NEUTRON ACTIVATION ANALYSIS OF Ag/Ca, Co/Ca, Cr/Ca, Fe/Ca, Hg/Ca, Rb/Ca, Sb/Ca, Sc/Ca, Se/Ca, and Zn/Ca MASS FRACTION RATIOS IN INFLAMED BONE

V. Zaichick[1], S. Zaichick[1,2]

[1] Medical Radiological Research Centre,
Koroleva str., 4, Obninsk, 249036, Russia,
e-mail: vezai@obninsk.com
[2] Current address: University of Illinois College of Medicine Chicago, IL 60612, USA

Fundamental Interactions & Neutrons, Nuclear Structure, Ultracold Neutrons, Related Topics.
JOINT INSTITUTE FOR NUCLEAR RESEARCH, Dubna, Russia, May 23 – 27, 2016

Introduction (1)

The roles of trace elements in the development and inhibition of diseases have a complex character and have risen many questions because of their essential and toxic effects on human health. The effects of trace elements are related to content and recorded observations range from a deficiency state, to function as biologically essential components, to an unbalance when excess of one element interferes with the function of another, to pharmacologically active doses, and finally to toxic and even life-threatening levels.[1,2] Thus, in normal environmental and health conditions there is a trace element homeostasis in tissues and fluids of human body and an unbalance of trace element contents could be a causative factor for many diseases.[2] On the other hand pathological condition can effect on contents and relationships of trace elements in tissues and fluids and it is possible to use these changes as markers of disease.[2]

It is well known that the tissues of human body differ greatly in their contents of trace elements. Our detailed previous studies have shown this using a chemical composition analysis of bone tissue.[3-29] Bone diseases can derive from all the tissue components of bone (cartilage, osteoid, fibrous tissue, and bone marrow elements). Each tissue can be subject to inflammation, benign or malignant tumors.

Osteomyelitis is a difficult-to-treat bone infection characterized by progressive inflammatory destruction of the bone, with necrosis and new bone formation.[30] Osteomyelitis occur most commonly in children, and the overall prevalence is 1 case per 5000 children.[31,32] Osteomyelitis typically affects the most rapidly growing ends of long bones and is more common in the lower extremity, the metaphysis of the distal femur and of the proximal tibia being the most common sites of infection.[33,34] The limbs are affected in 90 % of cases, and specifically lower limbs in 70 %.[35] There is a male predilection[32] as well as a difference in incidence according to racial origin and the geographic region.[35] The diagnosis of childhood osteomyelitis can be challenging.[36] Moreover, early diagnosis, essential for timely appropriate treatment and reduction of complications, can be very difficult.[37] Although imaging is essential in the diagnostic process, a bone biopsy is necessary to confirm a diagnosis of osteomyelitis. This also helps determine the type of organism, typically bacteria, causing the infection so the right medication can be prescribed. The etiology and pathogenesis of osteomyelitis is not well understood, however significant interest and effort in this bone disease led to the identification of numerous etiologic agents.[32,35] Osteomyelitis can be either acute or chronic. Factors that favor the development of acute bone infection are those that predispose to bacteremia. The major mechanism for the development of acute osteomyelitis is injuries due to penetrating bites and puncture wounds of the foot which may serve to infect bone directly.[38] People with diabetes, HIV, sickle cell anemia, chronic granulomatous or peripheral vascular disease are more prone to chronic osteomyelitis.[38,39]

Aims of the study

To our knowledge, no data are available for the trace element content to Ca content ratios in bone affected by osteomyelitis, to permit conclusion about the role of trace elements in etiology, pathogenesis, and diagnostics of this disease.

The aim of the study was to compare the ratios of selected trace element contents to Ca content in two groups of samples (normal bone and bone affected by osteomyelitis). For this purpose, the Ag/Ca, Co/Ca, Cr/Ca, Fe/Ca, Hg/Ca, Rb/Ca, Sb/Ca, Sc/Ca, Se/Ca, and Zn/Ca mass fraction ratios were determined in the two groups of samples using nondestructive instrumental neutron activation analysis (INAA) with high resolution spectrometry of short-lived radionuclides (INAA-SLR) and long-lived radionuclides (INAA-LLR).

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk.

Materials

Thirty-seven children, adolescents and adults were included in this study. The subjects were divided into two groups: reference and osteomyelitis. The reference group consisted of 27 patients with intact bone (12 females and 15 males, aged from 16 to 49 years) who had died from various non bone related causes, mainly unexpected from trauma. The intact cortical bone samples of femur, femoral neck, tibia and iliac crest were collected at the Department of Pathology, Obninsk City Hospital. Samples from 10 patients with osteomyelitis (3 females and 7 males, 9 to 21 years old) were obtained from open biopsies or after operation from resected specimens. All patients with bone diseases were hospitalized at the Medical Radiological Research Centre. In all cases the diagnosis was confirmed by clinical and histological data.

Methods

A titanium tool was used to cut and to scrub samples.[40,41] All bone samples were freeze dried, until constant mass was obtained, and homogenized. Then samples weighing about 100 mg for INAA-SLR were sealed separately in thin polyethylene film washed beforehand with acetone and rectified alcohol. The sealed samples were placed in labeled polyethylene ampoules. The mass fraction of Ca was determined by INAA-SLR using a horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor. The information of used nuclear reaction, radionuclide, gamma-energie, neutron flux, spectrometer and other details of the analysis including the quality control of results were reported by us before.[12, 14-20]

After INAA-SLR bone samples were taken out from polyethylene ampoules and polyethylene film and were wrapped separately in high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule. A vertical channel of the WWR-c research nuclear reactor was applied to determine the mass fraction of Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn by INAA-LLR. The quartz ampoule with bone samples, standards, CRM, and SRM was soldered, positioned in a transport aluminum container and exposed to a 100-hour neutron irradiation in a vertical channel with a thermal neutron flux about 10^{13} $n \cdot cm^{-2} \cdot s^{-1}$. The information of used nuclear reactions, radionuclides, gamma-energies, and other details of the analysis including the quality control of results were reported in our previous studies.[22,24,25,42]

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol–formaldehyde resins and aliquots of commercial, chemically pure compounds were used. Corrected certified values of BSS element contents were reported by us before.[43] Ten certified reference material (CRM) IAEA H-5 (Animal Bone) sub-samples and ten standard reference material (SRM) NIST 1486 (Bone Meal) sub-samples weighing about 100 mg were analyzed in the same conditions as bone samples to estimate the precision and accuracy of the results.

A dedicated computer program of INAA mode optimization was used.[44] Using the Microsoft Office Excel software, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels were calculated for different mass fraction ratios. The reliability of difference in the results between intact bone and bone affected by osteomyelitis was evaluated by Student’s t-test.

Results and discussion

The non-destructive INAA was used in this research study because this method has many definite advantages over other analytical methods, particularly, in the clinical chemistry. For example, after non-destructive INAA-SLR of Ca content in bone samples the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions were measured in the same samples. Moreover, non-destructive INAA there is a possibility to receive additional information about other trace element contents by destructive analytical methods such as atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry, inductively coupled plasma mass spectrometry and so on, using the same bone samples. Moreover, if a deep-cooled channel of nuclear reactor is available, the non-destructive INAA allows determining trace element contents in the fresh bone samples and combining trace element study with histological investigation. It is also necessary to keep in mind that the non-destructive methods are the current gold-standard solution to control destructive analytical techniques.[2] The destructive analytical methods are based on measurements of processed tissue. In such studies tissue samples are ashed and/or acid digested before analysis. There is evidence that certain quantities of chemical elements are lost as a result of such treatment.[2,41,45] There is no doubt that every method available for the measurement of chemical element contents in bone samples can be used. However, when using destructive analytical methods it is necessary to control for the losses of chemical elements, for complete acid digestion of the sample, and for the contaminations by trace elements during sample decomposition, which needs adding some chemicals.

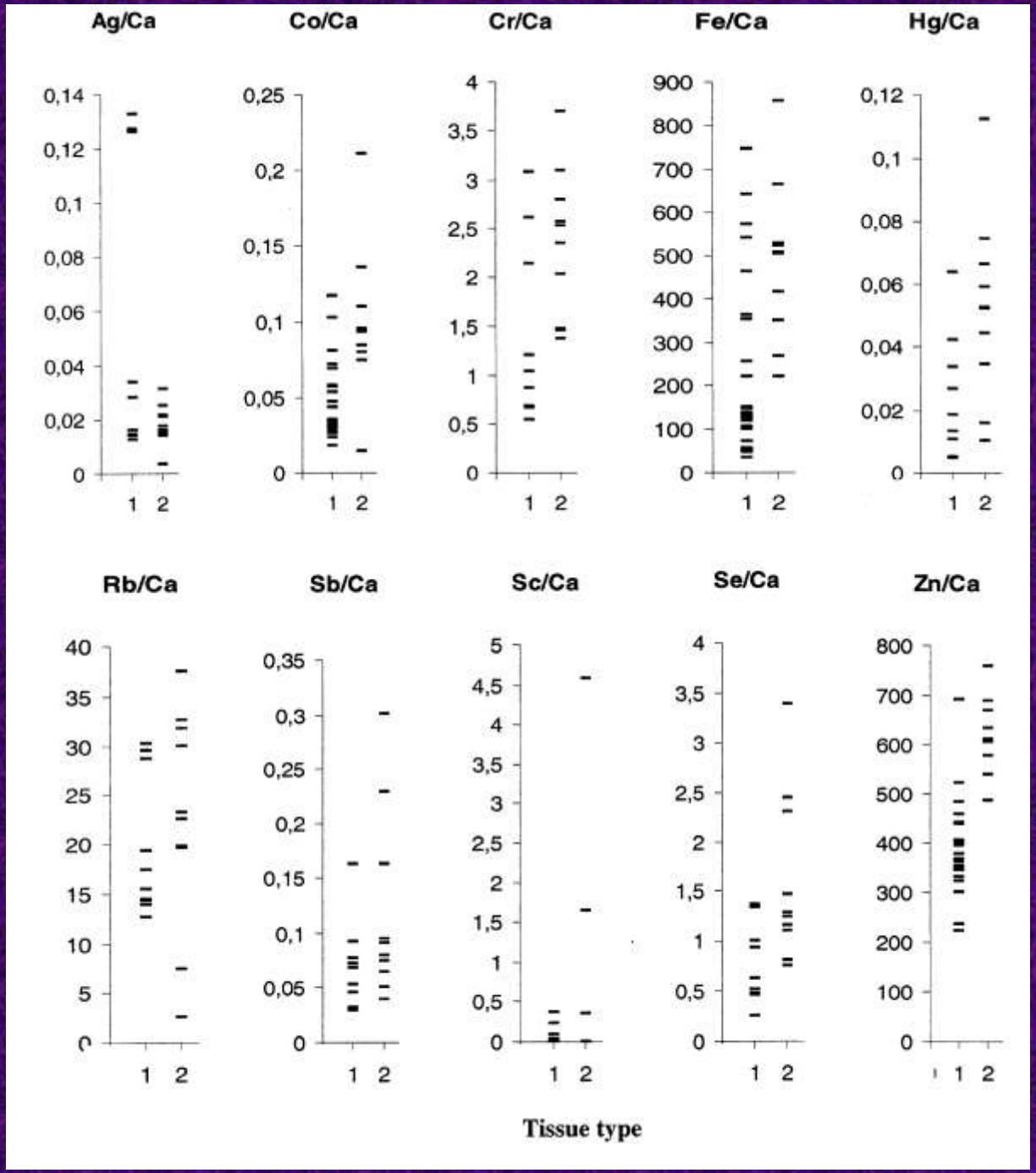

Fig. 1. Individual data sets for Ag/Ca, Co/Ca, Cr/Ca, Fe/Ca, Hg/Ca, Rb/Ca, Sb/Ca, Sc/Ca, Se/Ca, and Zn/Ca mass fraction ratios ($\times 10^6$) in intact (1) and inflamed bone (2).

Table 1. Basic statistical parameters for Ag/Ca, Co/Ca, Cr/Ca, Fe/Ca, Hg/Ca, Rb/Ca, Sb/Ca, Sc/Ca, Se/Ca, and Zn/Ca mass fraction ratios in tissue of intact bone and bone affected by osteomyelitis

Ratio×10^6	M	SD	SEM	Min	Max	Med	P0.025	P0.975
Intact bone, n=27								
Ag/Ca	0.0146	0.0070	0.0023	0.0013	0.0282	0.0139	0.0035	0.0262
Co/Ca	0.0465	0.0246	0.0049	0.0182	0.117	0.0352	0.0212	0.108
Cr/Ca	1.43	0.94	0.31	0.542	3.08	1.03	0.569	2.99
Fe/Ca	231	213	44	35.7	745	135	41.9	686
Hg/Ca	0.0242	0.0195	0.0065	0.0045	0.0636	0.0186	0.0047	0.0593
Rb/Ca	19.6	7.1	2.3	12.6	30.3	16.4	12.8	30.1
Sb/Ca	0.070	0.041	0.014	0.0296	0.163	0.0684	0.0300	0.149
Sc/Ca	0.073	0.124	0.039	0.0023	0.361	0.0034	0.0024	0.332
Se/Ca	0.78	0.40	0.13	0.256	1.37	0.633	0.295	1.36
Zn/Ca	384	93	19	222	693	366	231	590
Osteomyelitis, n=10								
Ag/Ca	0.0187	0.0074	0.0024	0.0036	0.0315	0.0193	0.0059	0.0300
Co/Ca	0.098	0.050	0.016	0.0141	0.211	0.089	0.0277	0.194
Cr/Ca	2.34	0.77	0.24	1.36	3.69	2.44	1.38	3.55
Fe/Ca	483	187	59	220	854	506	230	812
Hg/Ca	0.0522	0.0294	0.0093	0.0105	0.112	0.0523	0.0118	0.104
Rb/Ca	22.7	11.1	3.5	2.46	37.5	22.8	3.56	36.4
Sb/Ca	0.118	0.086	0.027	0.0385	0.301	0.0849	0.0410	0.285
Sc/Ca	0.66	1.47	0.46	0.0027	4.58	0.0033	0.0027	3.92
Se/Ca	1.59	0.85	0.27	0.751	3.39	1.26	0.764	3.18
Zn/Ca	624	78	25	487	758	622	499	742

M arithmetic mean, *SD* standard deviation, *SEM* standard error of mean, *Min* minimum value, *Max* maximum value, *Med* median, *P0.025* percentile with 0.025 level, *P0.975* percentile with 0.975 level

In the osteomyelitis group the means of Ag/Ca, Co/Ca, Cr/Ca, Fe/Ca, Hg/Ca, Rb/Ca, Sb/Ca, Sc/Ca, Se/Ca, and Zn/Ca mass fraction ratios are higher than in the normal bone tissues (Table 2). However, in the osteomyelitis group only the mean mass fraction ratios of Co/Ca ($p \leq 0.011$), Cr/Ca ($p \leq 0.037$), Fe/Ca ($p \leq 0.0027$), Hg/Ca ($p \leq 0.026$), Se/Ca ($p \leq 0.017$), and Zn/Ca ($p \leq 0.00000021$) are significantly increased when compared with those in normal bone.

Table 2. Means, ratio of means and the reliability of difference between mean values of Ag/Ca, Co/Ca, Cr/Ca, Fe/Ca, Hg/Ca, Rb/Ca, Sb/Ca, Sc/Ca, Se/Ca, and Zn/Ca mass fraction ratios ($\times 10^6$) in tissue of intact bone and bone affected by osteomyelitis

Ratio$\times 10^6$	Bone			Ratio
	Intact bone M±SEM	Inflamed bone M±SEM	Student's *t*-test $p \leq$	Inflamed bone to Intact bone
Al/Ca	0.0146±0.0023	0.0187±0.0024	0.23	1.28
Co/Ca	0.0465±0.0049	0.098±0.016	**0.011**	2.11
Cr/Ca	1.43±0.31	2.34±0.24	**0.037**	1.64
Fe/Ca	231±44	483±59	**0.0027**	2.09
Hg/Ca	0.0242±0.0065	0.0522±0.0093	**0.026**	2.16
Rb/Ca	19.6±2.3	22.7±3.5	0.47	1.16
Sb/Ca	0.070±0.014	0.118±0.027	0.13	1.69
Sc/Ca	0.073±0.039	0.66±0.46	0.24	9.04
Se/Ca	0.78±0.13	1.59±0.27	**0.017**	2.04
Zn/Ca	384±19	624±25	**0.00000021**	1.63

M arithmetic mean, *SEM* standard error of mean, *bold* statistically significant

Our findings showed that the mean of the Fe/Ca mass fraction ratio in the inflamed bone samples was almost two times greater than in normal bone tissues (Table 2). It is well known that Fe mass fraction in sample depends mainly from the blood volumes in tissues. The blood supply to the affected area is increased substantially during the inflammatory response.[48] Thus, it is possible to speculate that bone affected by osteomyelitis is characterized by an increase of the mean value of the Fe mass fraction because the level of blood is higher than that in normal bone.

Inflammatory processes are initiated and regulated by a great variety of inflammatory mediators including metalloproteases.[49] Most metalloproteases require Zn, but some use Co. Inflammation may induce a high perturbation in the intracellular and intercellular homeostasis as well as an imbalance between pro-oxidant and antioxidant enzyme activities in bone tissue.[50] Recent studies indicate that transition metals contents, including Cr, associated with the levels of oxidative stress in tissue.[51] Because of that elevated levels of Co/Ca, Cr/Ca, and Zn/Ca in inflamed bone tissue may result from oxidative stress in osteomyelitis.

In the inflamed bone tissue the mean Se/Ca mass fractions is 2.04 times higher ($p \leq 0.017$) than in normal bone (Table 2). The cause of increased Se in inflamed tissues remains unknown, but in general it is accepted that certain proteins containing Se can mediate the protective effects against oxidative stress. However the role of Se in inflamed bone tissue is not completely understood and requires further studies. Moreover, there are many other trace elements associated with the levels of oxidative stress in tissue. Thus, further studies are needed to increase the number of bone samples affected by osteomyelitis and to extend the list of trace elements investigated.

Conclusions

The combination of INAA-SLR and INAA-LLR is a satisfactory analytical tool to determine non-destructively the Ag/Ca, Co/Ca, Cr/Ca, Fe/Ca, Hg/Ca, Rb/Ca, Sb/Ca, Sc/Ca, Se/Ca, and Zn/Ca mass fraction ratios in human intact bone samples and samples of intraosseous lesions weighing about 100 mg. In the bone affected by osteomyelitis the mean mass fraction ratios of Co/Ca, Cr/Ca, Fe/Ca, Hg/Ca, Se/Ca, and Zn/Ca are significantly higher than in normal bone tissues. Thus, if we accept the ratio of trace element mass fraction to Ca mass fraction in the intact bone as a norm, we have to conclude that in inflamed bone the trace element homeostasis is significantly disturbed. The studies on the role of trace elements in the etiology of osteomyelitis have to be continued.

References

1. Ulmer D.D. Federation Proc., 1973, *32*, 1758-1762 . **2.** Zaichick V. J. Radioanal. Nucl. Chem., 2006, *269*(3) 653–659. **3**. Zherbin EA, Zaichick V. Several aspects of applied neutron activation in medicine: The present state and development of activation analysis in the Institute of Medical Radiology. 2nd Meeting on New Nuclear-Physical Methods Used in Solving Scientific-Technical and National Economic Problems. Join Institute of Nuclear Research, Dubna, Russia, 1976, pp 104–126 (In Russian). **4**. Kalashnikov V.M., Zaichick V. Voprosy med. khimii, 1977, *23*(1), 122- 127 (In Russian). **5**. Zaichick V. J. Radioanal. Nucl. Chem., Articles, 1993, *169*, 307-316. **6**. Zaichick V. J. Radioanal. Nucl. Chem., Articles, 1994, *179*, 295-303. **7**. Zaichick V, Ovchjarenko NN. J. Trace and Microprobe Techniques, 1996, *14*, 143-152. **8**. Zaichick V, Morukov B. Appl. Radiat. Isot., 1998, 49, 691-694. **9**. Zaichick V. J. Trace Elements in Experimental Medidicne, 1998, *11*(4), 509-510. **10**. Zaichick V, Ovchjarenko N, Zaichick S. Appl. Radiat. Isot., 1999, *50*, 283-293. **11**. Zaichick V, Snetkov A. Bone composition in children with rickets-like diseases before and during treatment. In: Mengen- und Spurenelemente. 20 Arbeitstagung (Anke M et al., editors). Friedrich-Schiller-Universitat, Jena, 2000, pp 1109-1117. **12**. Zaichick V, Dyatlov A, Zaichick S. J. Radioanal. Nucl. Chem., 2000, *244*, 189-193. **13**. Sastri C.S. et al. Fresenius J. Anal. Chem., 2001, *370*, 924-929. **14**. Zaichick V, Tzaphlidou M. Appl. Rad. Isotop., 2002, *56*, 781-786. **15**. Tzaphlidou M, Zaichick V. Appl. Rad. Isotop., 2002, *57*, 779-783. **16**. Tzaphlidou M, Zaichick V. Biol. Trace Elem. Res., 2003 *93*, 63-74. **17**. Zaichick V, Tzaphlidou M. Appl. Rad. Isotop., 2003, *58*, 623-627. **18**. Zaichick V. J. Radioanal. Nucl. Chem., 2004, *259*, 351-354. **19**. Zaichick V. Sex and age related Ca/P ratio in trabecular bone of iliac crest of healthy humans. In: Macro and Trace Elements. 22 Workshop (Anke M et al., editors). Friedrich Schiller University, Jena, 2004, Vol. 1, pp 248-255. **20**. Tzaphlidou M, Zaichick V. J. Radioanal. Nucl. Chem., 2004, *259*(2), 347-349. **21**. Zaichick V. J. Radioanal. Nucl. Chem., 2007, *271*(3), 573-576. **22**. Zaichick V, Zaichick S. J. Radioanal. Nucl. Chem., 2009, *281*, 47-52. **23**. Zaichick V. J. Radioanal. Nucl. Chem., 2009, *281*, 41-45. **24**. Zaichick S., Zaichick V. Journal of Trace Elements in Medicine & Biology, 2010, *24*, 1-6. **25**. Zaichick S, Zaichick V. Biol. Trace Elem. Res., 2010, *137*, 1-12. **26**. Zaichick S, Zaichick V. J. Radioanal. Nucl. Chem., 2012, *293*(1), 241-246. **27**. Zaichick V. Biol. Trace Elem. Res., 2013, *153*, 84-99. **28**. Zaichick V. Radiat. Environ. Biophys., 2013, *52*, 65-85. **29**. Zaichick V, Zaichick S. Biol. Trace Elem. Res., 2014, *159*(1-3), 32-38. **30**. De Boeck H. Acta Orthopædica Belgica 2005, *71*, 505-515. **31**. McCarthy JJ, Dormans J, Kozin SH. et al. Journal of Bone & Joint Surgery, 2004, *86-A*(4), 850-863. **32**. Kremers HM, Nwojo M.E, Ransom JE, et al. Journal of Bone & Joint Surgery, 2015, *97*(10), 837-845. **33**. Perlman MH, Patzakis MJ, Kumar PJ, et al. Journal of Pediatric Orthopaedics, 2000, *20*, 40-43. **34**. Maraqa NF, Gomez MM, Rathore MH, et al. Journal of Pediatric Orthopaedics, 2002, *22*, 506-510. **35**. Labbé JL, Peres O, Leclair O, et al. Orthopaedics & Traumatology: Surgery & Research, 2010, *96*(3), 268-275. **36**. Van Schuppen J, van Doom MM, van Rijn RR. Insights, 2012, *3*(5), 519-533. **37**. Hankin D, Bowling FL, Metcalfe SA, et al. Foot & Ankle Specialist , 2011, *4*(2), 100-105. **38**. Wald ER. The American Journal of Medicine, 1985, *78*(6Suppl 2), 206-212. **39**. Lavery L.A., Peters E.J.G., Armstrong D.G. et al. Diabetes Research and Clinical Practice, 200, *83*(3), 347-352. **40**. Zaichick V, Zaichick S. J. Anal. Chem., 1996, *51*(12), 1200-1205. **41**. Zaichick V. Sampling, sample storage and preparation of biomaterials for INAA in clinical medicine, occupational and environmental health. In: Harmonization of Health-Related Environmental Measurements Using Nuclear and Isotopic Techniques. International Atomic Energy Agency, Vienna, 1997, pp. 123-133. **42**. Zaichick V, Kolotov VP, Dogadkin N. Comparative analysis of major and trace elements in bone reference materials, IAEA H-5 (animal bone) and NIST SRM 1486 (bone meal). In: Macro and Trace Elements. 21 Workshop (Anke M et al., editors). Friedrich Schiller University, Jena, 2002, pp. 39-47. **43**. Zaichick V. Fresenius J. Anal. Chem., 1995, *352*, 219-223. **44**. Korelo A and Zaichick V. Software to optimize the multielement INAA of medical and environmental samples. In: Activation analysis in environment protection. Join Institute of Nuclear Research, Dubna, Russia, 1993, pp. 326–332. **45**. Zaichick V. Trace Elements in Medicine, 2004, *5*(3), 17-22. **46**. Zaichick S., Zaichick V. J. Radioanal. Nucl. Chem., 2012, *293*(1), 241-246. **47**. Zaichick V., Zaichick S. International Journal of Orthopedics and Rehabilitation, 2015, *3*(1), 1-9. **48**. Rankin JA. AACN Clinical Issues, 2004, *15*(1), 3-17. **49**. Henrotin Y, Deby-Dupont G, Reginster JY, Revue Medicale de Liege, 2001, *56*(6), 433-442. **50**. Philippe C., Wittrant Y. Oxidative Stress in Musculoskeletal Disorders – Bone Disease. In: System Biology of Free Radicals and Antioxidants, 2014, pp. 2951–2959. **51**. Ercal N, Gurer-Orhan H, Aykin-Burns N. Carent Topics in Medical Chemistry,2002, *1*(6), 529-539.