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Trace and Toxic Elements in Meat of Maral (Red Deer) Grazing in Kazakhstan.

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ABSTRACT

The presence of trace elements (Al, Fe, K, Ca, Mg, Cu, Na and Zn) and toxic elements (Cd, Co, Pb, Sr, Cr) determined in the maral (red deer) meat collected from East Kazakhstan region, during June, 2013 was examined. The elements were determined by inductively coupled mass-spectrometry (ICP-MS). The means of elements in maral meat were 36.58 mg/kg for Al, 38.39 mg/kg for Fe, 3045.3 mg/kg for K, 77.28 mg/kg for Ca, 224.07 mg/kg for Mg, 1.4 mg/kg for Cu, 217.94 mg/kg for Na and 30.04 mg/kg for Zn. A comparison of obtained data for toxic elements in the maral meat was made with some other types of meat (beef, lamb, horse meat and chicken). According to these data the Pb concentration in maral meat is one of the lowest (0.008 mg/kg) was found. However the concentration of Sr (1.07 mg/kg) and Cr (3.08 mg/kg) in maral meat were higher compared with other meat. In the same time the Pb, Cd and Cr concentrations in maral meat were not exceed or within the permissible levels, set by Technical regulation of the Customs Union TR CU No. 034/2013, Commission Regulation (EC) No. 466/2001, and U.S. FDA Food Contact Substances.

Keywords: maral meat, trace element, mass-spectrometry, livestock

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INTRODUCTION

It is a common knowledge that 70% of pollutants (ecotoxins and xenobiotics) are accumulated in the human body through the food [1, 2]. The main threat for human is the maintenance of xenobiotics in the foodstuff [3]. This includes heavy metals, pesticides, radioisotopes, nitrates and nitrites etc. According to [4] among these pollutants, radionuclides and heavy metals have mutagen and carcinogen effect. In the Republic of Kazakhstan, radiological considerations are an integral part of food safety due to the ecological situation. In the East Kazakhstan region, the main risks are associated with the radioactive fallout from 40 years of nuclear bomb testing [5]. Semipalatinsk Nuclear Test Site (SNTS) left a huge mark on the history of the nuclear race in the period of the Cold War. On August 29, 1949 the Soviet Union conducted the first nuclear explosion on the territory of Kazakhstan. From this moment, the forty-year story of SNTS began [6]. Soviet Union conducted 456 nuclear explosions at SNTS, including 116 aboveground and 340 underground explosions [6]. This led to radioactive contamination not only inside the test site but also outside, where people live [7]. Depending on the rate of equivalent dose, contaminated territories were subdivided on the next zones: zone of extreme radiation risk, zone of maximal radiation risk, zone of high radiation risk, zone of minimal radiation risk, and territory with privilege social-economic status [6]. Also, excess content of cadmium, lead, copper and zinc in the environment, primarily due to the operation of industrial enterprises, which contaminate adjacent territories [8]. Metal contamination raises environmental concerns, such as influences on the food chain, which can be potentially harmful to humans [9]. In the human diet, meat is seen as a major source of fat, and especially of saturated fatty acids (SFAs), which have been implicated in diseases associated with modern life (various cancers and coronary heart disease), mostly in developed countries [10]. Muscle and organ meats of game animals are traditionally valued as natural food that is both tasty and nutritious. There is also a current preference for wild foods, which may be seen as ‘organic’, over conventionally reared farm produce using intensive conditions which can be perceived as artificial [11]. Game meat is a natural food product, which has a lot of advantages [11]. Wild animals feeding in conjunction with nature’s cycle go through natural selection and preserve natural instincts, live free with no stress [12]. Maral is one of the easternmost subspecies of red deer that is native to areas in Kazakhstan, China, Mongolia and Russia [13]. Marals live in the Altai-Sayansk mountains, south-east of Kazakhstan and in Predbaikal (Russia). It is of great value for human as a source not only of meat but and of pharmacological material [14]. In the Republic of Kazakhstan marals are mainly inhabited in east part of Kazakhstan and as for 2012, there were about 3500 marals [15]. In the literature have been found a publication disused a wide range of trace elements in red deer meat from the north-eastern Poland [16]. The habitats territory of marals in the East Kazakhstan is belong to the zone of heightened and minimum radiation risk, according to the Republic of Kazakhstan’s social protection regulations from December 18, 1992.

The objective of this study is to determine the trace and toxic elements in maral meat and to compare them concentrations with those determined in beef, sheep, horse, and chicken meat.

MATERIALS AND METHODS

The chemicals such as nitric acid (HNO_3) and hydrogen fluoride (HF) were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA).

The experiment was carried out after collection of samples from 6 hunted red deer (*Cervus elaphus*) grown under the same environmental and climatic conditions. The experiments were performed with animals aged between 18 and 24 months. The marals were reared in the farm "Bagration" (East Kazakhstan region, Ulan area, village of Privolnoye, 50° 06' N, 81° 32' E) (Figure 1.). The farm location, the climate including the temperature fluctuations, the average annual precipitation, and thickness of the snow, the soils, and the vegetation were described in detail by [12]. The maral meat samples were collected during June, 2013 in game processing factories of Semey. Totally 6 animals (3 females and 3 mails) of maral meat were collected. The nature of the study required the collection of tissues post mortem is not contrary to ethical norms. Therefore it is consistent with worldwide accepted practice for animal welfare. Samples of the muscles of about 200 g (20 g each x 10 repetitions) were collected during examination by veterinary service. The materials for this study were collected following recommendations of [22].

Samples of beef, lamb, horse meat, and chicken meat were collected from the markets of Semey city. Totally 50 samples (5 samples x 200 g each x 5 repetitions, from other type of meat) were collected. The samples were transported to the laboratory and storage in the refrigerator at -18 to - 20ºC until the analysis. They were homogenized, and then, 1 - 2 g of the sample was placed in high-pressure Teflon containers. The samples were combusted at the temperature of 400ºC for 4 h and then to 600ºC for 2 h in a muffle furnace. For the digestion, a representative 1 g (dry weight) sample is digested with additions of 3 cm^3 HNO_3 and 2 cm^3 of HF in a microwave for 20 min in a Milestone microwave system. After microwave digestion the samples were diluted with 1% HNO_3 in a 10 cm^3 vessel.

Figure 1: The geographical location of the sampling sites

The content of elements in muscle samples was determined with the inductively coupled plasma–mass spectrometric method (ICP-MS, Varian-820 MS, Varian Company, Australia). The method was validated with certified reference materials. Calibration standards Var-TS-MS, IV-ICPMS-71A (Inorganic Ventures Company, USA) were used for calibrating the mass-spectrometer. The sensitivity of the mass-spectrometer was tuned up using a diluted calibration solution Var-TS-MS with concentration of Ba, Be, Ce, Co, B, Pb, Mg, Tl, Th of 10 μg/L. Three calibration solutions were used for the detector calibration. They were IV-ICPMS-71A of Cd, Pb, Cu, Zn elements diluted to 10, 50 and 100 μg/L. Discrepancies between the certified values and concentrations quantified were below 10 %. The operating parameters of the inductively coupled plasma mass spectrometer Varian ICP 820 –MS were as follows: plasma flow 17.5 L/min; auxiliary flow 1.7 L/min; sheath gas 0.2 L/min; nebulizer flow 1.0 L/min; sampling depth 6.5 mm; RF power 1.4 kW; pump rate 5.0 rpm; stabilization delay 10.0 s.

All analyses were performed in triplicate, and the results are presented in Table 1 as the means of measurements expressed in mg/kg wet weight.

The obtained results were statistically analyzed using SAS/STAD™ software (SAS Institute Inc. 100 SAS Campus Drive Cary, NC 27513-2414, USA). Initially, was determined the Normal distribution of the data [16]. The concentrations of elements had normal distribution. The differences were tested with parametric methods as was describe by [22]. For statistical comparisons between the particular groups, the Tukey test was used. The differences were considered to be significant at $p \leq 0.05$.

RESULTS

The results obtained showed the maral meat is a good source of microelements (Table 1). It is very suitable source of such micronutrients for the human body such as: potassium (K) - 3045.30 mg/kg, phosphorus (P) - 592.12 mg/kg, magnesium (Mg) - 224.07 mg/kg, sodium (Na) - 217.94 mg/kg, calcium (Ca) -

77.28 mg/kg, iron (Fe) - 38.39 mg/kg, aluminium (Al) - 36.58 mg/kg, zinc (Zn) - 30.04 mg/kg, manganese (Mn) - 6.92 mg/kg, copper (Cu) - 1.40 mg/kg, and nickel (Ni) - 0.30 mg/kg.

It was also shown that traces of rubidium (Rb) < 0.300 mg/kg, selenium (Se) < 0.011 mg/kg, silver (Ag) < 0.005 mg/kg, vanadium (V) and beryllium (Be) < 0.003 mg/kg are also present in the muscles of maral from East Kazakhstan (Table 1).

Table 1: The concentrations of trace elements in maral meat samples

Elements	Sample repetitions, mg/kg						Mean ± SD
	1	2	3	4	5	6	
Al	38.89±1.24	21.79±0.63	31.88±1.11	42.08±1.30	39.12±1.33	45.74±1.69	36.58±1.22
Be	< 0.003	< 0.001	< 0.002	< 0.004	< 0.003	< 0.005	< 0.003
V	< 0.003	< 0.003	< 0.002	< 0.003	< 0.002	< 0.004	< 0.003
Fe	41.54±1.49	30.39±0.97	32.76±1.08	35.66±1.21	43.48±1.39	46.54±1.62	38.39±1.29
K	3152.90±44.9	3127.2±42.8	3109.4±44.3	2675.5±27.2	3043.3±37.5	3163.6±43.1	3045.3±39.9
Ca	106.35±41.50	52.75±18.90	57.41±23.00	62.77±23.80	93.18±31.70	91.22±34.7	77.28±28.9
Mg	231.97±8.11	221.35±7.97	235.02±9.63	154.34±4.93	242.88±8.26	258.89±8.02	224.07±7.82
Mn	6.80±0.23	5.63±0.21	5.99±0.21	6.52±0.25	7.94±0.29	8.62±0.33	6.92±0.25
Cu	1.32±0.05	1.13±0.04	0.95±0.03	0.69±0.03	1.93±0.06	2.37±0.07	1.40±0.05
Na	227.22±8.17	177.29±6.02	201.31±6.84	161.60±5.81	272.98±9.83	267.23±9.62	217.94±7.72
Ni	0.32±0.01	0.24±0.01	0.25±0.01	0.36±0.01	0.35±0.01	0.31±0.01	0.30±0.01
Rb	< 0.33	< 0.30	< 0.32	< 0.21	< 0.31	< 0.33	< 0.30
Se	< 0.011	< 0.004	< 0.010	< 0.027	< 0.005	< 0.007	< 0.011
Ag	< 0.001	< 0.006	< 0.003	< 0.001	< 0.013	< 0.007	< 0.005
P	611.93±20.82	576.71±19.61	623.32±21.20	403.33±12.50	653.29±22.21	684.17±24.63	592.12±20.16
Zn	30.30±0.97	30.70±1.17	31.60±1.10	20.62±0.70	32.39±1.10	34.64±1.12	30.04±1.03

Mean ± SD - Mean value ± standard deviation
($p \leq 0.05$) - Statistically significant difference amongst the samples

The heavy metal content in the meat of red deer with relatively low and does not exceed the hygiene standards set by major regulations in the world (U.S. FDA Food Contact Substances: 21 CFR sections 174-189; Commission Regulation (EC) No. 466/2001; The Technical Regulations of the Customs Union - TR CU 021/2011 on Food Safety, and TR CU 034/2013 on the Safety of Meat and Meat Products) (Table 2) [17, 18, 19, 20]. It has been found that the concentration of chromium (Cr) is 1.58 mg/kg at a limit value < 10.00 mg/kg. The Sr concentration is a 1.35 mg/kg. For the remaining three heavy metals are found the following: the concentration of Cu is < 0.076 mg/kg, and those of Pb and Cd < 0.007 mg/kg. The lead levels are several times lower than the limit ≤ 0.100 mg/kg for children younger than 3 years, and respectively, of eligible ≤ 0.200 mg/kg for children over 3 years. A similar situation was established and the concentration of cadmium (Cd), which is considerably lower than indicated as the critical levels of ≤ 0.300 mg/kg (Table 2).

Table 2: The concentrations of toxic elements in maral meat

Elements	Sample repetitions, mg/kg						Mean ± SD	Permitted levels, mg/kg
	1	2	3	4	5	6		
Cd	< 0.005	< 0.004	< 0.005	< 0.004	< 0.008	< 0.006	< 0.007	≤ 0.03
Pb	< 0.004	< 0.003	< 0.008	< 0.003	< 0.016	< 0.014	< 0.007	≤ 0.10 children < 3 years ≤ 0.20 children > 3 years
Cr	2.24±0.69	2.03±0.69	3.08±1.08	1.66±0.57	6.33±2.34	4.67±1.59	1.58±0.57	≤ 10.00 for canned food in chrome package
Co	< 0.062	< 0.042	< 0.061	< 0.048	< 0.063	< 0.073	< 0.076	
Sr	1.12±0.04	0.85±0.03	1.07±0.04	0.94±0.03	0.83±0.03	1.35±0.04	1.32±0.05	

Mean ± SD - Mean value ± standard deviation
($p \leq 0.05$) - Statistically significant difference amongst the samples

A comparison of obtained data for toxic elements in the maral meat was made with some other types of meat (beef, lamb, horse meat and chicken). According to these data the concentration of Pb in maral meat is 26 times smaller than that found in beef; 15 times lower than horse meat; 3.5 times lower than chicken and is commensurate with that of lamb (Figure 2).

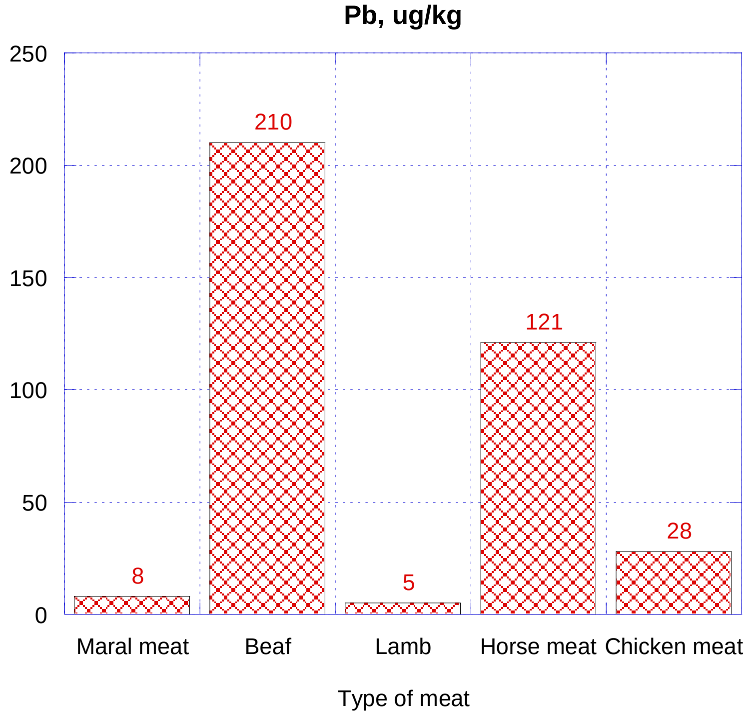

Figure 2: Comparison of the lead (Pb) concentration in maral meat, beef, lamb, horse meat and chicken

Slightly different ratios are established on the concentration of Cd in the maral meat. It was 3.2 times smaller than that found in beef but 5 times higher than that of the horse meat, chicken and especially lamb (Figure 3).

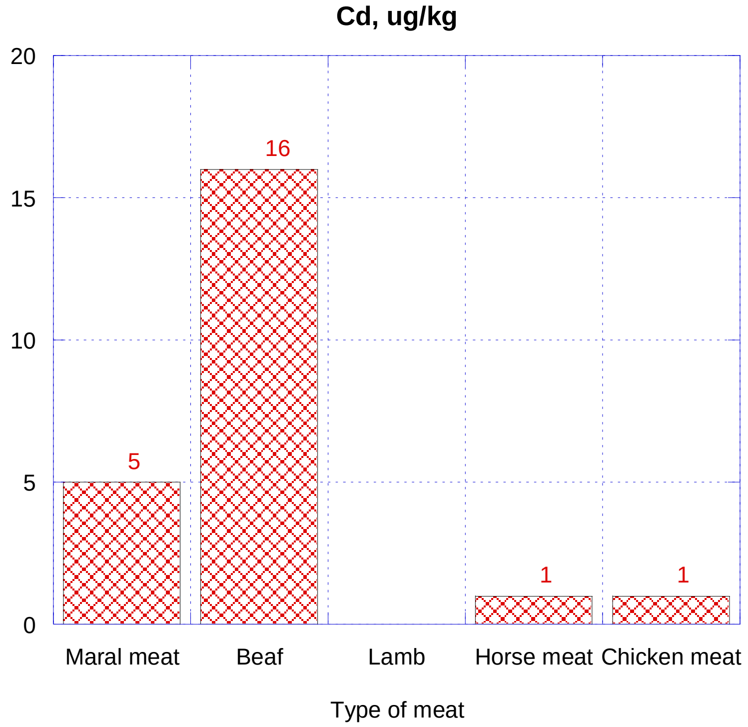

Figure 3: Comparison of the cadmium (Cd) concentration in maral meat, beef, lamb, horse meat, and chicken

However, it was found that the concentration of Co (Figure 4), Cr (Figure 5), and Sr (Figure 6) into the maral meat were several times greater than that found in the other four kinds of meat. In the same time the concentrations of the Pb, Cd and Cr in maral meat were not exceed or within the permissible levels, set by Technical regulation of the Customs Union TR CU No. 034/2013 [20], Commission Regulation (EC) No. 466/2001 [18], and U.S. FDA Food Contact Substances [17].

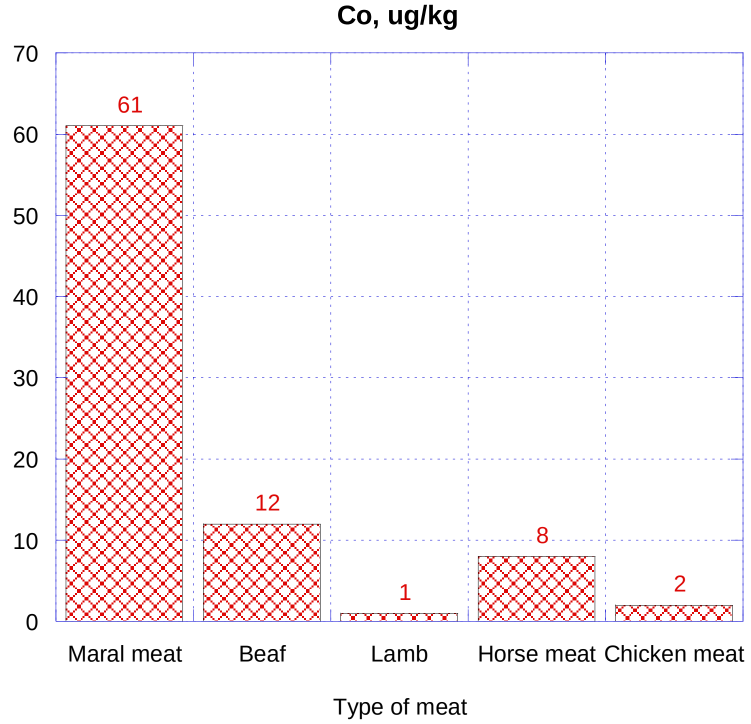

Figure 4: Comparison of the cobalt (Co) concentration in maral meat, beef, lamb, horse meat, and chicken

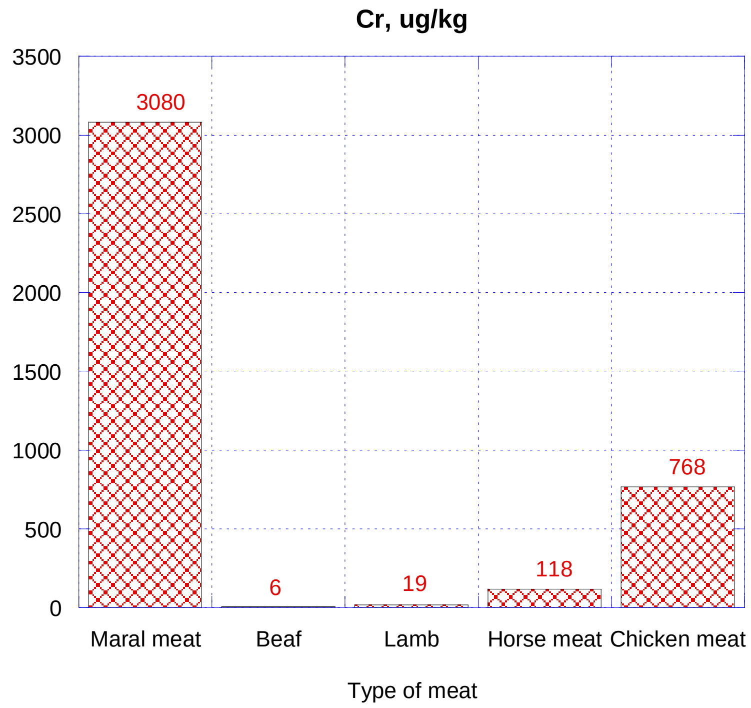

Figure 5: Comparison of the chromium (Cr) concentration in maral meat, beef, lamb, horse meat, and chicken.

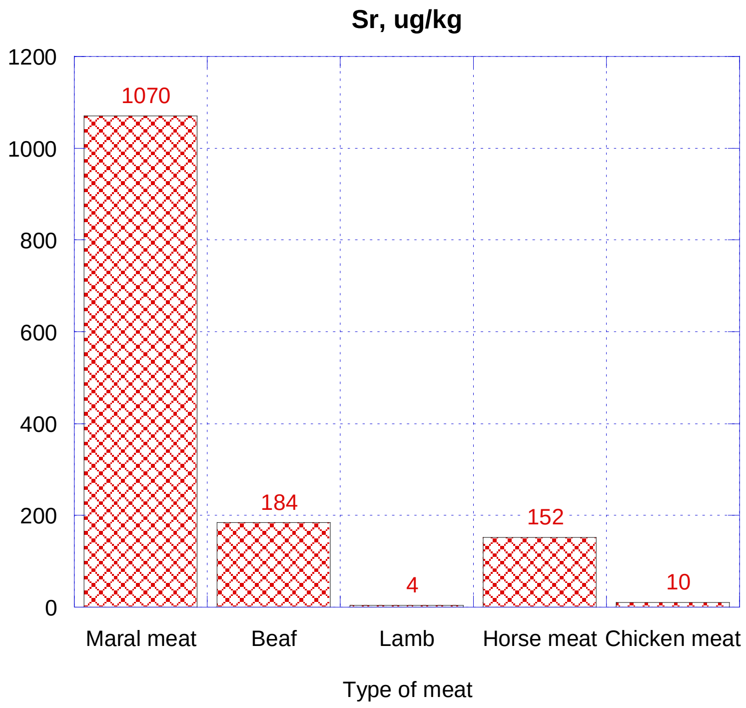

Figure 6: Comparison of the strontium (Sr) concentration in maral meat, beef, lamb, horse meat, and chicken.

DISCUSSION

Similarly to our results [21] were considered the muscle meat of red deer hunted in Poland as a very good source of essential microelements such as Co, Cr, Cu, Mo, Mn, Se and Zn in the human diet.

A comparison between our data and those published by [22] , and [21] shows that the concentrations of Cu, Rb and Se in the maral meat from East Kazakhstan are lower than their concentrations determined in meat from red deer hunted in North-eastern and South-western Poland. In terms of human nutritional needs, a relatively high selenium content of muscles can be beneficial [21].

Contrary to this, the concentration of Zn in maral meat from Eastern Kazakhstan is higher by approximately 5.50 mg/kg compared to the its concentration in meat of red deer hunted in North-eastern and South-western Poland [22].

Our results concerning the content of Cu and Zn in maral meat are in good agreement with those reported by [23] about red deer meat from Slovakia, and with finding of [24] which showed copper concentration of muscle tissue of red deer hunted in Warmia and Mazury, Great Lakes Land region in north-eastern of Poland of 1.9-6.4 mg/kg, and zinc concentration between 19-64 mg/kg.

Nowadays, toxicologists draw the attention to Cd. This heavy metal is an antagonist of Zn, P, Cu and other essential elements, blocking important tissue ferments. In the human body Cd enters into competitive relationships with essential for human elements, such as Fe, Cu, Zn and Ca, and if there are micronutrient deficiencies of these elements, Cd accumulation is increasing [25]. Identified by us traces of cadmium (in concentration ≤ 0.03 mg/kg) confirmed the data up 0.01 to 0.04 mg/kg reported by [26] in muscles of free ranging red deer from hunting grounds in four counties of North-east Croatia, [23] in Slovakia, and [27] - 0.0584 mg/kg in Croatia. Our results are slightly lower than those represented by [19] and [21] for the cadmium concentrations of muscle tissue of red deer caught in Warmia and Mazury regions of the North-eastern of Poland; with overall means of 0.10 and 0.07 mg/kg wet weight, respectively. According to [21] Cd exists as a chemical element present at trace levels in plants and mushrooms in deer's food chain in uncontaminated areas.

Pb causes chronic poisoning, effects on the central nervous system, blood, protein synthesis and genetic. All Pb compounds affect in the same way, and difference of toxicity depends basically on the irregular solubility in body fluids, especially in gastric juice [28, 29]. The determined by us traces of Pb < 0.007 mg/kg wet weight are similar of data described by [23] for Slovakian red deer meat; by [27] - 0.171mg/kg for muscles of red deer hunted in four regions of North-east Croatia; and by [26] who reported that the renal Pb concentration ranged was 0.058 - 3.770 mg/kg, but hepatic Pb concentration was 0.077 - 0.108 mg/kg. The Pb concentration in the East Kazakhstan maral meat is less than the result (0.22 mg/kg wet weight) reported for polish red deer meat [24, 21, 22].

As expected, because of longstanding nuclear tests in the area of East Kazakhstan concentration of strontium in maral meat is 8.25 times higher (1.32 mg/kg) than that (0.16 mg/kg) found in meat from red deer in Poland [21].

Lead was considered as toxic, and the concentrations found in maral meat (via the food chain intake) were well below the European Union tolerance limit. Furthermore, concentration of Cd and Cr are not exceed or within the accessible levels. Due to Single Sanitary Requirements (The Technical Regulation TR CU 021/2011of the Russia-Kazakhstan-Belarus Customs Union (CU) on Food Safety; Commission Regulation (EC) No. 466/2001; U.S. FDA Food Contact Substances) allowable concentrations of toxic elements in the slaughter meat are: for Pb – 0.5 mg/kg and for Cd – 0.05 mg/kg. Technical regulation TR TS 034/2013 of the Russia-Kazakhstan-Belarus Customs Union (CU) specifies the permissible levels for next toxic metals as shown in Table 2.

CONCLUSION

The results obtained enable more precise figure of macro- and microelement composition of meat from murals, grazing in East-Kazakhstan. The data obtained will be useful in further research of designing and developing new functional foods and forecasting of formulation according to the current ecological situation in the region and the requirements of nutrition science. Food safety becomes an urgent problem and its importance grows each year. Food safety investigations of meat and meat product have taken major relevance because it will increase the production volume of meat products in the Republic of Kazakhstan and will stimulate exports/imports of meat. The export procedures require more stringent requirements on food safety and must be applicable very careful veterinary sanitary expertise of the meat products.

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