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ACTIVITY CONCENTRATION OF URANIUM, THORIUM AND POTASSIUM IN THE URINE OF ROE DEER (*C. CAPREOLUS*) IN VOJVODINA (NORTHERN PROVINCE OF SERBIA)

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Abstract. *The naturally occurring radionuclides are the major source of radiation to which humans are exposed to. A total of 21 samples of roe deer (Capreolus capreolus) from different locations in Vojvodina were tested. The samples were prepared for measuring by microwave digestion. The content of potassium in the samples was determined using atomic absorption spectrometry (AAS) The total content of uranium and thorium were determined using inductively coupled plasma mass spectrometry (ICP-MS). The concentration of the activity of the tested radionuclides was calculated based on their specific activities. It can also be concluded that potassium-40 is the predominant natural radionuclide in urine as compared to other radionuclides and it moved in the range of 128-381 Bq/L. The results of urine analysis showed wide ranges in radionuclide activity concentrations: 18.4-231.9 mBq/L for ^{238}U, 0.94-11.90 mBq/L for ^{235}U and 1.03-89.02 mBq/L for ^{232}Th.*

Key words: *ICP-MS, potassium, thorium, uranium, urine*

1. INTRODUCTION

Peacetime uses of nuclear energy (nuclear weapons testing, nuclear reactor accidents, industrial and medical use of radioactive compounds) and the application of phosphate mineral fertilizers in agricultural production lead to substantial environmental contamination. Land contaminated with radionuclides represents the first link in the food chain hence the radioactive contamination of crop and livestock production. The largest source of radiation activity in the biosphere is the natural radionuclide potassium-40 [1]. Some other natural radionuclides, which have always been present on Earth, also include radioactive elements such as ^{232}Th, ^{235}U and ^{238}U. The exposure to low-level radiation originating from these natural elements has always been affecting all living beings on Earth, This is considered to be background radiation or natural phon. Radioactive radiation can cause cancerous illnesses and other changes in the health of humans and the whole living world in general, thus substantially influencing the changes in the environment.

The "technologically enhanced naturally occurring radioactivity" is attributed to uranium, which is brought to the environment through diverse technological procedures and agro technical measures [2]. Technological development resulted in substantial increase of natural soil radioactivity, predominantly through intensive application of agro technical measures based on the use of artificial phosphate fertilizers that contain substantial amounts of natural uranium. Moreover, mining of ore containing heavy metals (zinc, copper, led) from deep layers of lithosphere and processing thereof, as well as the thermal power stations producing huge amounts of solid waste (ash, cinder) that contain natural radionuclides such as uranium, thorium and their prodigy [3], increase the levels of soil radioactivity. Uncontrolled application of phosphate fertilizers implicates substantial potential for undermining the ecological balance as they represent the most powerful source of ^{238}U and ^{232}Th content in the soil, and hence in plants and other links within the food chain. In a superphosphate, uranium is deposited as highly water-soluble uranyl sulphate – UO2(SO4) and urano-sulphate – U(SO4) [4]. In addition, there is a generally accepted public belief that during the 1999 bombing, the area of Vojvodina was polluted with depleted uranium. All of the above factors point to the great current concern and the importance of considering the state of the radioactivity of the environment in Vojvodina [5]. As opposed to other radioisotopes, radioactive decay of ^{238}U and ^{232}Th results in formation of the series of u nstable nuclei, ^{226}Ra and ^{222}Rn being the most dangerous members of uranium chain. ^{226}Ra is a long half-life α-emitter, manifesting affinity for accumulating in bones, while gaseous 222Rn is responsible for internal irradiation of lung tissues. Emissions of those radionuclides into the environment represent potential significant risk factors for the exposure of local inhabitants to ionizing radiation, as well as for increased levels of natural radiation in particular regions.

The involvement of particular radionuclides into biological cycle is associated with plants' ability to

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absorb radioactive elements from the soil via their root systems. The amount of radioactive material from the soil absorbed by plants is directly proportional to the pollution emission density in a particular territory [6]. The transfer of radionuclides from the soil into the plants is substantially dependant on the soil type, i.e. its physico-chemical properties that significantly affect the resorption rate of radioactive material. The most important physico-chemical properties include chemical composition (concentration of minerals and content of organic matter), structure (mechanical composition), pH, moisture content and crop density [7].

Roe deer (Capreolus capreolus) are the most numerous representatives of large game in hunting grounds in Serbia. They feed on the so-called. “soft food” (grass, green or dry forest or meadow plants and fruits), the so-called. “tough food” (branches of trees, shrubs, bark trees) as well as on the so-called “hard food” (acorn, chestnuts, beech). In addition to the listed nutrients, roe deer also unwillingly consume the remains of soil, stones and dry needles of conifers. They can live up to 15 years [8]. Roe deer, whose digestive tract is poorly adapted to cellulose digestion, prefer young summer plants, medicinal plants and fruit-bearing plants with narrow leaves which contain much fewer polluting particles from the air. There favourite feed is fresh meadow grass, young wheat grains and ivy, poplar leaves, and blackberries. They also like to eat acorn and nibble on the bark of forest vegetation [9].

A game that lives in certain geographical areas can be a very good bio-indicator of contamination by radioactive residues as it consumes unprocessed vegetable foods and their radius of movement is related to a particular habitat. Therefore, the basic aim of these tests is to determine the level of radioactive contamination of the ecological area by analyzing game urine. Based on these results, locations with increased content of natural radionuclides are determined. This would allow easier monitoring of the degree of contamination of an area in order to improve the environmental protection and preservation of protected and highly protected wildlife. In this respect it should be taken into account that the accumulation of radionuclides is influenced by the endogenous factors (age, sex, health condition of the animal) and exogenous factors (geographical position, hydrographic conditions, soil, climate, plant life) [10]. Considering the way of feeding and free movement in nature, deer – as a kind of game, can also usefully serve in environmental control as bioindicators of radioactive contamination.

The removal of harmful substances from the body is done through various organs and is most intensive through the urogenital organs and the digestive tract. The most important quantities of radionuclides, especially natural uranium and its isotopes ^{235}U and ^{238}U, are eliminated by urine and faeces. Uranium compounds, which are taken in through food, quickly pass from the lungs to blood and are eliminated from the body through the kidneys, which makes urine analysis useful for detection of very low contamination by those natural radionuclides for which the kidneys are the “critical organ”. The rate of excretion of radionuclides and their compounds from the organism depends on their physico-chemical properties, their concentration, as well as the states and functions of the organs through which the excretion is done. The the absorption excedes the elimination, there is an accumulation of the substances in the organism, and such substances are said to have “cumulative” properties.

2. Materials and methods

In order to determine the urine levels of natural radionuclides, the samples were collected from different localities (protected nature reserves) in the territory of Vojvodina. During 2016/17, a total of 21 samples of urine from roe deer were collected. Immediately after the shooting, the tearing of the hull and extraction of the internal organs of the game was carried out. Samples of urine were collected by puncture of the urinary bladder and they were promptly subjected to further laboratory treatment. The samples were prepared by wet digestion using Ethos, Labstation Microwave, Milestone [11]. Potassium content was determined using the method of AA spectrophotometry on Spectr AA–10, Varian, at a wavelength of 766.5 nm and using cesium as the ionization-suppressor. In nature, potassium-40 is found in a mixture of natural potassium isotopes (^{39}K, ^{40}K and ^{41}K) with a mass fraction of 0.0119%.The urine samples levels of potassium-40 activity were calculated from total potassium, using the mass activity value for potassium being 31.561 Bq/g K [12]. Concentrations of thorium and uranium in all the samples were analyzed by a technique of inductively coupled plasma with mass spectrometry, on the Agilent ICP-MS 7700.

Table 1. Instrument parameters for Agilent ICP-MS

RF Power	1550 W
Smpl Depth	7.4 mm
Carrier Gas	0.9 L/min
Nebulizer Pump	0.1 rps
Integration time (U)	1 sec/point
S/C Temp	2 °C
OctP RF	180 V
Readings/replicate	3
Detector mode	pulse
Integration time (Th)	0.1 sec/point

The activity levels of uranium-235 and uranium-238 in urine samples were determined according to the total uranium concentration using mass activity values 0.570 Bq/mgU for ^{235}U and 11.10 Bq/mgU for ^{238}U. The activity of thorium was obtained using a specific activity of 4.11 Bq/mgTh for ^{232}Th [12]. The five standard solutions were prepared to 1, 2, 3, 4 and 5 ng/ml, for both elements, Th and U. Standard curves with linearity of R=0.99 were obtained. The limit of detection (LOD) in urine samples for uranium, 0.6 ng/L and for thorium, 3 ng/L was achieved. Concentrations of ^{235}U and ^{238}U, were obtained by calculation, given the known natural abundance of $^{235}U/^{238}U$ is 0.72%/99.28%. The total uranium is the sum of the individual isotope concentrations [13]. Inductively coupled argon plasma mass spectrometry

(ICP-MS) is a highly useful and practical analytical tool, offering near-simultaneous determination of elemental isotopes with detection limits rivaling or exceeding those of such techniques as graphite furnace AAS and activation analysis. The sample analysis process, from sample preparation to counting and reporting, takes less than a day.

3. Results and discussion

Table 2 shows the results of the determination of potassium, thorium and uranium concentrations, as well as of the level of activity of ^{40}K, ^{232}Th, ^{235}U and ^{238}U natural radionuclides in tested urine samples. Based on the results shown in Table 2, it can be concluded that of all the elements tested, potassium is present in the largest quantities. Its serum concentration ranged from 4.05 (Kulpin) to 12.06 (Futog) g/L corresponding to ^{40}K activities of 128-381 Bq/L. The concentration of K in urine is very dependent on the amount of K in food, since all K from food is resorbed in the small intestine very quickly – it reaches the liver by blood flow and, from there, it is distributed throughout the body. Such K is extremely rapidly excreted in urine [14].

Table 2. The content of natural potassium, thorium and uranium and the activity of the potassium-40, thorium-232, uranium-238 and uranium-235 in samples of roe deer urine

Sample No.	**Potassium** [g/L]	**^{40}K** [Bq/L]	**Thorium** [μg/L]	**^{232}Th** [mBq/L]	**Uran** [μg/L]	**^{238}U** [mBq/L]	**^{235}U** [mBq/L]
1.	8.00	252	11.362	46.67	4.688	52.0	2.67
2.	9.94	314	1.362	5.60	2.356	26.2	1.34
3.	7.06	223	0.812	3.34	1.956	21.7	1.11
4.	12.06	381	0.750	3.08	2.288	25.4	1.30
5.	6.92	219	0.488	2.00	2.506	27.8	1.43
6.	9.00	284	0.512	2.10	2.662	29.6	1.52
7.	9.74	307	0.388	1.59	2.356	26.2	1.34
8.	7.95	251	0.362	1.49	1.656	18.4	0.94
9.	11.29	356	0.362	1.49	2.762	30.7	1.57
10.	10.52	332	25.775	105.9	2.831	31.4	1.61
11.	4.24	134	0.800	3.29	1.569	17.4	0.89
12.	5.81	183	14.888	61.14	20.894	231.9	11.90
13.	5.44	172	0.900	3.70	3.031	33.6	1.73
14.	8.51	269	0.388	1.59	3.288	36.5	1.87
15.	9.61	303	21.675	89.02	4.181	46.4	2.38
16.	6.51	206	0.738	3.03	4.088	45.4	2.33
17.	5.94	187	0.388	1.59	3.825	42.5	2.18
18.	8.72	275	8.925	36.66	3.731	41.1	2.13
19.	6.52	206	0.412	1.69	2.856	31.7	1.63
20.	4.05	128	0.250	1.03	2.894	32.1	1.65
21.	10.62	335	0.188	0.77	3.575	39.7	2.04
Variation Interval	**4.05-12.06**	**128-381**	**0.188-25.775**	**0.77-105.9**	**1.569-20.894**	**17.40-231.9**	**0.89-11.90**

The measured concentrations of thorium in the roe deer urine in most of the tested samples are very low and uniform and are of the same order of magnitude as the values obtained in the kidneys of various wild bird species [15]. In all wild bird samples analyzed, the thorium content was very low and uniform and ranged in the range of 1.40-5.85 μg/kg with an activity of 5.8-24.0 mBq/kg. In some serum samples, the measured values for thorium content were significantly increased and amounted to 8.925 μg/L=36.66 mBq^{232}Th/L (Čenej); 11.362 μg/L=46.67 mBq^{232}Th/L (Čurug); 14.888 μg/L = 61.14 mBq^{232}Th/L (Nemanovci); 21.675 μg/L = 89.02mBq ^{232}Th/L (Futog) and 25.775 μg/L = 105.9 mBq^{232}Th/L (Čenej). In other urine samples analyzed, the thorium content was very low and uniform and in the range of 0.188 (Čenej)) -1.362 (B.Topola) μg/L with an activity of 0.77-5.60 mBq/L. Thorium is an ubiquitous element with only radioactive isotopes. Because of its radiation properties and the biokinetics of Thorium-232 following incorporation, it is one of the radioisotopes with the highest radiotoxicity [16].

The largest uranium content (20.894 μg/L) in the urine of the roe deer was measured at the Nemanovci site, with ^{238}U activity 231.9 mBq/L, while the ^{235}U activity was 11.90 mBq/L. The measured uranium content in all other urine samples was also low and uniform and in the range of 1.656 (Čurug) to 4.688 (Čurug) μg/L, with the activity of ^{238}U in these samples ranging from 18.4 to 52.0 mBq/L and the activity of ^{235}U ranging from 0.94 to 2.67 mBq/L. The measured uranium content in wild bird samples was also low and uniform and ranged in the range of 3.48-11.65 μg/kg, with the activity of ^{238}U in these samples ranging from 38.63-129.3 mBq/kg and the activity of ^{235}U in the range from 1.98 to 6.64 mBq/kg [15]. The obtained values for the concentration of the activities of the analyzed radionuclides: ^{40}K, ^{232}Th, ^{238}U and ^{235}U can be successfully used to define the natural background of the investigated areas [17], which enables the registration of “technologically increased natural radioactivity”, primarily due to the application of modern agro-technical measures based on the use of artificial fertilizers of phosphate origin [4].

4. Conclusion

In all tested urine samples, potassium-40 is the dominant natural radionuclide with an average concentration of activity for all sites, $^{40}K_{sr}$ = 253 ± 72 Bq/L. At locations 1. (Čurug), 10. and 18. (Čenej), 12. (Nemanovci) and 15. (Futog), the measured thorium activity-232, $^{232}Th_{sr}$ = 67.9 ± 25.9 mBq/L was significantly higher than at other locations, $^{232}Th_{sr}$ = 2.34 ± 1.21 mBq/L. Also, the activity of uranium-238 and uranium-235, as biologically important radionuclides, in urine samples from 1. (Čurug), $^{238}U_{sr}$ = 141 mBq/L and 12. (Nemanovci), $^{235}U_{sr}$ = 31.8 mBq/L it was higher compared to the other sites, $^{238}U_{sr}$ = 31.8 ± 8.3 mBq/L and $^{235}U_{sr}$ = 1.63 ± 0.43 mBq/L.For Vojvodina, as an area with great opportunities for the production of health-safe food, it is extremely important to systematically monitor the level of radioactivity of the environment, because if it is contaminated with substances with a technologically elevated level of natural radioactivity, the dose received by the population from radioactive radiation may increase. Method ICP-MS has proved to be a very sensitive method for quantitative determination of Th and U concentration in urine samples. Therefore, ICP-MS offers a useful alternative for monitoring of thorium and uranium in environmental samples. Research in this area and the results obtained contribute to a better understanding of the translocation of radioactive residues from food to certain tissue of game. By determining the level of accumulation of natural radionuclides in the internal organs of wild animals it is possible to estimate the ecological state of geographical areas from which the investigated game originates.

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