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Research Article

Some Heavy Metals' Concentrations in the Metacarpal Bones of Paleontological Cattle from Azmashka Settlement Hill

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ABSTRACT

The aim of the present study was to investigate and determine concentrations of some heavy metals in the cattle metacarpal bones, found from Azmashka settlement hill. They belonged to four periods: Early Neolith (EN), Early Halkolith (EH), Late Halkolith (LH) and Early Bronze (EB). The natural bone material was obtained from the archaeological site Azmashka village mound, found 6 km east of Stara Zagora (Bulgaria) and also from the territory of Hrishteni village, following radiocarbonic analysis. In the sampling an atomic absorption spectrophotometry was used. The samples have been burned dry and dissolved in acid until solution with optimal element concentration. Higher concentrations of iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), lead (Pb), chrome (Cr) and magnesium (Mg) were observed. The quantity of Fe, Cu, Zn, Mn and Pb were with higher values at Early Halkolith, compared to the same in the other periods. The highest heavy metals' concentrations were found, as following: iron, cooper, manganese, lead – at Early Halkolith and zink, chrome and magnesium – at Late Halkolith. The lowest heavy metals' concentrations were found, as following: iron, manganese, chrome, magnesium – at Early Neolith and cooper, zink and lead– at Early Bronze. Differences in the other elements' concentrations from the studied periods weren't significant. Qualitative differences influenced by the heavy metals in the bone structure weren't found. The content of heavy metals in the studied metacarpal bone material is considerably high compare to the normal values, mentioned by some researchers studied other species. The trend of concentration increasing is from Early Neolith to Early Bronze. This is due to the metacarpal bone contamination with soil, as which has been polluted from many years by the industrial manufacture of the nitrogen fertilizer.

Key Words: Heavy metal, bones, osteoarchaeology

ÖZET

AZMASHKA YERLEŞİM HÖYÜĞÜNDEKİ PALEONTOLOJİK SIĞIRLARIN METAKARPAL KEMİKLERİNDE BAZI AĞIR METALLERİN KONSANTRASYONLARI

Bu çalışmanın amacı Azmashka yerleşim tepesinde bulunan sığır metacarpal kemiklerindeki bazı ağır metal konsantrasyonlarının tespitinin araştırılmasıdır. Kemikler dört döneme aitti: Erken Neolitik (EN), Erken Kalkolitik (EH), Geç Kalkolitik (LH) ve Erken Tunç (EB). Doğal kemik materyali Stara Zagora'nın 6 km doğusunda bulunan Azmaska köyü höyüğündeki arkeolojikal alandan ve ayrıca Hrishteni köyü bölgesinden temin edildi ve aşağıdaki radyokarbon analizleri yapıldı. Örneklemede atomik absorbsiyon spektrofotometresi kullanıldı. Örnekler solüsyonda uygun element konsantrasyonuna ulaşıncaya kadar kuru ve çözünmüş asit içerisinde yakıldı. Yüksek konsantrasyonda demir (Fe), bakır (Cu), çinko (Zn), manganez (Mn), kurşun (Pb), Krom (Cr) ve magnezyum (Mg) tespit edildi. Fe, Cu, Zn, Mn ve Pb değerlerinin erken kalkolitik dönemde, diğer dönemler ile karşılaştırıldığında yüksek düzeyde olduğu bulundu. En yüksek ağır metal konsantrasyonları şu şekilde bulundu: demir, bakır, manganez ve kurşun- Erken Kalkolitik ve çinko, krom ve magnezyum- geç kalkolitik dönem. En düşük ağır metal konsantrasyonları şu şekilde bulundu: demir, manganez, krom, magnezyum-erken Neolitik ve bakır, çinko ile kurşun-Erken Tunç. Çalışma kapsamındaki dönemlerde diğer elementlere ait konsantrasyonların farklılıkları önemli bulunmamıştır. Kemik yapısında ağır metallerin etki ettiği kalitatif farklılıklar bulunmamıştır. Bu çalışmadaki kemik materyalinde ağır metal düzeyi, bazı araştırmacıların çalıştığı diğer türlerdeki normal değerlerin üzerindedir. Konsantrasyonların yükselme eğilimi erken Neolitik'ten Erken Tunç dönemine doğrudur. Uzun yıllardır endüstriyal azotlu gübre sanayii üretimi ile kirlenmeden dolayı metakarpal kemikler, kum ile kontamine olmuştur.

Anahtar Kelimeler: Ağır metal, kemikler, osteoarkeoloji

Introduction

The bone is a basic component in the archaeological and paleontological discoveries. Its mineral content and solubility is an important parameter when measuring the degree of conservation and preservation of the bone structure in the soil. The extraction of this information depends on the understanding the stability of the bones' components over time (Berna et al., 2004). Diagnosis is a geochemical term, describing the changes in the bone following individual death. It includes all processes influencing the demineralization as of the soil, as of the organic material without the impact of the high temperature and atmospheric pressure.

From archeological findings it is known, that some heavy metals, e.g. Pb have been widely used by humans. Prehistoric data for the accumulation of the Pb in the human body are from 2nd century B.C. as 90-95% of the Pb has been stored in the bones (Drasch, 1982).

The chemical content of the human and animal bones from different archaeological sites could provide the anthropologists and anatomists a wide range of information, including relative and chronological dating (Oakley, 1969; Taylor, 1987), diet (Ericson, 1985; Klepinger, 1984; Lambert et al., 1984; Price et al., 1985), climatic specifics (Longinelli, 1984; Luz et al., 1984).

Despite that the postpartum chemical contamination of the bones from the surrounding soil complicates a lot the applications of these particular methods. It has been accepted that the presence of metal traces play significant impact onto the synthesis, the cross-binding, calcification and the diseases of the connective tissue (O'Dell et al., 1966; Schiffmann et al., 1966).

In attempt to control these postmortem changes in the bone, some researchers utilize the chemical analysis of the soils, located in the archaeological sites. The chemical exchange between the soil and the bone mineral content have been subject of many comparative investigations of the mineral concentration in the archaeological sub-fossils and surrounding soil (Keeley et al., 1977; Kyle, 1986; Lambert et al., 1979; Lambert et al., 1984; Nelson and Sauer, 1984; Waldron et al., 1979; Waldron, 1983). Those methods are limited, due to the different mineral and salt dissolution in the soil and bones.

A lot of authors (Bohn et al., 1985; Milnes and Hutton, 1983) determine some heavy metals, such as Fe, Al, Ti, Mn are basic components of higher erosion soils, because of their insoluble state.

The aim of the study was to investigate and determine the levels of some heavy metals, stored in the bones from different prehistoric periods. That could serve as a base for comparative analysis with other findings from archaeological sites in the region.

Materials and Methods

There were cattle metacarpal bones samples (eighteen from Early Neolith, seventeen from Early Halcolith, nineteen from Late Halcolith and sixteen from Early Bronze) were studied. The natural bone material was obtained from the archaeological site Azmashka village mound, found 6 km east of Stara Zagora (Bulgaria) and also from the territory of Hrishteni village, following radiocarbonic analysis. The land was a relatively flat area of 10 acres, surrounded by "Azmack" (where the name comes from) and was accessible only from northwest. The height measured at "Azmack" was 7.90 m and the thickness of the culture layer – around 7 m. The cultural layer belongs to four prehistorical periods: Early Neolith (EN) – (6000–5500 B.C.), Early Halkolith (EH) – (4900–4100B.C.), Late Halkolith (LH) and Early Bronze (EB) – (2550–2200 B.C.).

Mean samples from the cattle metacarpal bones were obtained for examination of heavy metals contain. In the sampling an atomic absorption spectrophotometry was used by AOAC (2007). The samples have been burned dry and dissolved in acid until solution with optimal element concentration. For the study a light source with dry cathode was used. Each sample was atomized in flame of air – acetylene at 2000–3000°C. Through the atomic cloud a ray of light was passed, whose frequency correlated to the atom's energy transitions. This intensity decreasing of the light source was measured in optical density units, that gives relation to the concentration of the determined atoms. The content of the studied element was calculated with the following formula:

$$X\ (\text{mg /kg. light}) = \frac{C.100.1000.P}{m.1000}$$

The data were statistically processed by one-way ANOVA Statistica 7 (data analysis software system) version 7 (2004). The appropriate differences among treatment means were compared by Fisher LSD test. Differences were considered significant at $P<0.05$.

Results

The concentration of heavy metals (Fe, Cu, Zn, Mn, Pb, Cr, Mg) in the metacarpal bones of prehistoric cattle are shown in Table 1. In all of the studied period Fe was with highest concentrations, compared to the other elements. In Early Halkolith period its values were highest compared to Early Neolith. In Early Neolith period Fe concentration was 99.59±0.02 ppm, in Early Halkolith period – 291.29±0.01 ppm, in Late Halkolith period – 170.81±0.03 ppm and Early Bronze – 149.37±0.09 ppm. The lowest concentrations measured in the metacarpal bone matrix were those of Cu. In Early Neolith period Cu concentrations were 9.06±0.01 ppm, in Early Halkolith – 11.09±0.01 ppm, in Late Halkolith – 9.4±0.00 ppm and Early Bronze – 5.72±0.01 ppm. Highest values of Zn were measured in Late Halkolith-130.24 ppm and lowest concentrations were detected in Early Bronze – 70.22±0.01 ppm. In Early Neolith period its concentrations were 103.91±0.01 ppm and in Early Halkolith – 123.03±0.01 ppm. Mn values were highest in Early Halkolith-153.17±0.04 ppm and lowest were in Early Neolith-46.23±0.00 ppm. Its concentrations in the other periods varied as following - in Late Halkolith-130.32±0.05 ppm, and in Early Bronze-70.23±0.01 ppm. Pb concentrations were with close values in all periods. In Early Neolith period it was 13.86±0.01 ppm, in Early Halkolith –14.53±0.01 ppm, in Late Halkolith –

13.90±0.00 ppm, and in Early Bronze-12.00±0.01 ppm. Cr was also with similar values in different periods. Highest level of its concentration was measured in Late Halkolith-23.92±0.00 ppm, lowest in Early Neolith - 18.52±0.00 ppm and other periods was 20.56 ±0.01 ppm in Early Halkolith, and 22.86±0.01 ppm. Mg was with lowest value in Early Neolith-1.56 ±0.00 ppm and highest in Late Halkolith-1.82±0.00 ppm. In the other periods its concentrations were as following: in Early Halkolith – 1.72±0.00 ppm and Early Bronze – 1.64±0.01 ppm.

Table 1. Some heavy metal values (ppm) in the metacarpal bones of the prehistoric cattle from the different periods Early Neolith (EN), Early Halkolith (EH), Late Halkolith (LH) and Early Bronze (EB).

Tablo 1. Erken Neolitik (EN), Erken Kalkolitik (EH), Geç Kalkolitik (LH) ve Erken Bronz (EB) gibi farklı periyotlardaki prehistorik sığırların metakarpal kemiklerinde bazı ağır metal değerleri (ppm).

Haevy Metal Sample		**Iron (Fe)**		**Coopper (Cu)**		**Zink (Zn)**		**Manganese (Mn)**		**Lead (Pb)**		**Chrome (Cr)**		**Magnesium (Mg)**	
Periods	**N**	**MV**	**SD**	**MV**	**SD**	**MV**	**SD**	**MV**	**SD**	**MV**	**SD**	**MV**	**SD**	**MV**	**SD**
EN	18	99.59	0.04	9.06	0.03	103.91	0.02	46.23	0.01	13.86	0.04	18.52	0.01	1.56	0.01
EH	17	291.29	0.02	11.09	0.01	123.03	0.03	153.17	0.09	14.53	0.01	20.56	0.01	1.72	0.01
LH	19	170.81	0.08	9.42	0.01	130.24	0.09	130.32	0.01	13.90	0.01	23.92	0.01	1.82	0.01
EB	16	149.37	0.12	5.72	0.01	70.22	0.02	70.23	0.02	12.00	0.02	22.86	0.01	1.64	0.01

N – Number of samples; MV – Mean Value; SD – Standard Deviation; P<0.05 - Level of significance

Discussion

Many researchers (Martiniakova et al., 2011) studied the heavy metals in the bones of *Myodes glareolus* and *Microtus arvalis*, as they determined the point limitations of the heavy metals as follows: Cd - 0.005 ppm, Ni - 0.12 ppm, Fe - 0.02ppm, Cu - 0.01 ppm, Zn - 0.13 ppm, and Pb - 0.15 ppm. In the relation to the underground living, the particular rodents are a good biomarker of long-term accumulation of heavy metal in the bones. In comparison, our results showed significant increase of heavy metals in the bones.

Our results corresponded to the study of Drasch (1982) about the accumulation of some heavy metals in the bone structures. That is perhaps the wide use of these elements in different archeological periods.

The chemical content of the studied heavy metals could provide a wide source of information necessary for adequate chronological dating as done by Oakley (1969) and Taylor (1987).

During the Early Neolith the heavy metal concentration had been lower compared to the other eras. This is perhaps due to the fact that the bones are the deepest in the ground. The iron (Fe) had values twice lower than these from the rest of the periods, which is close to the data determined in the bones (58.4 ppm - 116.3 ppm) of some water birds (Kalisinska et al., 2007).

Other authors (Rai and Behari, 1986) found in contemporary animals that the presence of Cu was from 5 to 50 ppm and Fe, Mg and Pb - from 50 ppm to 5000 ppm. Also in contemporary cattle, Kumar et al. (2008) research Cu, Fe and Zn in dry mineralized metacarpal bones' organic matrix. The authors found mean value concentrations of Cu - 2.7±0.4 ppm. In the same bone samples Fe was also detected. Its average concentrations were found to be 8.4±3.7 ppm. Compare to these results, our data for the prehistoric cattle show significant differences, compared to the contemporary ones.

Similar to the studies of many authors (Keeley et al., 1977; Kyle, 1986; Lambert et al., 1979; Lambert et al., 1984; Nelson and Sauer, 1984; Waldron et al., 1979; Waldron, 1983) the present study had a topic to control the postmortal changes in the metacarpal bones, located in the archaeological regions.

The presence of heavy metals in the bones should not be taken separate from the soil content investigation. Soil from the area of Azmashka settlement hill established values of some heavy metals, including Cu and Pb (Eneva and Todorova, 2004). The values of copper had been on the upper scale from 38.8 ppm to 47.9 ppm, which were taken as a result of treatment of the existing vineyards with Copper chemicals. The values for lead varied from 16.9 ppm to 29.5 ppm, which is lower from the background concentration.

The content of heavy metals in the studied metacarpal bone material is considerably high compared to the results of some researchers studied other species. The trend of concentration increasing is from Early Neolith to Early Bronze. We assume that this is due to the metacarpal bone contamination with soil, as wich has been polluted from many years by the industrial manufacture of the nitrogen fertilizer.

For the first time such study of archeological bone material was carried out in Azmashka settlement hill and territory of Hrishteni village. Heavy metals' accumulation in cattle metacarpal bones in different prehistorical periods give information for animal feeding and breeding in Early Neolith, Early Halkolith, Late Halkolith and Early Bronze.

REFERENCES

Berna, F., Matthews, A., Weiner, S., 2004. Solubilities of bone mineral from archaeological sites: the recrystallization window. Journal of Archaeological Science 31, 867-882.

Bohn, H., McNeal, B., O'Connor, G., 1985. Soil Chemistry. Wiley Interscience, John Wiley & Sons, Inc, New York.

Drasch, G., 1982. Lead burden in prehistorical, historical and modern human bones. Science of the Total Environment 24 (3), 199-231.

Eneva, C., Todorova, M., 2004. Content of heavy metals leached cinnamon forest soils in the Agrobiochim EAD-Stara Zagora. Journal of Agricultural Science and Forest Science 3 (2), 65-67.

Ericson, J., 1985. Strontium isotope characterization in the study of prehistoric human ecology. Journal of Human Evolution 14 (5), 503-514.

Kalisińska, E., Salicki, W., Kavetska, K., Ligoki, M., 2007. Trace metal concentrations are higher in cartilage than in bones of scaup and pochard wintering in Poland. Science of the Total Environment 388 (1-3), 90-103.

Keeley, H., Hudson, G., Evans, J., 1977. Trace - element content of human bone in various states of preservation: 1, The soil silhouette. Journal of Archaeological Science 4, 19-24.

Klepinger, L., 1984. Nutritional assessment from bone. Annual Review of Anthropology 13, 75-96.

Kumar, R., Shoab, S., Fakruccin, K., Ahmad, A., 2008. Identification of trace elements in bovine metacarpus bone by spectro chemical analysis. Journal of King Abdulaziz University Science 20 (2), 17-26.

Kyle, J., 1986. Effect of post-burial contamination on the concentrations of major and minor elements in human bones and teeth - the implications for palaeodietary research. Journal of Archaeological Science 13 (5), 403-416.

Lambert, J., Simpson, S., Szpunar, C., Buikstra, J., 1984. Ancient human diet from inorganic analysis of bone. Accounts of Chemical Research 17 (9), 298-305.

Lambert, J., Szpunar, C., Buikstra, J., 1979. Chemical analysis of excavated human bone from Middle and Late Woodland sites. Archaeometry 21 (2), 115-129.

Longinelli, A., 1984. Oxygen isotopes in mammal bone phosphate: A new tool for paleohydrological and paleoclimatological research? Geochimica et Cosmochimica Acta 48 (2), 385-390.

Luz, B., Kolodny, Y., Howowitz, M., 1984. Fractionation of oxygen isotopes between mammalian bone - phosphate and environmental drinking water. Geochimica et Cosmochimica Acta 48 (8), 1689-1693.

Martiniaková, M., Omelka, R., Jančová, A., Stawarz, R., Formicki, G., 2011. Concentrations of Selected Heavy Metals in Bones and Femoral Bone Structure of Bank (*Myodes glareolus*) and Common (*Microtus arvalis*) Voles from Different Polluted Biotopes in Slovakia. Archives of Environmental Contamination and Toxicology 60 (3), 524-532.

Milnes, A., Hutton, J., 1983. The Soils; an Australian Viewpoint: Calcretes in Australia. CSIRO, Melbourne/Academic Press, London.

Nelson, D., Sauer, N., 1984. An evaluation of postdepositional changes in the trace element content of human bone. American Antiquity 49 (1), 141-l47.

Oakley, K., 1969. Analytical methods of dating bones. In: Brothwell, D., Higgs, E., (Ed), Science in Archaeology. New York, Thames and Hudson, pp. 35-45.

O'Dell, B., Elsden, D., Thomas, J., Partridge, S., Smith, R., Palmer, R., 1966. Inhibition of the biosynthesis of cross links in elastin by a lathyrogen. Nature 209, 401-402.

Price, T., Schoeninger, M., Armelagos, G., 1985. Bone chemistry and past behavior: an overview. Journal of Human Evolution 14 (5), 419-447.

Rai, D., Behari, J., 1986. Biophysical characterization of osteoporotic bone. Fluorescence spectra and morphology of bone. Environmental Research 40 (1), 68-83.

Schiffmann, E., Corcoran, B., Martin, G., 1966. The role of complexed heavy metals in initiating the mineralization of 'elastin' and the precipitation of mineral from solution. Archives of Biochemistry and Biophysics 115 (1), 87-94.

Statistica 7.0, 2004. Copyright© 1984-2004. StatSoft. Inc, Tulsa, Oklahoma, USA.

Taylor, R., 1987. Radiocarbon Dating: An Archaeological Perspective. Academic Press, New York.

Waldron, H., Khera, A., Walker, G., Wibberley, G., Green, C.H., 1979. Lead concentrations in bones and soil. Journal of Archaeological Science 6 (3), 295-298.

Waldron, H., 1983. On the post - mortem accumulation of lead by skeletal tissues. Journal of Archaeological Science 10 (1), 35-40.