THE POSSIBILITY OF DIRECT ANALYSIS OF BIOLOGICAL TISSUES OF A FEW MILLIGRAMS BY SRXRF METHOD

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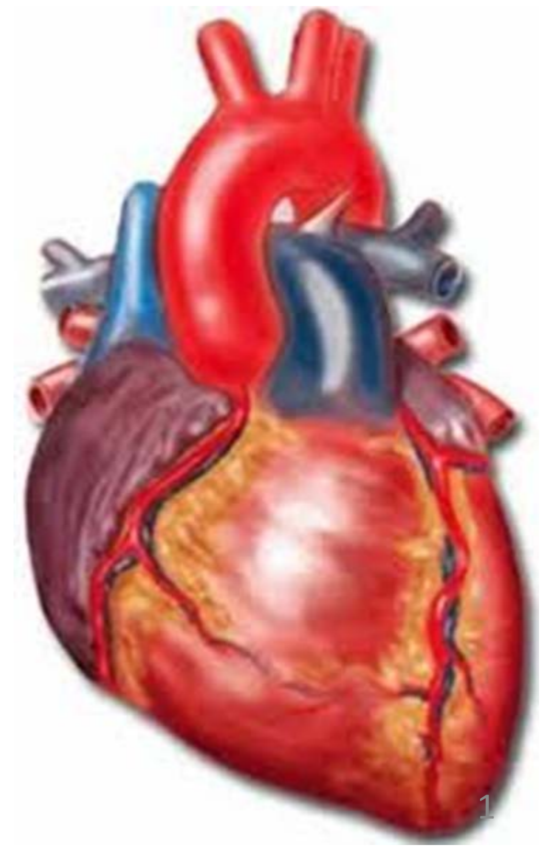


The investigations were done in

Nikolaev Institute of Inorganic Chemistry SB RAS

in collaboration with

Budker Institute of Nuclear Physics SB RAS
and
Meshalkin Institute of pathology of circulation of the blood

The main stages of analysis by SRXRF tissues samples

At each stage it is necessary to select the optimum conditions for the analysis of specific samples.

1. Sampling

lyophilization

Freezing

Chemical fixation

2. Sample preparation

Pressing of crushed material

Drying under load

Pressing without rubbing

3. Selection of reference samples

Known concentration analysis. elements

Identity matrix

Accounting corrections to the absorption (μ)

4. Measurements of the spectra SRXRF

Normalization on inside standard

Other modes of normalization

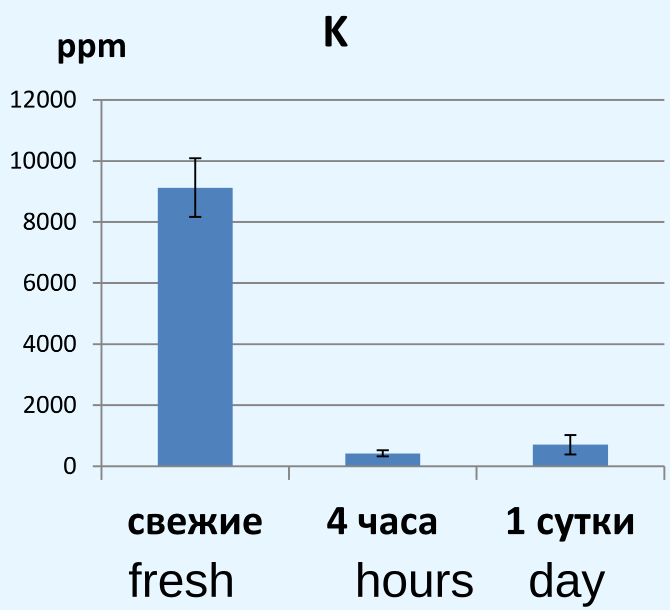
Normalization on Compton

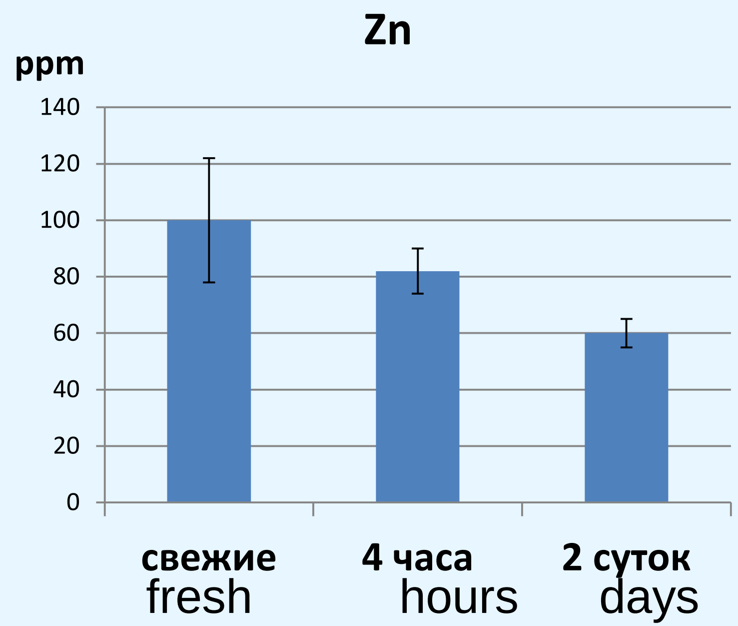
5. The calculation of the concentration of elements determined

1. Sampling

Study changes the elemental composition of the samples of biological tissue by chemical fixation. It is very important to make the correct sampling, especially when samples are very small mass (<20 mg).

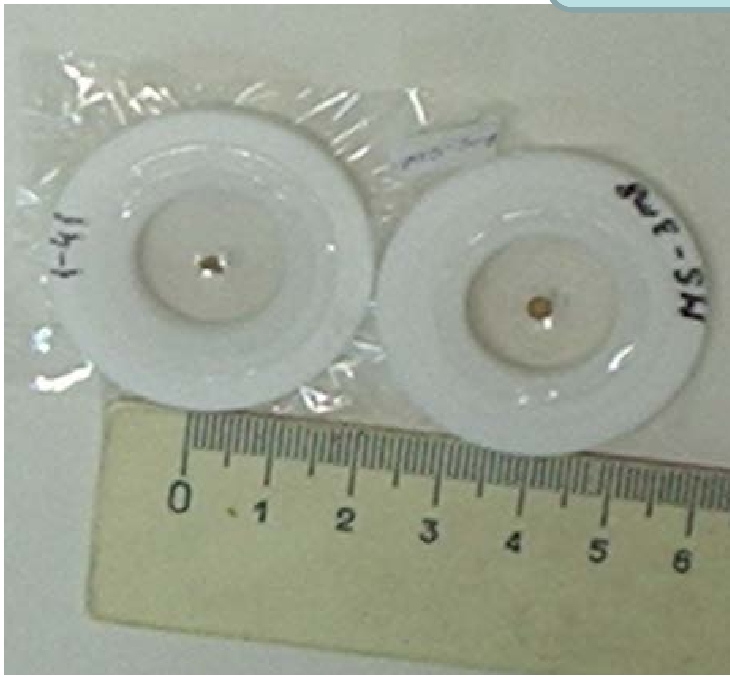
Experimental conditions: fragments of rat heart. Formalin fixation samples (pH = 7**) from 4 hours to 6 days**. Determining elements: Cl, K, Ca, Fe, Cu, Zn, Se, Br, Rb, Sr.





Content of element (K, Zn) fall over time. The result of the experiment was shown, what it is advisable to use when sampling is freezing the samples to obtain the most accurate picture of the elemental composition of the tissue.

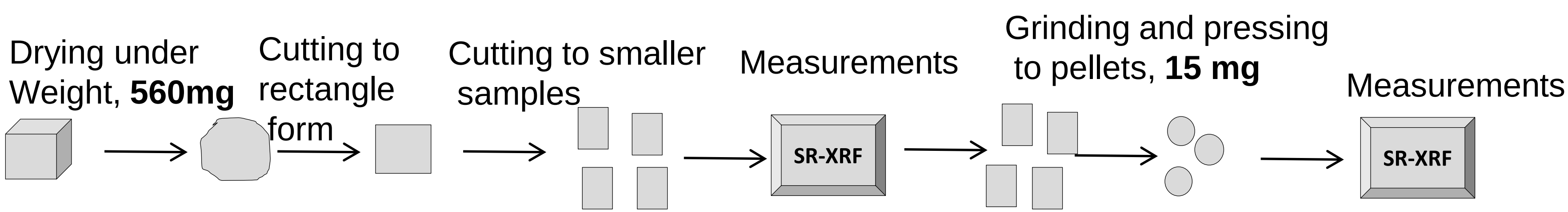
2. Sample preparation



You see the finished sample 2: the standard and the test sample. We must be sure that the tablet is about 3 mg of the standard sample and dry film (3 mg) are identical. **Experiment on identity**: experimental sample weight**-560** mg. Drying time **- 14** days from the mass of dry samples **15** mg to **1** mg.

Sample preparation thin fragment of tissue (wet tissue), drying **without heating** (within a day), while under pressure by **slow drying**.

Packaging samples Mylar film, after step sample preparation **- dry tissue - from 0.5 to 15 mg.**

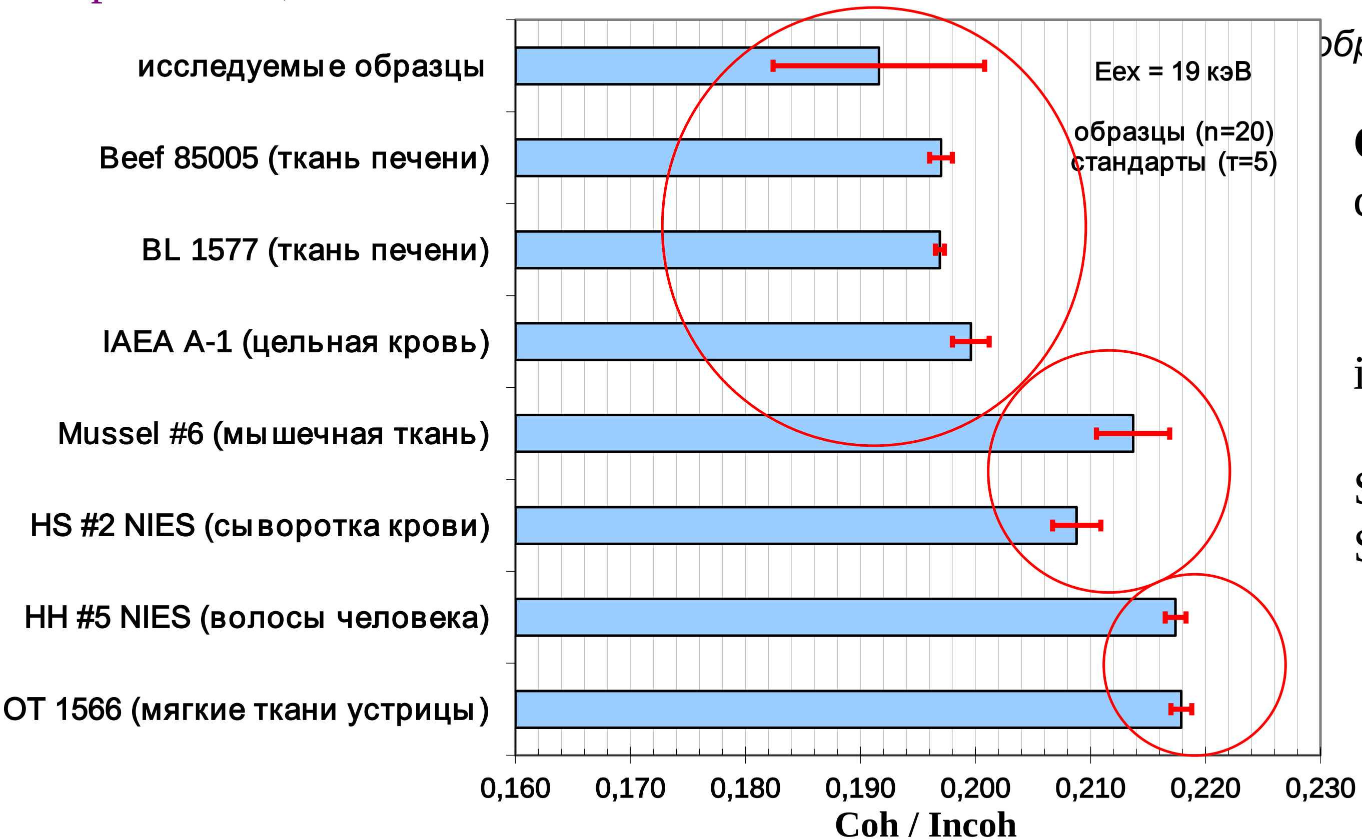


This method of sample preparation may be the **only available**, if necessary, quantitative analysis of samples of tissues of small mass, including biopsy material, where the weight of the unit mg.

3. Selection of reference samples

Specimen: 1- infarct zone, 2-the zone of periphery of infarct, 3-myocardium of left ventricle, 4-myocardium of right ventricle, 5-myocardium of left auricle, 6-myocardium of right auricle

Samples **n=20**, Standarts **n= 7**.



бразцы) = 5%.

Coh - peak area of coherent scattering,

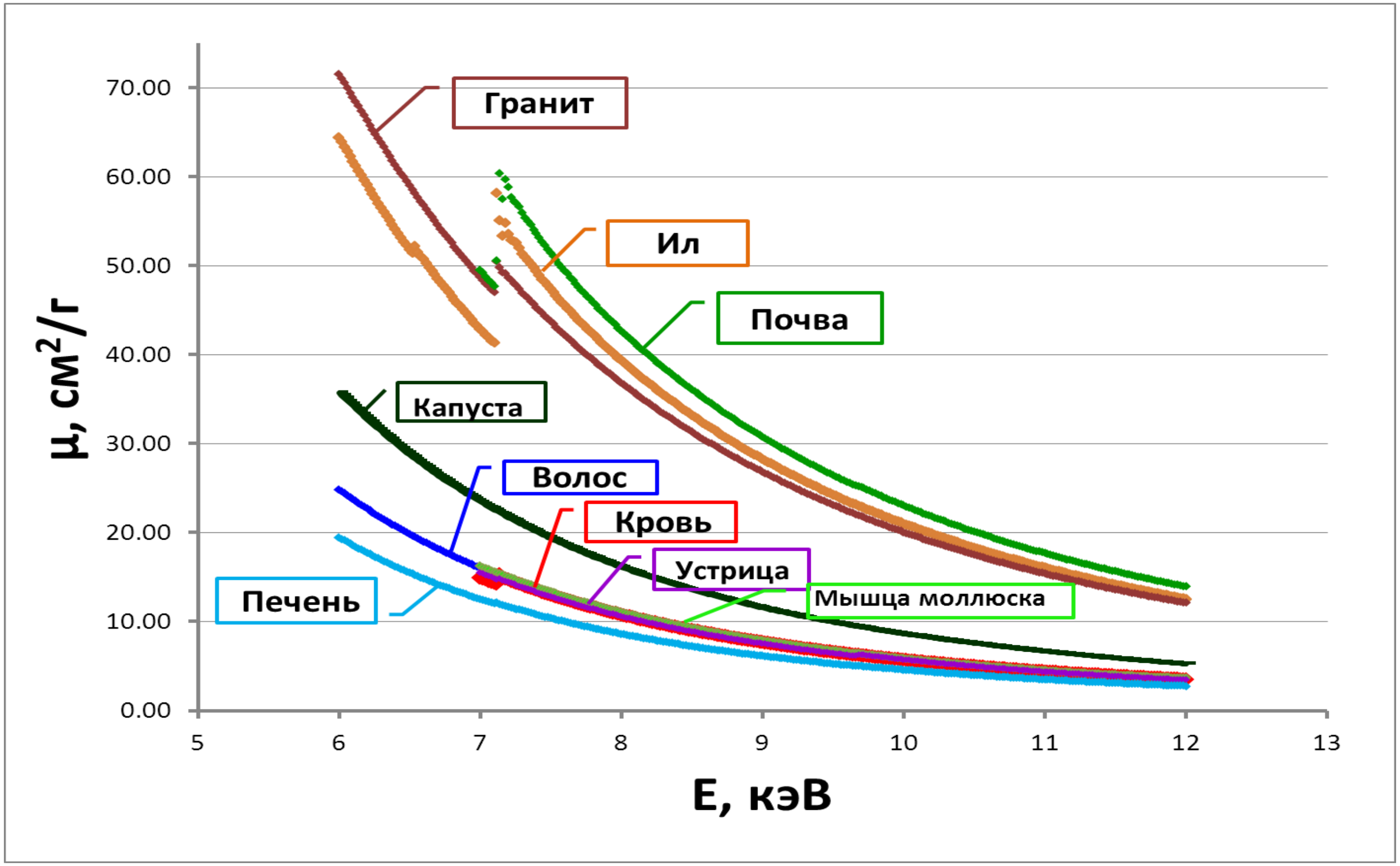
Inoh - peak area of incoherent scattering.

Sr (stand.) = 0.2 – 1.5%
Sr (samp.) = 5%

The value Coh / Incoh varies slightly (Sr = 5%) for the study of muscle tissue, and reference materials.

That confirms in this case, the validity of the use of different standards with a matrix different from the sample matrix.

Mass attenuation coefficients of samples with biological and geological matrices



Geological standards:
granite
silt
soil

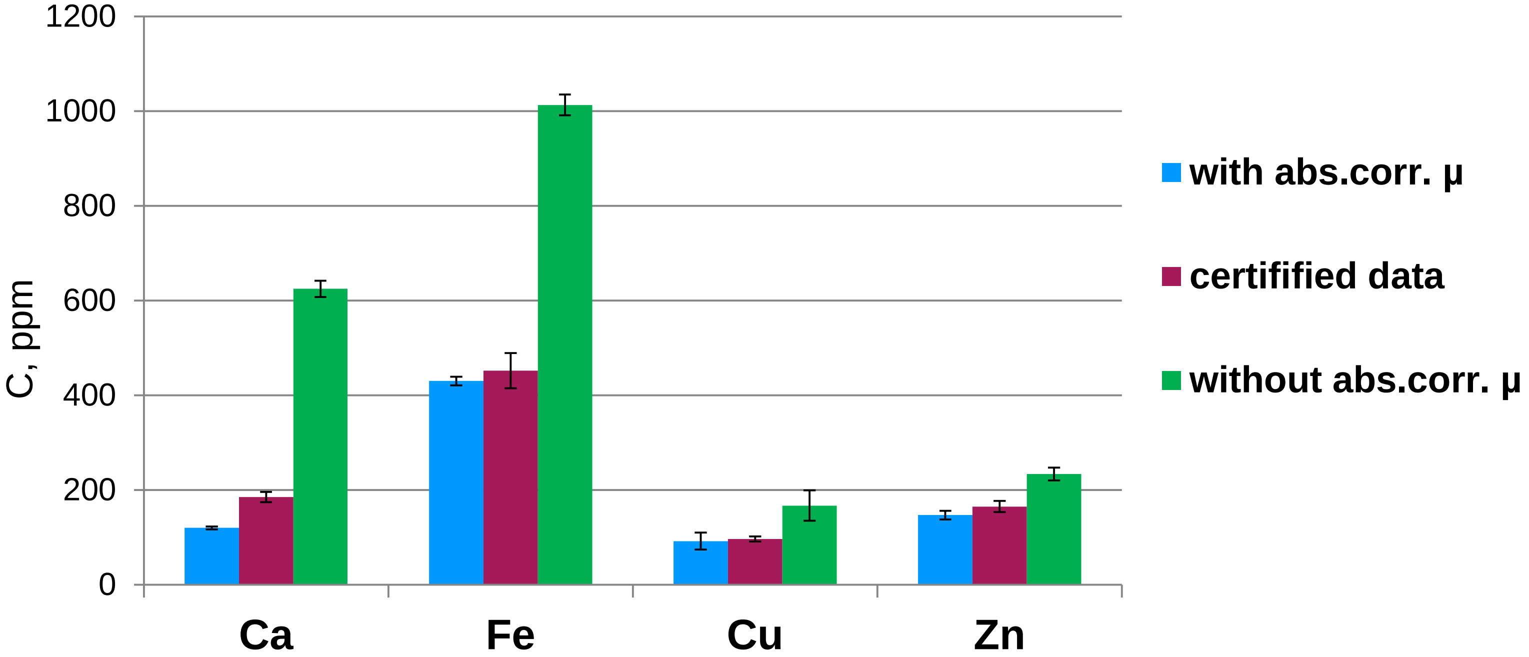
Biological standards:
cabbage
hair
blood
liver
oyster
mollusca muscle

Curves mass attenuation coefficients of samples with biological and geological matrices were measured and plotted.

For mollusk muscle tissue standards and serum, despite the fact that their biomatrix different in nature, there is no significant difference for both the scattering characteristics, and the relative sensitivity coefficients spectrometer. While the concentrations of many elements differ by an order!

Calculation of concentrations (ppm ± SD) K, Ca, Mn, Fe, Co, Cu, Zn, Br, Rb, Sr, Mo sample in NCS ZC 85005 Beef liver- beef liver (0.0196 g / cm2) of the sample relative to the standard IAEA Soil- 7 - the soil (0.0508 g / cm2)

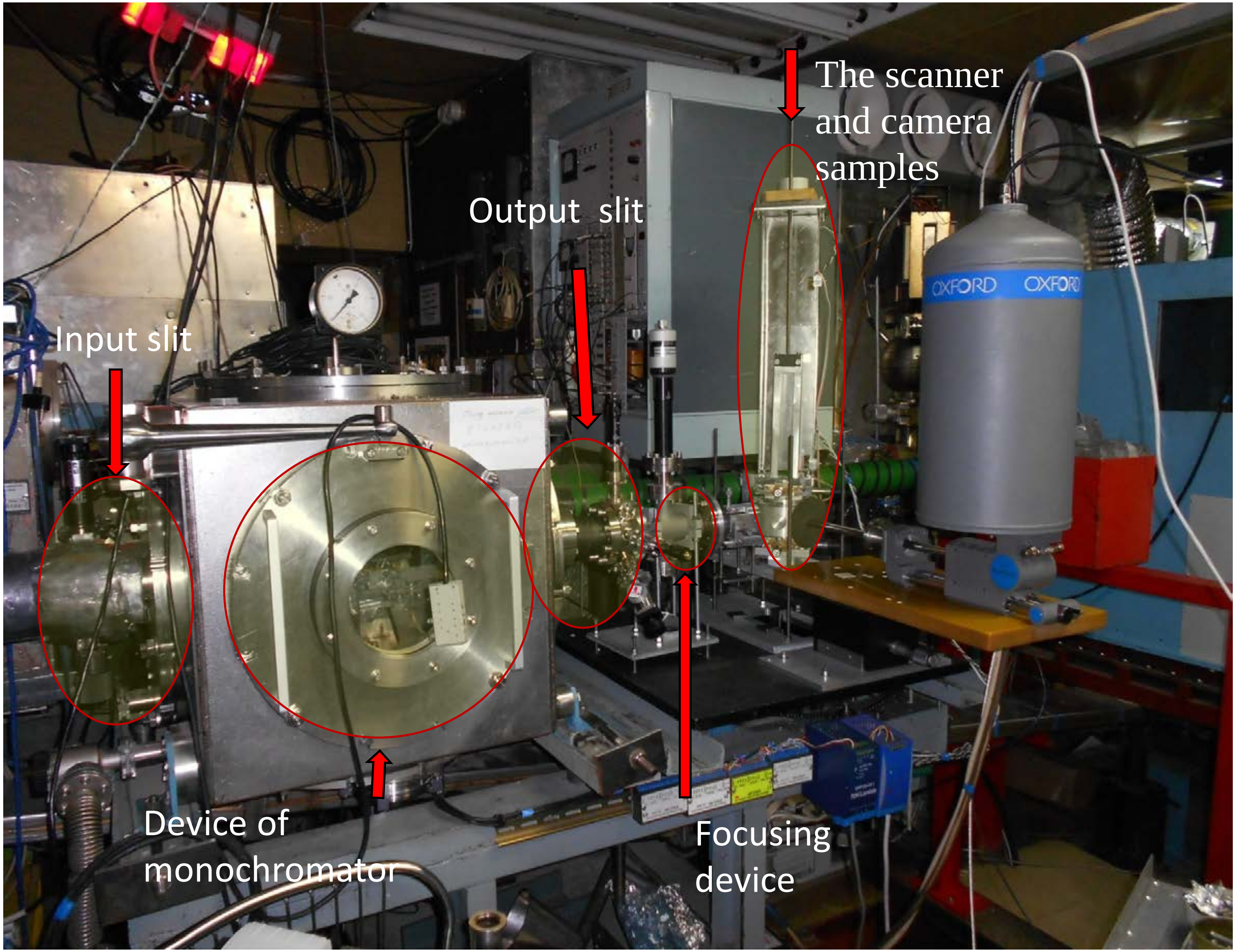
with and without correction for absorption (μ).



When the difference between the mass attenuation coefficients of the analyzed sample and a standard in 6 times vary is possible to obtain correct results of the analysis adjusted for absorption and peak normalization on the Compton scattering.

Trunova V.A., Sidorina A.V., Zolotarev K.V. Using external standard method with absorption correction in SRXRF analysis of biological tissues // **X-Ray Spectrom. – 2015.** – V. 44, N. 4. – P. 226-229.

Experimental Station SRXRF analysis BINP SB RAS



<u>VEPP-3</u>

E_{ex} = 2 GeV, B =2T, I_e=100 mA;

<u>Station</u>

Monochromator

Si (111) ;

Square beam 2×5 mm^2 ;

The excitation energy

8 - 42 keV

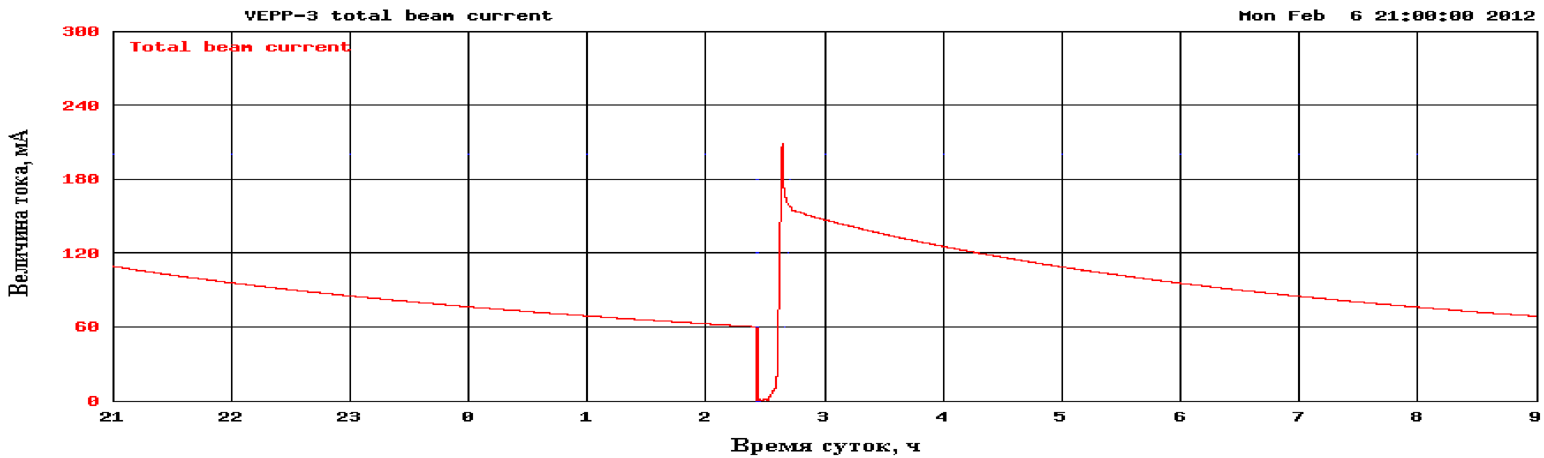
Determined elements: from S to U

Si(Li) detector (OXFORD, 10 mm^2),

energy resolution (at 5.9 keV - 135 eV)

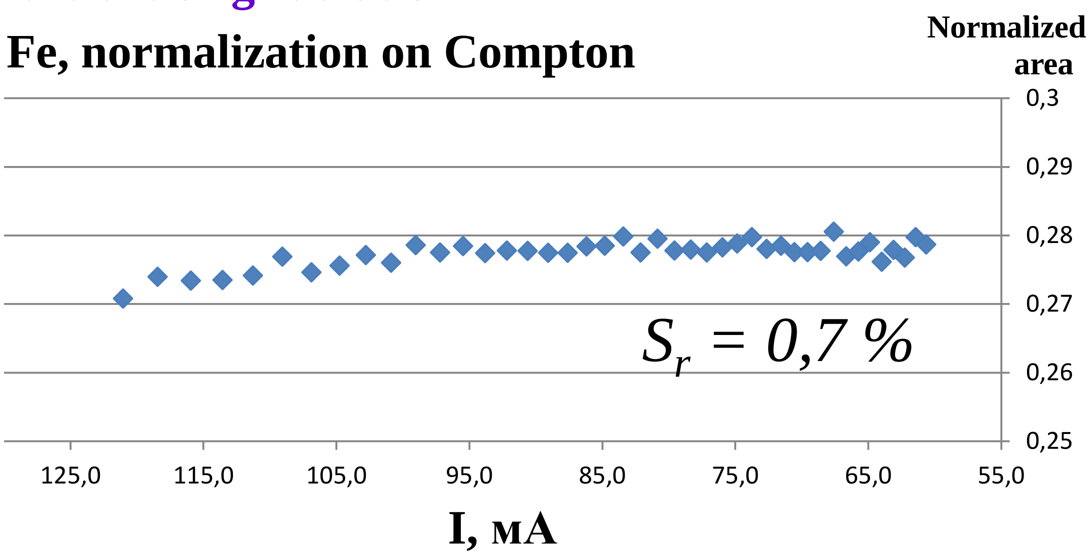
4. Measurements of the spectra SRXRF

The fall of the current storage ring with time
(≈ 1 mA for elemental analysis VEPP-3 during one measuring station).



Needed normalization of the measured spectra to account for changes in the intensity of the exciting radiation

Fe, normalization on Compton



Peak area Kα line Fe with normalization on the peak area of Compton scattering at current values 120-60 mA 43 measurement for standard sample

The metrological characteristics of the method were identified: reproducibility, detection limit and accuracy.

The reproducibility of the results of SRXRF analysis and uniform distribution of the chemical elements in samples of myocardium

Reproducibility (S_{r1}%) SR-XRF analysis of myocardial tissue sample.

The degree of inhomogeneity of distribution of chemical elements in the sample (S_{r2}%).

Элемент	S_{r1} % (n=8)	S_{r2} % (n=8)
K	1.0	6
Ca	1.3	10
Cr	4	17
Mn	17	29
Fe	0.8	5
Co	11	25
Ni	20	27
Cu	2.3	5
Zn	0.6	6
As	26	78

The detection limits (C_{min}):

$$C_{min} = 3.29 \cdot C_{st} \cdot \frac{\sqrt{N_{bgr}}}{N_p - N_{bgr}}$$

P=0.95

Np - the integrated peak area (Ka-line) defines the elements,
Nbgr - background area,
Cst – concentration determined element in the standard sample.

C_{min}, мкг/г		
	K	Ca
13 кэВ		
Mussel NIES №6	14.2	7.2
Bovine liver NIST 1577	8.9	4.1
18 кэВ		
Mussel NIES №6	11.7	5.0
Bovine liver NIST 1577	8.8	3.2
23 кэВ		
Mussel NIES №6	17.1	8.2
Bovine liver NIST 1577	11.6	6.8

VALIDATION ACCURACY OF THE RESULTS OF SRXRF ANALYSIS OF TISSUES SAMPLES (m = 8-10 mg) by t-criterion.

Element	SRXRF, ppm, liver	АЭС-ДДП, ppm, liver
K	5400 ± 600	-
Ca	91 ± 21	86 ± 7
Mn	5.4 ± 0.9	6.8 ± 0.5
Fe	1290 ± 230	1050 ± 90
Cu	14.4 ± 2.7	11.0 ± 0.9
Zn	98 ± 18	106 ± 11
Se	5.0 ± 0.9	-
Br	30 ± 3	-
Rb	30 ± 5	-
Sr	0.090 ± 0.018	0.110 ± 0.012

Comparison of test results a liver sample
2 methods: **SRXRF**,
two-jet arc plasma atomic-emission spectrometry
(**TJAP-AES**)

элемент	образец 1		образец 2	
	миокард левого желудочка сердца (ишемия)			
	C, мкг/г (сухой вес)			
	РФА-СИ	ИСП-АЭС	РФА-СИ	ИСП-АЭС
	доверит. интервал (*P* = 0,95)			
S	2800 – 5100		1400 – 2400	
Cl	780 – 1200		350 – 550	
K	1000 – 1600	800 – 920	550 – 850	660 – 820
Ca	620 – 980	1100 – 1300	390 – 610	1100 – 1300
Cr	0.3 – 0.5		0,1 – 0,3	
Mn	1,1 – 1,8	1,4 – 1,6	1,4 – 2,2	1,5 – 1,7
Fe	120 – 160	140 – 200	170 – 230	220 – 280
Ni	0,8 – 1,2		1,1 – 1,9	
Cu	6,9 – 9,1	8,0 – 10	6,0 – 8,0	9,0 – 11
Zn	1200 – 1400	1600 – 1800	1900 – 2200	2000 – 2200
Se	0,33 – 0,49		0,55 – 0,85	
Br	7,9 – 10		9,6 – 12	
Rb	1,4 – 1,7		1,4 – 1,8	
Sr	1,3 – 2,1	2,5 – 3,5	1,6 – 2,5	1,5 – 2,5

Comparison of test results 2 samples
2 methods: **SRXRF**, inductively coupled plasma atomic-emission spectrometry (**ICP-AES**)

Joint research with Meshalkin Institute of pathology of circulation of the blood

System studies lasted for 9 years, was published in print for more than 30 publications in foreign and domestic journals.

Material:

pathologic – autopsy, biopsy, operating

Advantage of synchrotron radiation:

low-mass analysis of samples,

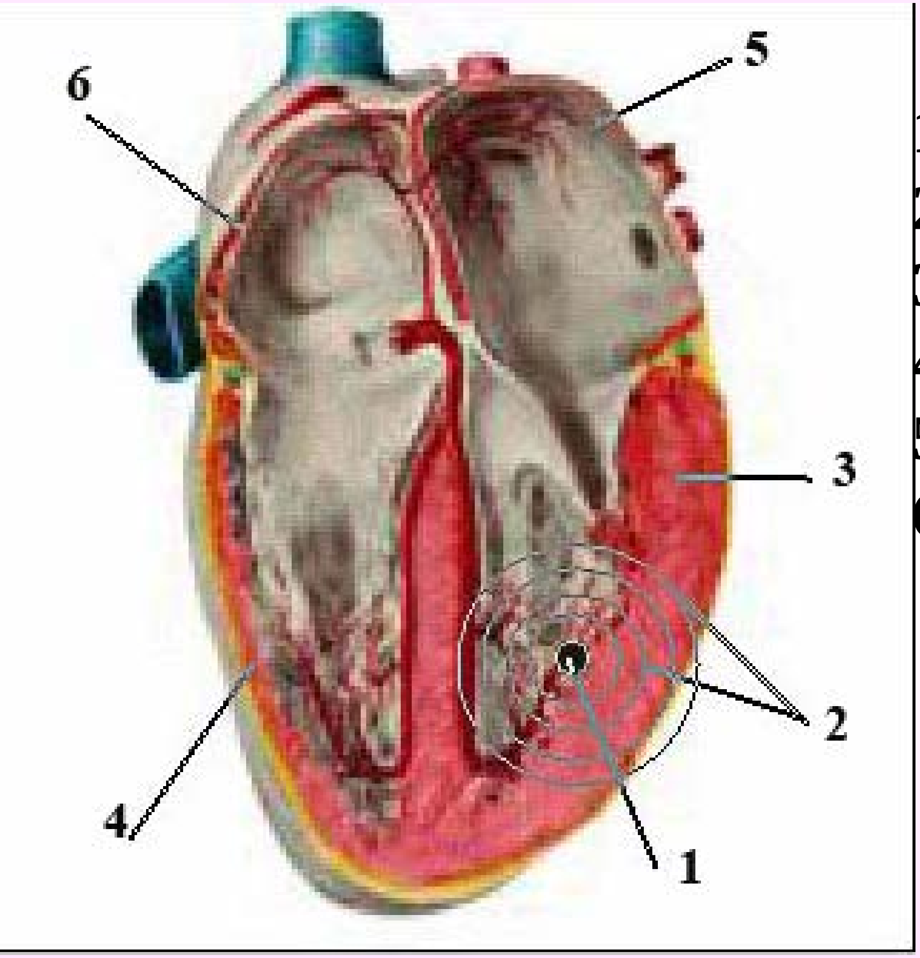
lower detection limits,

measurements with variation of energy of the exciting photons.

Complexity analysis:

1) the amount of material for analysis are limited (difficulty biopsy);
2) direct analysis of biological objects is often not possible:
    a) effect of the matrix,
    b) low concentrations of trace elements in the samples,
    c) the high volatility of some chemical elements (halogens, S, Se, Hg, As);
3) the complexity of using an internal standard (direct analysis);
4) selection of external standard with a matrix array of such sample

The sampling of myocardium tissue



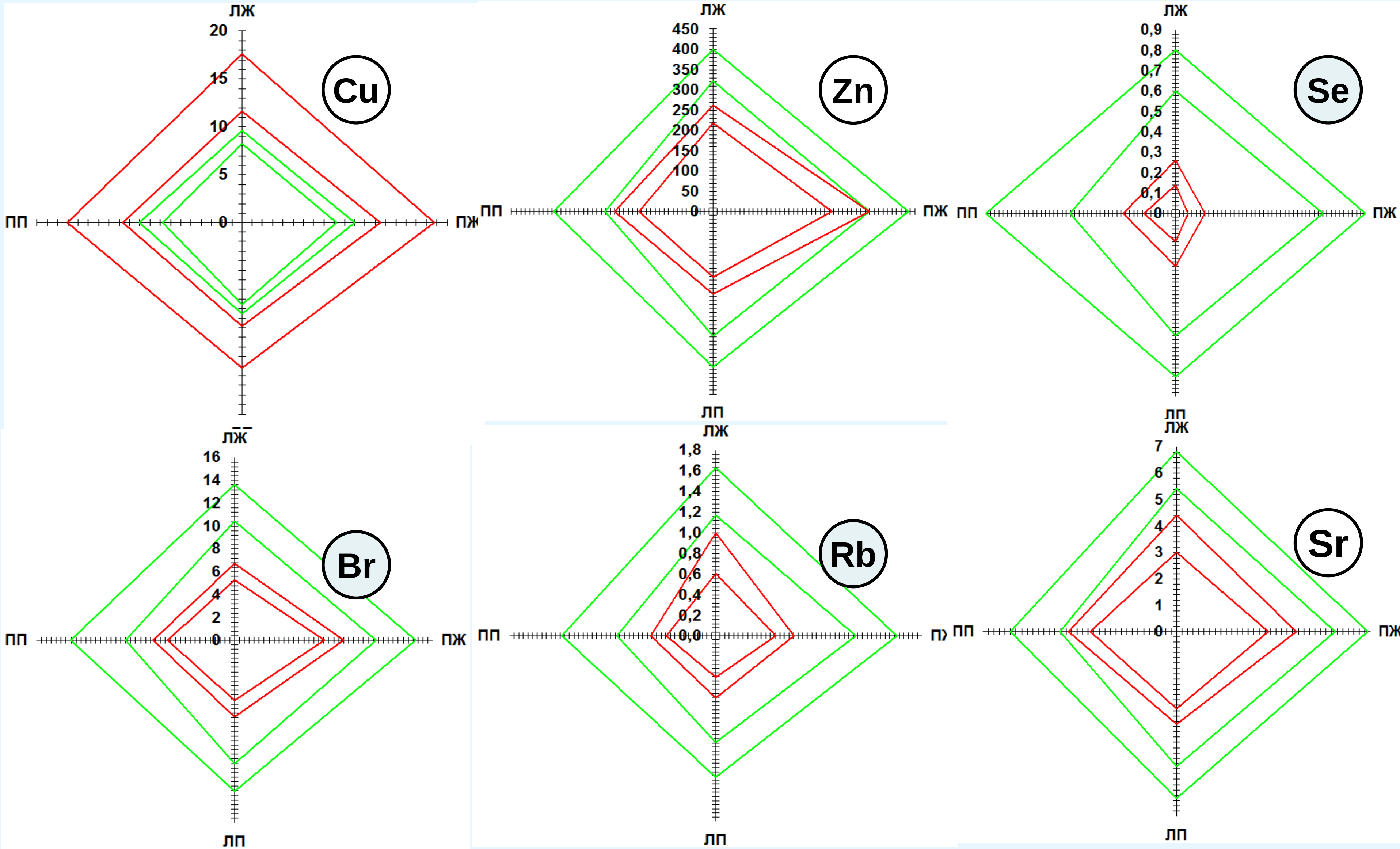
The parts of myocardium, which were investigated:

1- infarct zone
2-the zone of periphery of infarct
3-myocardium of left ventricle
4-myocardium of right ventricle
5-myocardium of left auricle
6-myocardium of right auricle

Different pathology:

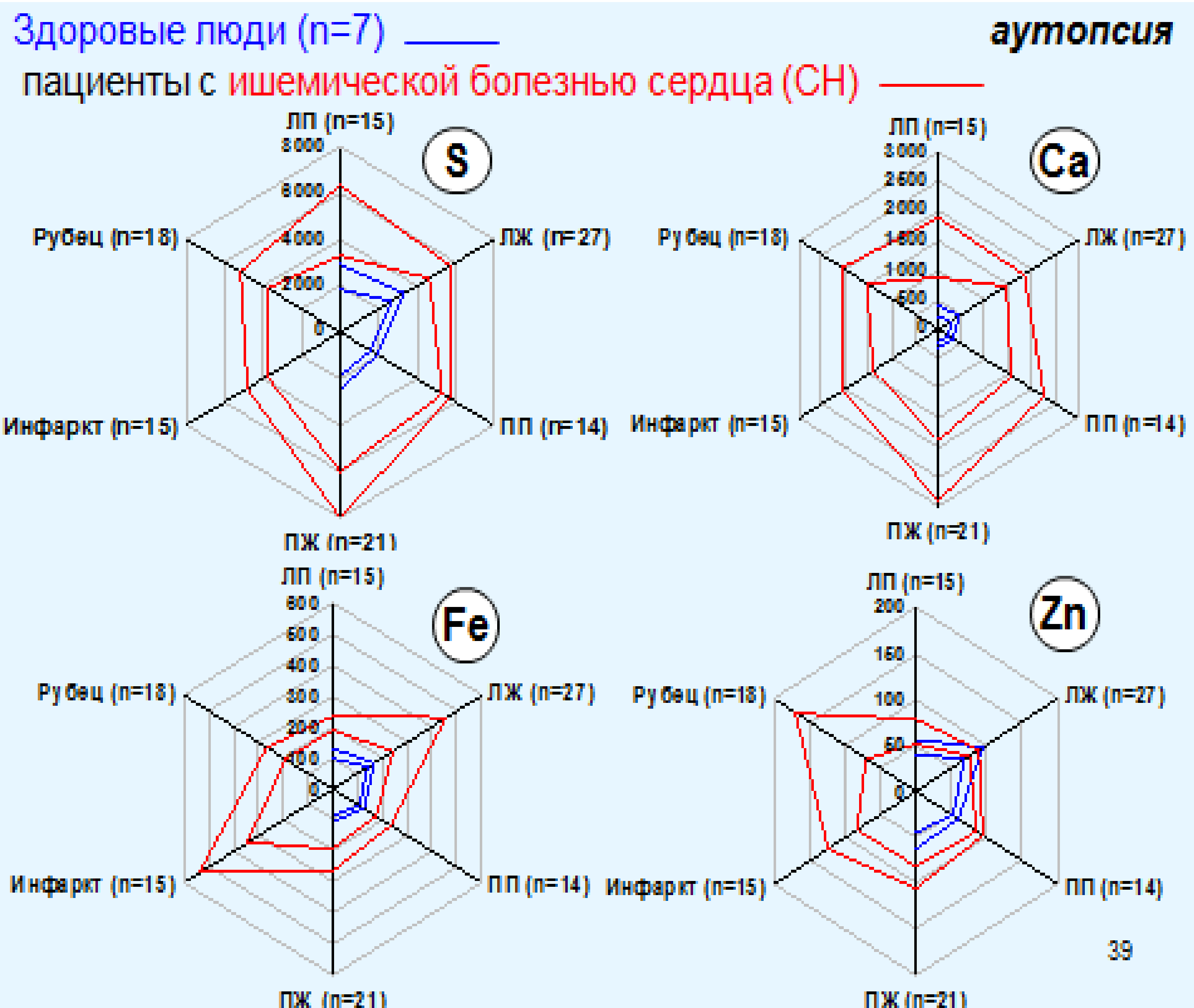
- congenital heart defect,
- acquired heart diseases,
- cardiac ischemia,
- vascular disease (aortic aneurysm, aortic dissection)
- cardiomyopathy **(heart transplantation**).

Healthy children (n = 5) and children with congenital heart disease (TMS) (n = 20)



These diagrams show the different parts of the heart the difference between the content elements in a healthy and pathological myocardium, congenital heart disease. Only Cu increasing the concentration is almost an order of magnitude

Cardiac ischemia, n – 90, Healthy people, n – 7

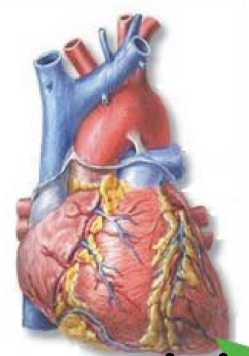


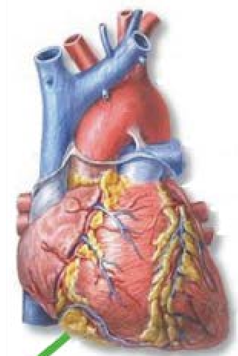
1- infarct zone
2 - myocardial scarring
3- myocardium of left ventricle
4- myocardium of right ventricle
5- myocardium of left auricle
6- myocardium of right auricle

All elements have increased the content of even one order of magnitude in patients with myocardial infarction. Fe and Zn have maxima in the area of infarction and scarring.

Such distribution pattern of trace element concentrations in different parts of the heart at different pathologies physiologists obtained for the first time.

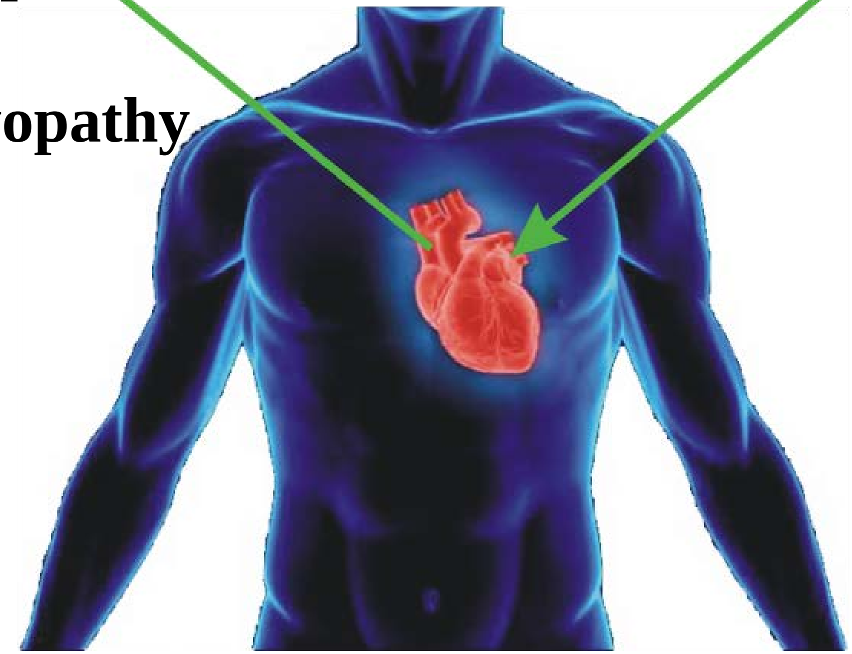
Human
heart transplantation
Studied 60 biopsy samples



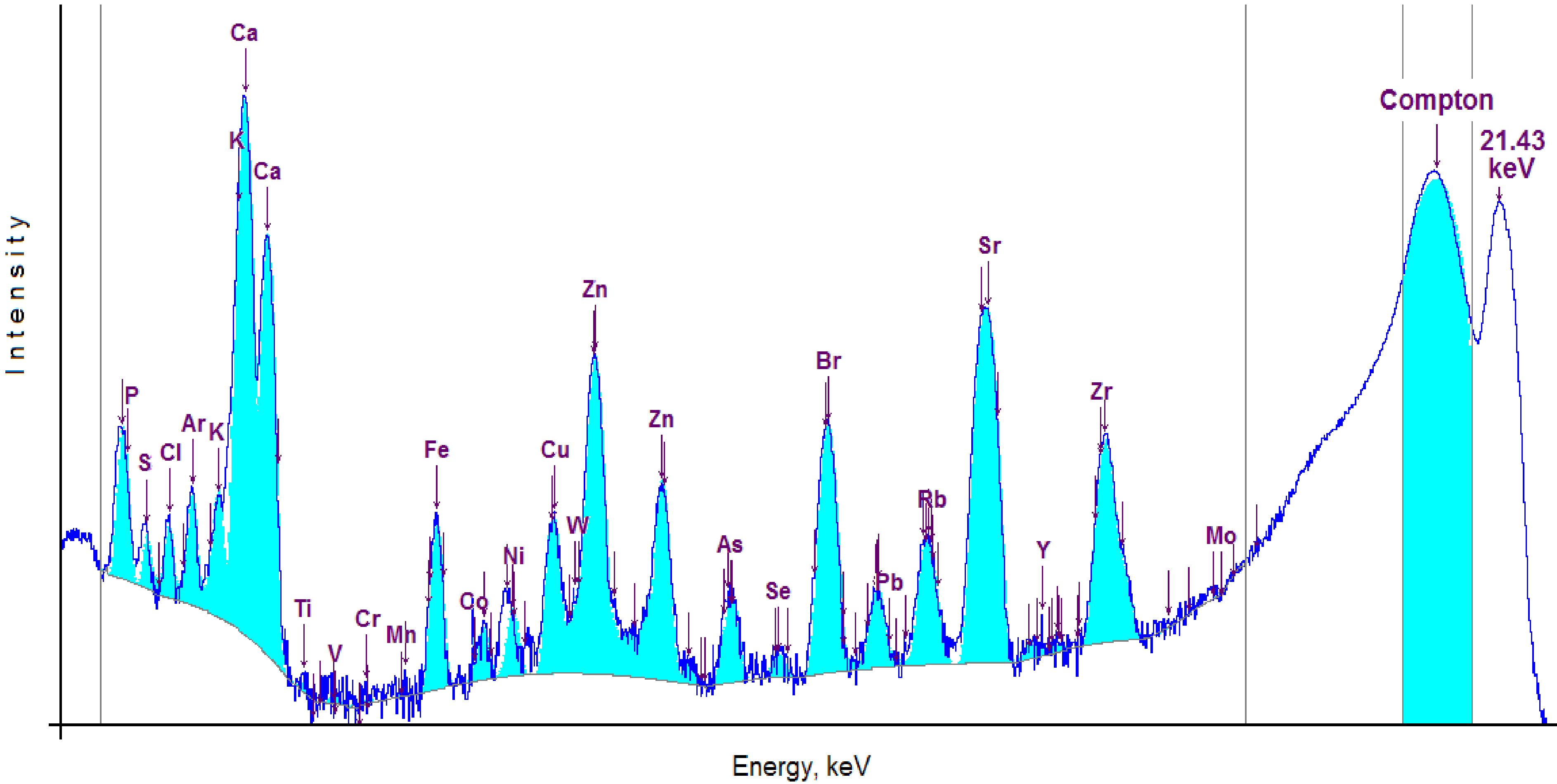


Own heart recipient
(autopsy)
dilated cardiomyopathy

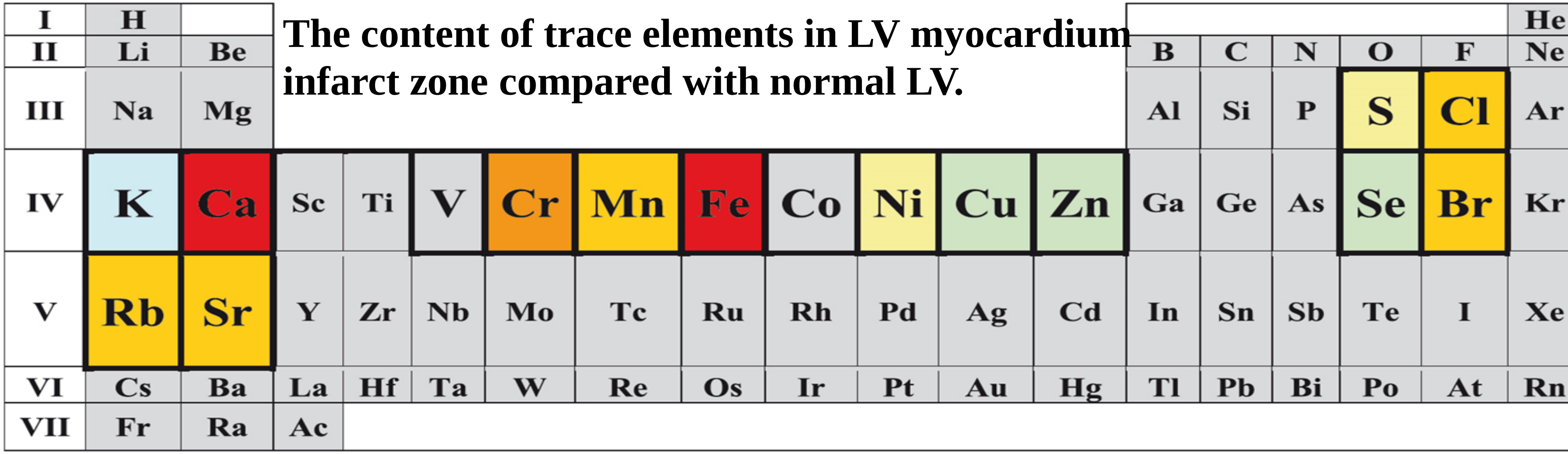
The donor heart
(Biopsy, autopsy)

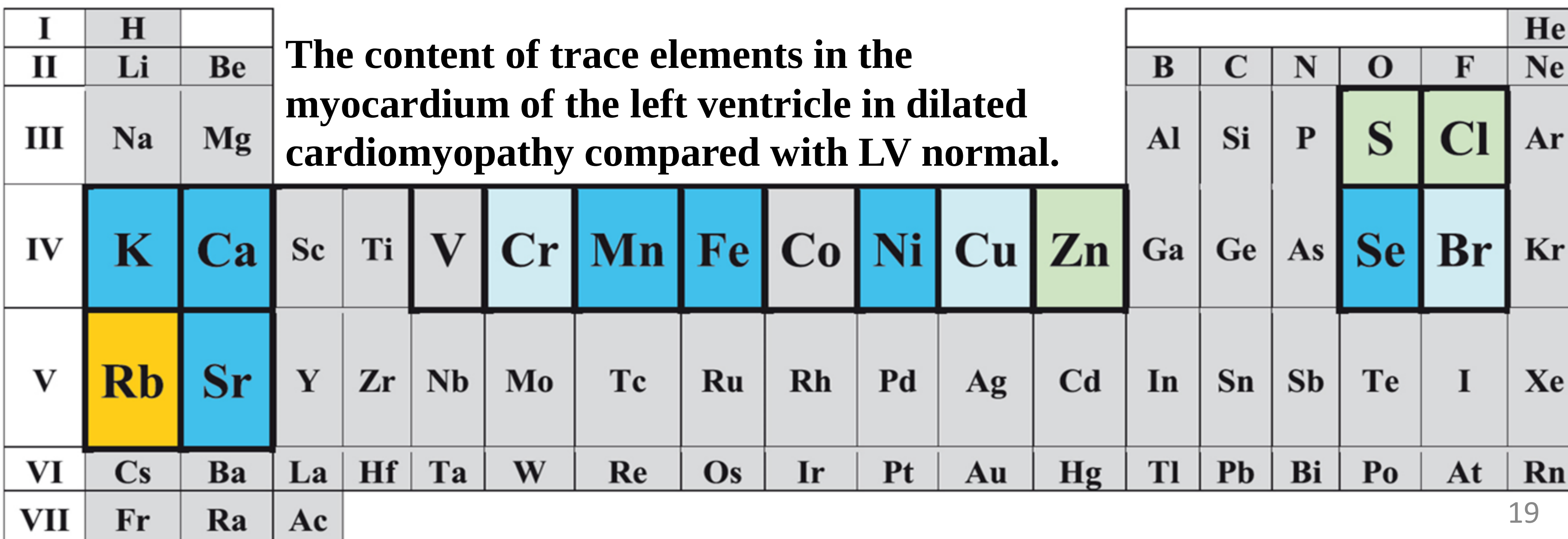


Heart pathology - heart transplantation (biopsy) sample weight of 0.5 mg



- 200% и <	- 150%	- 100%	- 50%	-25% - +25%	+50%	+100%	+150%	+200% и >	не известна концентрация

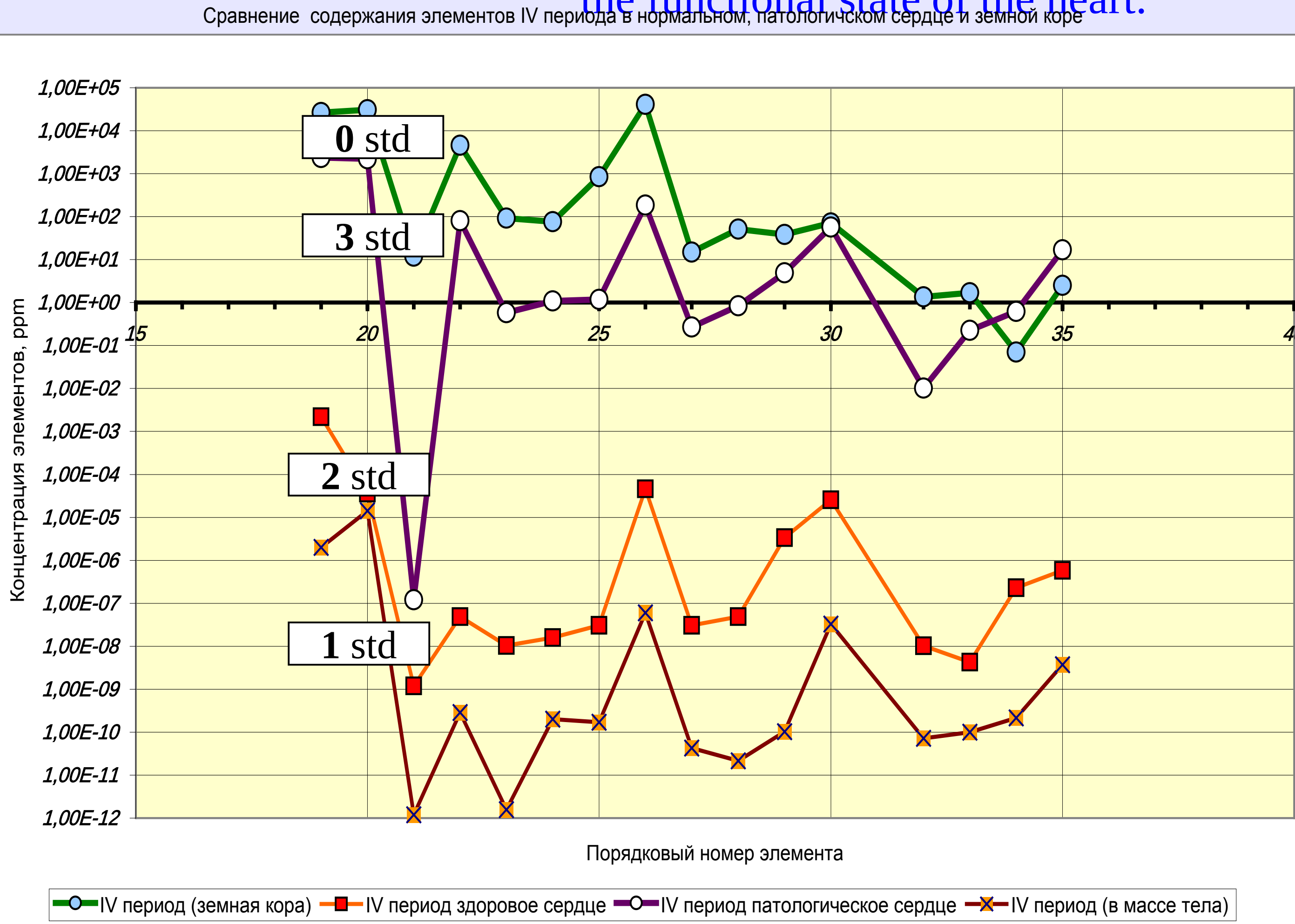




Method of evaluating the functional state of the heart

Patent №2466389

Using data on the distribution of chemical elements in the earth's crust, healthy and diseased heart were calculated the Pearson correlation coefficient, and provides a method of evaluating the functional state of the heart.



Designations

0-std-Earth's crust
1-std- the human body
2-std- normal heart
3-std- cardiac disease
X1- own heart
X2- donor heart biopsy
X3- donor heart autopsy

K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ge, As, Se, Br

R01 between **0Std и 1Std**, **R02** between **0Std и 2Std**, **R03** between **0Std и 3Std.**

Comparison of the Pearson correlation coefficient for the number of elements studied (n=18) by the example of the patient's disease cardiomyopathy (heart transplantation)

- Pearson's correlation coefficient for the **18** elements studied is $\mathbf{R_{03} = 0.144}$ in a patient with a transplanted heart, there is **no correlation.**

- The same pattern is observed for the donor heart and to the heart of the recipient in the biopsy specimens and autopsy: $\mathbf{R_{0X1} = 0.022}$ $\mathbf{R_{0X2} = 0.106}$ $\mathbf{R_{0X3} = 0.020}$
- There is no correlation between a healthy body and heart recipient, between the healthy and the donor heart biopsy: $\mathbf{R_{1X1} = 0.114}$ $\mathbf{R_{2X2} = 0.030}$

- **But there is a correlation between pathological cardiac and remote recipient's heart, the donor heart biopsy and autopsy:** $\mathbf{R_{3X1} = 0.753}$ $\mathbf{R_{3X2} = 0.746}$ $\mathbf{R_{3X3} = 0.935}$
- **And the same is a high correlation between the biopsy and autopsy donor heart:** $\mathbf{R_{X2X3} = 0.925}$

As a result of analysis by SRXRF fragments autopsy and biopsy material physiologists to the following conclusions:

- The absence of the Pearson correlation coefficient in the transplanted donor heart (biopsies) and a healthy heart counted for 18 investigated elements makes it possible to assume that in the donor heart does not exist detected lesions. Perhaps it was one of the reasons for the unsuccessful outcome of transplantation of donor heart.

- **Cu** deficiency can provoke Marfan syndrome, aortic aneurysm formation.
- **Zn** deficiency leads to the development of heart disease.
- **Se** deficiency increases the risk of coronary heart disease, myocardial infarction and cardiomyopathy.

- In the myocardium of patients with ischemic heart disease due to lower content to increased content of CE, especially **Ca, Fe, Rb**.
- A high content of **Fe** in the arterial wall may indicate the early development of atherosclerotic processes.
- Dilated cardiomyopathy (**heart transplantation**) - reduced content of basic CE: K, Ca, Sr, Fe, Ni, Se compared with the normal **2** times. The only possible marker of disease cardiomyopathy is increased to **2** times the content of **Rb**.
- This is a direct non-destructive analysis of biological tissues **SRXRF** (weight less than <4 mg), we developed a methodological approach, which allowed for the first time to get the data presented here.


Thank You for attention