to environmental contamination, lead concentration in studied bones was lower than in modern bone samples. According to our results we concluded that the probable main dietary components of individuals excavated from Naklice burial site from Early Mediaeval period were leafy vegetables, legumes and small amounts of cereals.

Keywords: elemental status, ancient bones, diet, mediaeval period, Naklice, atomic absorption spectrometry

INTRODUCTION

Naklice lie near Split (Figure 1) and were inhabited from the 5th to the 4th century BC. Naklice burial site was excavated during 1980s and 1990s by archaeologists from the Museum of Croatian Archeological Monuments in Split. This particular burial site is important in understanding the early process of migration and social organization of ancient population of that geographical area (living habits, types of food they consumed and organization of their society...).

The total absorbed metals dose could be objectively determined by checking the element status in biological samples.[1–3] Content of heavy metals in a diet could correlate with heavy metals content in human bones[4,5], therefore, determining heavy metals concentrations, and their relationship in human bone, could be used to reconstruct the basic diet.[6] Cereals and vegetables were the main nutrition in the early medieval period. In spite of the closeness to the sea shore, fish was not included as a substantial nutrient.

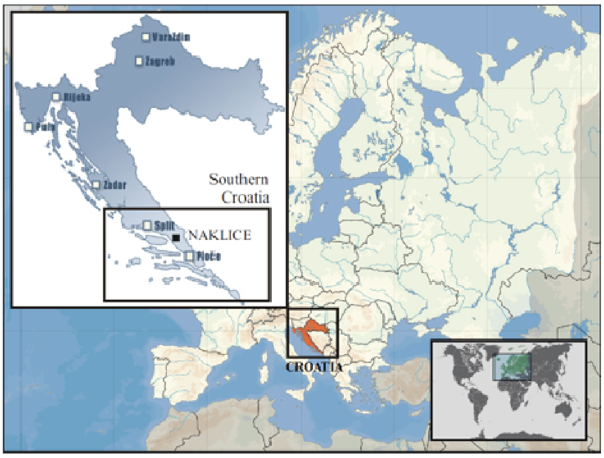

Figure 1. Geographical location of Croatia Republic and excavation zone Naklice burial site, in Southern Croatia.

Determining heavy metals concentrations in human remains is well recognized in the science world. Other authors previously described how elements give

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clues to the diets of ancient populations. Studies of humans demonstrate that anthropogenic exposures to metals are reflected in bone and teeth 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.[7] In reality we evaluated two subsets of metals: Pb, Cd and Hg which represent the environmental pollution, while the rest of the metals (Ca, Sr, Cu, Zn, Fe, Mn) reflect the proposed paleodiet regiments. To be able to conclude about the pollution in the past and the modern pollution, we analyzed the burial site soil.

Vuorinen *et al.* determined heavy metals concentrations from remains from Ficana, Italy, between 8^{th} and 6^{th} century BC.[8–10] They found good correlation between zinc and strontium according to the age and gender where higher concentrations were found in older populations, of both men and women. Jankuhen *et al.* measured elemental status in ancient human bones from 6^{th} to 8^{th} century AD.[11] Results showed strong positive correlation between P-Ca; Na-Sr; and Mn-Fe concentrations, which were also in good correlation with concentration of Mn-Fe in the soil environment. The negative correlation was established between: Ca-Fe and Ca-Sr. However, the surprisingly positive Ca-Sr correlation must be of completely different origin because of the general fact that Ca is substituted by Sr and therefore a negative correlation between Ca and Sr is expected. No explanation could be given from the biological or from the geological point of view. It should be mentioned that the potential dietary significance of Sr might be an explanation of this positive correlation. The determination of natural concentrations of metals in tissues, even though it is difficult due to massive increase of metal concentrations in the environment, enables the assessment of anthropogenic element loading of the human body.[12] Nonetheless, Martinez-Garcia *et al.* proposed that the metals in bone remains were a consequence of two entry routes: inhalation of atmospheric aerosols and ingestion of diverse contributions in the diet.[13]

One of the goals of this study was to suggest that testing metal concentration levels of the bone material excavated from ancient burial sites becomes a standard procedure in the archeological and anthropological algorithm in the future investigations of human remains. That step could aid in better understanding of medieval living habits.

MATERIALS AND METHODS

Reagents used for the extraction, measurement and standard metal solutions (1 mg/L), were suprapur (Merck, Darmstadt, Germany). The conductivity of deionised water used in the experiment was 0.06 µS/cm. Standard solutions were prepared in range of expected concentration values.

Samples preparation

After anthropological and before chemical analysis bone samples (Table 1) were collected, washed in tap water, mechanically cleaned with a plastic brush and a mechanical grinder to remove all bone surface material. A part of a dense cortical bone was sawed for analysis. Using plastic forceps samples were transferred into a clean vial filled with 5 % sodium hypochlorite and were shaken occasionally to remove the residual fat. The sample is then washed again 3 times in redistilled water and acid leached (in 5 % nitric acid; HNO_3) to minimize the influence of post mortem contamination. Then the bone was leached gain in dd water and dried at room temperature. Bone pieces were crushed into small fragments using razor blades and stored in sterile polypropylene tubes at −20 °C until analyzed. After drying to a constant weight the samples were soaked in 6 ml 65 % HNO_3 over night, than were washed in distilled water and were dried in room temperature. Approximately 0.5 g each was than wet-ashed in 65 % nitric acid and hydrogen peroxide in the teflon-TFM vessels. The samples with added reagents were left to stand at room temperature in open tubes overnight. Vials were then sealed into the digestion bombs and the automated (temperature regulated) microwave digestion (CEM, USA Model Mars 5-2004 with 1600 W power) protocol was initiated.[14] The microwave program was in two steps as follows: ***Step 1*** (a) ramp to 125 °C; (b) 15-min to reach preset pressure of 200 psi (1 psi= 6895 Pa); (c) 20-min to hold preset pressure of 200 psi. ***Step 2*** (a) ramp to 150°C; (b) 10-min to reach preset pressure of 300 psi; (c) 20-min to hold preset pressure of 300 psi. The diges-

Table 1. Presumed gender and age

No.	Sample	Gender	Age
1P	Skull	Female	Adult
3P	Humerus	Male	Adult
4P	Femur	-	Adult
5P	Femur	Male	Adult
6P	Tibia	Female	Adult
7P	Tibia	Female	Adult
8P	Femur	Male	Adult
9P	Femur	-	-
13P	Mandible	Female	Child
15P	Tibia	Female	Adult
17P	Humerus	Male	30−35
18P	Femur	Female	15−20
19P	Femur	Male	-
20P	Tibia	Male	30−35
21P	Humerus	Female	About 25
24P	Ulna	Female	-

Table 2. Experimental conditions for trace metal determinations by AAS

Metal	Pb	Cd	Fe	Mn	Cu	Zn	Ca	Sr
Wavelength / nm	283.3	228.8	248.3	279.5	324.8	213.9	422.7	460.7
Slit width / mm	0.5	0.5	0.8	0.2	0.5	0.5	0.5	1.2

Table 3. Mass fractions (mg/kg except for Ca %) determined by AAS measurements

Sample	Dry weight (%)	*w*(Pb) (mg/kg)	*w*(Cd) (mg/kg)	*w*(Mn) (mg/kg)	*w*(Fe) (mg/kg)	*w*(Cu) (mg/kg)	*w*(Zn) (mg/kg)	*w*(Ca) (%)	*w*(Sr) (mg/kg)	*w*(Hg) (mg/kg)	$\frac{w(\mathrm{Sr})}{w(\mathrm{Ca})}$
1P	94.90	3.21	0.017	205.0	4513.0	9.11	51	30.46	481	0.0388	0.00158
3P	95.16	16.40	0.075	184.0	2716.0	18.29	110	36.84	267	0.0358	0.00072
4P	94.72	15.70	0.013	176.0	3180.0	16.47	104	32.97	278	0.1705	0.00084
5P	95.59	0.38	0.036	23.7	149.0	2.37	62	40.09	473	0.0221	0.00118
6P	95.18	0.87	0.047	195.0	1068.0	5.06	180	35.14	413	0.0173	0.00118
7P	96.83	4.28	0.028	220.0	2654.0	12.18	176	36.54	442	0.1102	0.00121
8P	94.26	3.73	0.035	82.6	1259.0	6.19	153	34.19	403	0.0360	0.00118
9P	93.74	1.01	0.049	341.0	1786.0	7.58	184	20.90	212	0.0207	0.00101
13P	95.51	0.91	0.015	41.5	754.0	1.81	288	24.17	468	0.0089	0.00194
15P	95.44	2.18	0.026	138.0	2279.0	7.90	255	24.35	582	0.0513	0.00239
17P	95.95	0.47	0.014	36.2	659.0	3.37	232	28.52	485	0.0233	0.00170
18P	94.83	0.29	0.009	41.8	171.0	1.55	131	24.64	469	0.0121	0.00190
19P	94.88	0.34	0.007	25.8	45.7	1.08	98	33.73	398	0.0049	0.00118
20P	94.70	0.46	0.014	29.0	423.0	1.77	75	31.47	467	0.0058	0.00148
21P	96.04	0.52	0.021	42.2	439.0	2.36	94	31.82	369	0.0157	0.00116
24P	96.34	0.61	0.098	7.8	197.0	5.99	99	32.73	222	0.0106	0.00068
Median	**95.17**	**0.87**	**0.024**	**62.40**	**911.00**	**5.53**	**121**	**32.28**	**428**	**0.0214**	**0.00118**
S.D.	0.79	5.36	0.025	98.0	1327.0	5.32	70	5.35	106	0.0440	0.00047
Max	96.83	16.40	0.098	341.0	4513.0	18.29	288	40.09	582	0.1705	0.00239
Min	93.74	0.29	0.007	7.8	45.7	1.08	51	20.90	212	0.0049	0.00068

tion took about 70 minutes and cooling another 30 minutes. Digested samples were diluted to 50 ml with deionised water.

Measurement

Element concentrations of manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), calcium (Ca) and strontium (Sr) were measured with an atomic absorption spectrometer (flame AAS, Analytik Jena AAS Vario 6, Germany) in flame mode (Table 2 experimental conditions).[15] The analysis of Pb and Cd were performed using Graphite Furnace Atomic Absorption Spectrometry Concentrations of Fe, Mn, Cu and Zn were determined using Flame Atomic Absorption Spectrometry with flame C_2H_2/Air. Ca and Sr were determined using Flame Atomic Absorption Spectrometry with flame C_2H_2/N_2O_2 in the same instrument. In each measurement Deuterium background correction was used. Mercury concentration was determined by three direct consecutive measured by mercury analyzer.[16]

RESULTS AND DISCUSSION

In this study, we analyzed the content of Pb, Cd, Mn, Fe, Cu, Zn, Ca, Sr and Hg in total of 16 individuals from five archaeological excavations which were found at Naklice burial site (Southern Croatia) from Early Mediaeval period. Anthropological measurement and sampling were done at the Department of Forensic Medicine, University Hospital Split. One sample each was taken from skull, mandible and ulna; three samples were taken from different humeri; four samples were from different tibias; six samples were taken from different femur bones (Table 1). In spite of DNA analysis it was not possible to determine gender of all individuals. Anthropological measurements did not successfully determine the presumed age of all individuals, except for a small portion of samples for which it was undoubtedly determined to whom they belonged to. In Figure 2

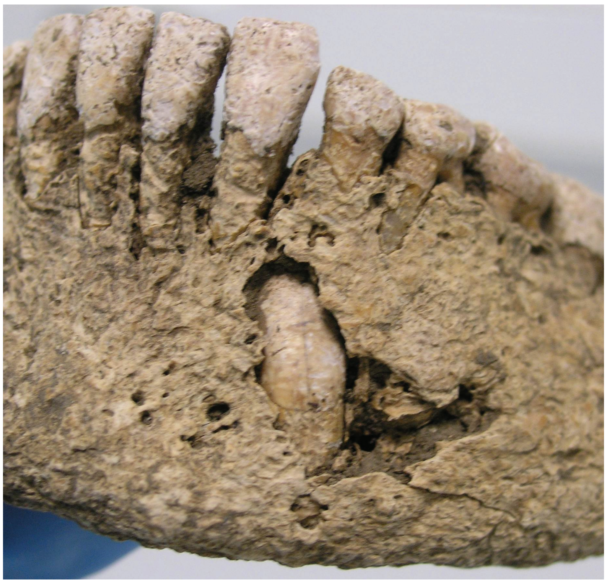

Figure 2. A child's mandible showing a second dentition tooth that has not sprouted yet.

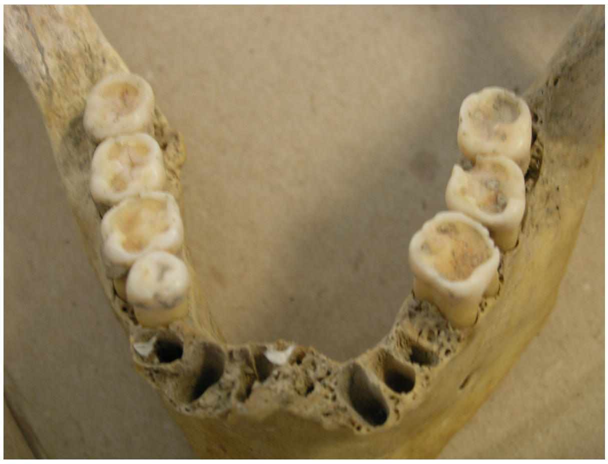

Figure 3. Dental erosion which is finding on one the excavated mandibles.

a child's mandible shows a second dentition tooth that has not sprouted yet.

Table 3 shows the statistics, median, standard deviation, and minimum and maximum values of different elements.

Human exposure to environmental metals can occur simultaneously from various sources. One exposure route is ingestion of metals through food and beverages which contain those elements, and second is the inhalation of atmospheric aerosols. Correlation analysis leads us to consider the presence of studied metals in the analyzed bone samples to be the consequence of analogous inputs.

In the effort to distinguish eventual sample contamination, we analyzed the soil from the burial sites. Table 4 shows only the average value of 4 soil samples which were all very alike in their composition. The limestone soil of Naklice burial site had a mildly alkaline pH (8.87). That kind of soil strongly binds heavy metals, therefore minimizing the possible diagenesis in the sample.[17]

When comparing our results to the modern bone heavy metal concentrations, as found by Ščančar *et al.*, it is obvious that Cu, Fe, Ca and Sr greatly exceeded the values while concentration of Zn and Pb were lower.[5] Concentration of Sr was about ten times higher than in modern bone samples. Due to environmental contamination, Pb concentration in studied bones was lower than in modern bone samples. Even though we used different methods from Vuorinen *et al.*, we can still report that the concentration of Sr was in good correlation with concentration determined from 9 child burials from the Ficana excavations (from the 8th to the 6th century B.C.) and Zn median concentration was identical to the same reference bone samples.[8,9] Zapata *et al.* revealed results from bone element analysis of two Late Roman populations compared with reference values for modern bone and with the element contents of the sediment samples. When compared to Zapata's results our Mn and Fe concentrations were higher. It could probably be due to the environmental contributors at each archaeological site and content of elements like Mn and Fe in the soil environment.

In correlation with elemental contents of various main food components known to have been used during the Early Middle Ages, we could reconstruct a menu.[6] With exception of milk, all main dietary components, such as roots and tubers, meat, cereals, legumes and leafy vegetables are rich in Zn, especially the vegetables. Our results showed that studied individuals had ten times higher concentration of Sr and five times higher concentration of Ca, then is commonly found in modern population bones. Those elements are one of the main components in leafy vegetables. There was a good correlation between Sr/Ca relationship in leafy vegetables (0.0018) and our bone results (0.0012).[6] Foods that are most saturated with Cu are legumes and cereals, in that order. Eating raw and poorly processed legumes and

Table 4. Metals mass fractions (mg/kg except for Ca %) in burial soil sample determined by AAS measurements

Sample	Dry weight (%)	w(**Pb**) (mg/kg)	w(**Cd**) (mg/kg)	w(**Mn**) (mg/kg)	w(**Fe**) (mg/kg)	w(**Cu**) (mg/kg)	w(**Zn**) (mg/kg)	w(**Ca**) (%)	w(**Sr**) (mg/kg)	w(**Hg**) (mg/kg)
soil	98.16	3.61	0.36	156	10745	10.10	38.1	27.78	597	0.018

cereals ultimately leads to significant dental erosion, which is an obvious finding on one the excavated mandibles (Figure 3).

According to our results we concluded that the probable main dietary components of individuals excavated from Naklice burial site from Early Mediaeval period, were leafy vegetables, legumes and small amounts of cereals, and that testing metal concentration levels of the bone material excavated from ancient burial sites can aid in better understanding of medieval living habits.

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SAŽETAK

Status elemenata u osoba iz nalazišta kod Naklica (južna Hrvatska): rekonstrukcija srednjovjekovne ishrane

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Analiziran je status elemenata u osoba iz srednjovjekovnih nalazišta kod Naklica (Južna Hrvatska) te je napravljena rekonstrukcija ishrane. Izuzeti su uzorci različitih koštanih ostataka od 16 osoba koje su umrle u 9. st. i ekshumirane u novije vrijeme. Sadržaj željeza, olova, kadmija, mangana, cinka, bakra, stroncija i kalcija određeni su plamenom tehnikom atomske apsorpcijske spektrofotometrije. Koncentracija žive određena je analizatorom žive trima uzastopnim direktnim mjerenjima. Usporedbom dobivenih rezultata s rezultatima uzoraka kostiju novijeg doba, uočljivo je da koncentracija Cu, Fe, Ca i Sr značajno premašuje vrijednosti, dok koncentracija Zn i Pb je niža. Koncentracija Sr je oko 10 puta viša nego koncentracija u uzorcima kostiju novijeg doba. Zbog zagađenja okoliša, koncentracija olova u ispitivanim uzorcima je niža od koncentracije određene u kostima iz novijeg doba. Iz dobivenh rezultata zaključujemo da je vjerojatno glavne namirnice, osoba iz ranog srednjeg perioda ekshumiranih iz nalazišta kod Naklica, bile: zeljasto povrće, leguminoze i mala količina žitarica.