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QUALITATIVE AND QUANTITATIVE ANALYSIS OF ARSENIC AND MERCURY IN HUMAN REMAINS OF THE XVI–XVII CENTURIES FROM THE MOSCOW KREMLIN NECROPOLISES BY NEUTRON ACTIVATION ANALYSIS AT THE **IREN** FACILITY AND THE **IBR-2** REACTOR OF **FLNP JINR**

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The death circumstances of some representatives of the Russian state highest nobility of the 15th – early 17th centuries still cause controversy among historians. This is due to the lack of accurate data in written sources or different interpretations of the information presented in these sources. Studies of burial places of the members of the Royal family of Ivan the Terrible, carried out in 1963–1964*, allowed us to obtain the data about the microelement composition of the remains of people of the Russian Middle Ages for the first time. However, the interpretation of these results, both in the 1960s and today, remains ambiguous, including the foreign historical literature [1].

In the 1990s and at the beginning of the 21st century, with the help of modern research methods, the burials of Russian Grand Duchesses and Tsarinas from the necropolis of the Ascension Cathedral of the Moscow Kremlin were studied. These studies allowed us to expand significantly the database on the microelement composition of bone tissue of people of XV–XVII centuries [2].

The main objective of this study is to check the results obtained previously by other methods of analysis (chemical, X-ray fluorescence) by the method of neutron activation analysis (recognized as primary in international analytical practice [3]). The main detectable elements are arsenic and mercury.

The samples for the study were transferred to the Frank Laboratory of Neutron Physics (FLNP) of the Joint Institute for Nuclear Research (JINR) in February 2017. Information about the names of historical figures, from whose burial places the samples were taken, was reported only after the completion of the study. Sample 1 (Fig. 1) is the rib fragment from the burial of the son of Tsar Ivan the Terrible, Tsarevich Ivan Ivanovich (died in 1581). Sample 2 (Fig. 2) is the rib fragment from the burial place of Prince Mikhail Vasilievich Skopin-Shuisky (died in 1610). Sample 3 (Fig. 3) are elements of hair of the first wife of Tsar Ivan Vasilievich the Terrible — the first Russian Tsarina Anastasia Romanovna (died in 1560).

*Expert information on the materials of the study of remains from the sarcophagus of Ivan the Terrible, his sons — Ivan and Fedor, as well as Skopin-Shuisky: In the Commission of the Ministry of Culture of the USSR on the opening of tombs in the Archangel Cathedral / State Scientific Research Institute of Forensic Medicine of the Ministry of Health of the USSR. Moscow. March 12, 1964 (in Russian).

Fig. 1. Sample 1 before cleaning

Fig. 2. Sample 2 before cleaning

Fig. 3. Sample 3 before cleaning

The samples were studied by neutron activation analysis in February–April 2017. We performed two independent experiments using the basic facilities of FLNP JINR — the IREN research facility [4, 5] and the IBR-2 reactor [6].

Two sets of samples were prepared for the experiments. Working with fragments of the skeleton and the hair required different approaches.

PREPARATION OF THE BONE TISSUE FOR IRRADIATION

Rib bones have a porous internal structure, so the samples required very careful cleaning to prevent the saturation by components of the detergent. Cotton wool soaked in distilled water was used to remove rough surface contaminants

(sand, dust, etc.). Bone surface soaked with water assisted to a partial swelling of contaminations of organic nature (decay). The bone fragments were carefully wiped with cotton wool soaked in a 3% solution of sodium bicarbonate for removal of such contaminations. The bone fragments were washed with large amount of distilled water (about 0.5 l) using a laboratory polypropylene vessel for washing to remove the remains of cleaning solution from the samples surface. The bone was held on weight using stainless steel tweezers. Remains of organic compounds were removed from the surface of the samples using cotton wool soaked in 96% ethyl alcohol. All operations for removal of contaminants were performed by repeated wiping of the samples using pieces of cotton wool, soaked with the appropriate cleaning solutions, until the termination of the wool contamination.

Since the porous ends of the bones could absorb a certain amount of cleaning solutions and the removed particles of contaminations, small sections of the ends of the ribs were removed after cleaning. Figs. 4 and 5 show the image of the bone tissue after cleaning.

Samples of bone tissue were dried in a drying oven during the day at temperature of 40 °C. This provided evaporation of maximum possible water quantity.

Grinding the samples to a powder was performed using a planetary mill, equipped with agate cup and balls. The grinded samples were dried for 72 h at temperature of 40 °C to constant weight.

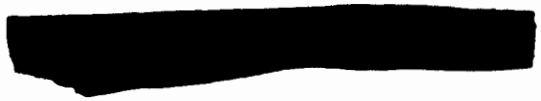

Fig. 4. Cleaned sample 1

Fig. 5. Cleaned sample 2

PREPARATION OF HAIR FRAGMENTS FOR IRRADIATION

To remove surface contaminations, the fragments of the hair (about 3.5 g) were divided into three parts. Each part was soaked three times in 100 ml of acetone during 10 min, every two minutes the content of the cup was gently mixed. Cleaned hair was placed between two sheets of filter paper and was kept for 24 h at room temperature according to the method used in [7].

To carry out the study, the standard materials from the National Institute of Standards and Technology (USA) [8] have been irradiated together with the samples. Masses of the standards (7 pieces) and samples for irradiation at the IREN research facility were approximately 1 g. Weighed samples were packed in plastic bags. Each packed sample was placed in an individual plastic container. Masses of the standards (10 pieces) and samples for irradiation at the IBR-2 reactor were about 0.1 g. Weighed samples were packed in aluminum capsules. All capsules were placed in one aluminum container.

The hardware-software tool for automation of samples weight registration [9], created at FLNP JINR, was used for weighing.

IRRADIATION

The first set of samples and standards was irradiated at the IREN facility in March 2017 for 115.5 h. The IREN installation worked in the following mode: the maximum energy of electrons was 55 MeV, the average current was 2.4 μA. The flux densities of the thermal and resonance neutrons at the facility were determined by the cadmium difference method. Copper samples served as indicators. The flux density of the thermal neutrons was about $\Phi_{\text{th}} = 6.0 \cdot 10^7$ cm$^{-2} \cdot$ s^{-1}, the flux density of the resonance neutrons at 1 eV was $7.6 \cdot 10^6$ cm$^{-2} \cdot$ s^{-1}.

The second set of samples and standards was irradiated in the third channel of the IBR-2 reactor. The installation for irradiation is described in [10]. The total irradiation time was about 15 days. The flux density of the thermal neutrons was about of $5.2 \cdot 10^{11}$ cm$^{-2} \cdot$ s^{-1}, $\Phi_{\text{res}\,1} = 7.5 \cdot 10^{10}$ cm$^{-2} \cdot$ s^{-1}.

DATA ACQUISITION AND ANALYSIS

Gamma spectra of induced activity of samples were measured twice after irradiation using the automation system for measurement of spectra (Fig. 6) developed at FLNP JINR. This automation system includes a high-purity germanium detector with high resolution produced by Canberra, sample changer, and software [11, 12]. The energy resolution of the detector is 1.8 keV for the 1173 keV line of ^{60}Co, relative efficiency is 40%.

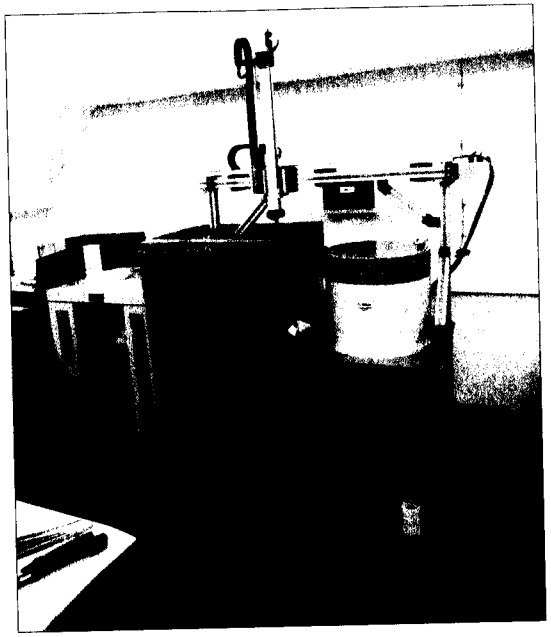

Fig. 6. Automation system for measurement of spectra

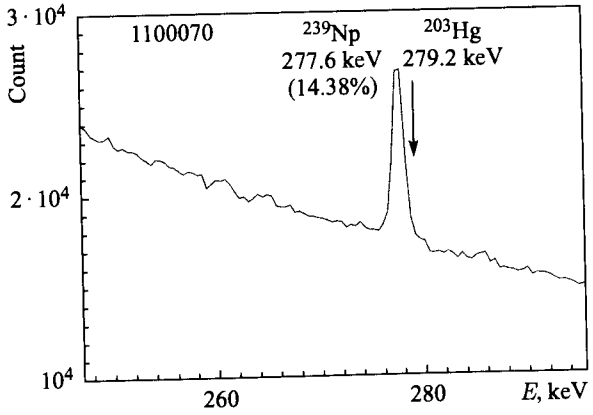

Fig. 7. The spectrum fragment of sample 1 irradiated at the IBR-2 reactor (the first measurement)

The first measurement (Fig. 7) was carried out a few hours after the end of irradiation at the IREN facility and several days after irradiation of the samples at the IBR-2 reactor (taking into account the radiation situation). The second

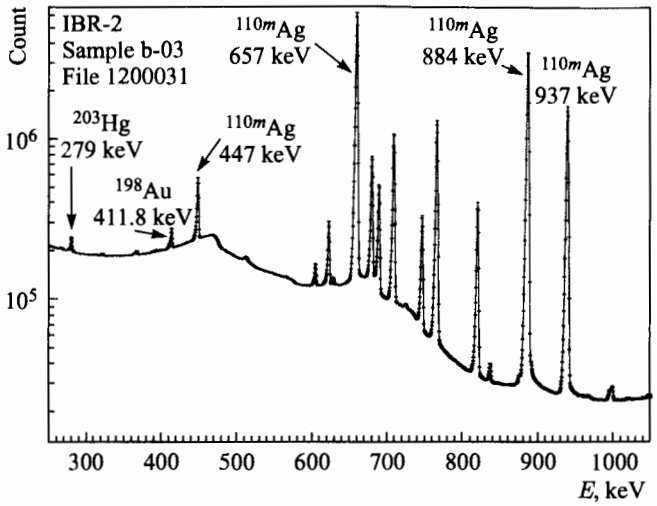

Fig. 8. The spectrum fragment of sample 3 irradiated at the IBR-2 reactor (the second measurement)

measurement (Fig. 8) was carried out about three weeks after the end of irradiation. The Genie-2000 software was used for measurements and analysis of the spectra.

Calculations of mass fraction of elements were made by two methods: the relative (using the standards, the elemental composition of which is well-known and certified) and the absolute one (based on the equation of activation) [13]. The Concentration software, created at FLNP JINR [14], was used to calculate the mass fraction of elements by relative method. Nuclear constants from [15,16] were applied. The results of the calculations are summarized in Table 1. The results of determination of mass fraction of additional elements are presented in Table 2.

Table 1. Mass fraction of arsenic and mercury in the samples

Sample number	Arsenic (As)		Mercury (Hg)	
	Mass fraction, mg/kg	Relative uncertainty, %	Mass fraction, mg/kg	Relative uncertainty, %
1	0.19	30	0.36	19.1
2	0.23	30	0.2	29.5
3	1.18	18.3	46.6	2.5

Table 2. Mass fraction of some additional elements in the samples

Sample number	Iron (Fe)		Zinc (Zn)		Silver (Ag)		Antimony (Sb)		Gold (Au)	
	Mass fraction, mg/kg	Relative uncert., %	Mass fraction, mg/kg	Relative uncert., %	Mass fraction, mg/kg	Relative uncert., %	Mass fraction, mg/kg	Relative uncert., %	Mass fraction, mg/kg	Relative uncert., %
1	1210	8	624	4	0.131	15	0.21	24	$7.5\cdot10^{-5}$	19
2	1373	7.3	460	4	4.8	10	<0.1	—	$1.3\cdot10^{-2}$	30
3	<1170	—	<100	—	3460	14	2.72	16	6.4	11

ANALYSIS OF THE RESULTS

As a result of the qualitative analysis, the presence of mercury and arsenic was established in all fragments of remains from burial places of the Moscow Kremlin.

Quantitative analysis of the presence of mercury fully confirmed the acute poisoning of the first Russian Tsarina Anastasia, as the mercury content in her hair was extremely high. The young woman (she died at the age of about 27) could not accumulate such quantity of harmful substance using medieval cosmetics and medicines. The Tsarina became a victim of intrigues of the court nobility, who tried to remove representatives of the Zakharins–Koshkins family (ancestors of the Romanovs) from the royal encirclement [17].

The increased content of mercury was detected in the bone remains of Tsarevich Ivan and Prince Mikhail, that correlates with the results of chemical analysis of materials from their burials in 1964* (mass fractions of arsenic and mercury in the hair and rib bones of contemporary people from [18] are given in Table 3). However, the reasons of the admission of mercury into the organisms of these young people were different, as well as the reasons of their death.

Table 3. Mass fraction of arsenic and mercury in the scalp hair and rib bones of modern humans

Sample type	Mass fraction, mg/kg	
	Arsenic (As)	Mercury (Hg)
Hair	$\leqslant 0.05$	0.145 ± 0.009
Rib bones	<0.1	$\leqslant 0.008$

*See note p. 1.

For many years Tsarevich Ivan was treated with mercury ointments from venereal disease (syphilis) [19]. Therefore, by the age of 27 (he died at this age from a craniocerebral trauma) he developed the chronic poisoning of the organism with this harmful element.

Prince Mikhail Skopin-Shuisky died suddenly at the age of about 23. The death of a young healthy man and well-trained warrior was explained as poisoning by his contemporaries. Signs of poisoning were showed up suddenly at the feast and indicate an acute poisoning in the gastrointestinal form (in this case, it was possible using the combined composition of the poison — mercury and arsenic).

The obtained results of neutron activation analysis give the opportunity to introduce into scientific circulation more accurate values of the content of mercury and arsenic in samples from the burial places of Russian historical figures of the second half of the 16th – early 17th century.

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