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Biomonitoring of 12 trace elements in blood and urine of children by inductively coupled plasma mass spectrometry

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Abstract: The authors determined mass concentrations of 12 elements (As, Cd, Cr, Cu, Mn, Ni, Pb, Se, Sr, Tl, V, Zn) in blood and urine taken from 100 rural children living in unremarkable condition in the West Ural region of the Russian Federation. We applied inductively coupled plasma mass spectrometry in conformity with Methodical Guidelines 4.1. 3230-14 (FR.1.31.2014.17064) designed by us and detailed in the paper. The article contains setting parameters for Agilent 7500cx quadrupole inductively coupled plasma mass spectrometer. The authors present grounds for an optimal scheme of samples preparation which helps to eliminate "matrix" effect and explain why it is advisable to use reaction/collision functioning mode as it enables interference overlaps suppression.

To prepare whole blood samples for analysis, we used acid dissolution in concentrated nitric acid followed by centrifugation. The arithmetic mean (AM) of element content in blood amounted to 0.34 µg/l (As); 0.39 µg/l (Cd); 6.41 µg/l (Cr); 866.11 µg/l (Cu); 13.73 µg/l (Mn); 5.86 µg/l (Ni); 23.65 µg/l (Pb); 87.43 µg/l (Se); 33.54 µg/l (Sr); 0.04 µg/l (Tl); 0.16 µg/l (V); 4713.48 µg/l (Zn). The validity of the results was confirmed by means of SERONORM™ Whole Blood L2 standard samples (Norway). Urine samples were directly analyzed after 1/10 (V/V) dilution with 1% nitric acid solution. The arithmetic mean of element content of urine corresponded to 18.99 µg/l (As); 0.12 µg/l (Cd); 1.91 µg/l (Cr); 13.28 µg/l (Cu); 0.96 µg/l (Mn); 1.84 µg/l (Ni); 0.83 µg/l (Pb); 22.55 µg/l (Se); 239.09 µg/l (Sr); 0.16 µg/l (Tl); 0.68 µg/l (V); 270.56 µg/l (Zn). The validity of the results was confirmed by analyzing the SERONORM™ standard urine samples (Norway). Regional differences from levels found in other countries included higher concentrations of Cr, Mn and Ni in blood and lower levels of Se and Zn levels in West Ural children. Urine concentrations of West Ural children showed higher levels of As and Sr.

Key words: essential and toxic elements, blood, urine, children, ICP-MS, reaction/collision cell (ORS technology), internal standard

1. Introduction

Studies dedicated to fundamental processes which occur in a human body under influence exerted by various environmental factors are among priority issues in contemporary scientific research. Human biological monitoring (HBM) data of give true information about overall contents of hazardous substances in a body or about their biological impacts at various introductions into it as well as about individual differences in exposure levels, metabolism and excretion speed [1]. Human biomonitoring data are the most important for assessing impacts on health in case of biologically accumulating or persistent contaminants. The report issued by WHO European Regional Office [2] gives a review on concepts and sphere of HBM techniques application and presents the results of the latest international and national research over the last 15 years; it also describes trends in distribution of specific contaminants, and lists priority issues of environmental protection and health protection basing on HBM data analysis. Toxic mercury (organic and non-organic compounds), cadmium, lead, and arsenic are the most strictly controlled among all elements. The authors of the report highlight that HBM data on East European region are insufficient; as for data on the Russian Federation, they are represented only by overall mercury content in hair and urine, lead content in umbilical blood, and cadmium and arsenic content (in mothers' urine). These data were obtained during pilot testing which took place in maternity hospitals in Moscow region [2, 3].

It is obvious for all analysts and physicians that if standardized approaches to research programs are applied it will help to achieve comparability of HBM data between countries, to determine groups with high exposure level and to provide surveillance over efficiency of activities aimed at negative impacts elimination. At the same time it hardly seems advisable to put only toxic elements in research shortlists. Microelements take active part in all metabolic processes as they are components of various enzymes and they catalyze biochemical reactions in a body. Thus, Fe, Mn, Cu, Zn, and Se are involved into endogenous antioxidation system functioning; they participate in transport proteins and receptors work. Toxic effects exerted by cadmium, mercury, and lead, can be neutralized by such essential elements as Zn and Se. It's clear that elements imbalance in a human body influences functioning of all systems and organs [4]. Cu, Zn, Mn, Ni, Se, Pb, Cr, As, Cd, Tl and V are known to be recommended diagnostic elements in blood and urine [5].

Significant problems remain in interpreting the significance of the concentration of elements detected in body fluids because of non-uniform methods of analysis and lack of internationally accepted reference values [6-8]. National HBM-I and HBM-II reference values have been introduced by the German Commission on HBM [9]. The HBM-I value represents a level of xenobiotics in human biological media that is considered to pose no adverse health risk such that no action is required. The HBM-I value is thus considered to serve as a control biomarker reference. A higher xenobiotic concentration may be designated HBM-II, a reference value that carries a negative health risk and, as such, a threshold value for action. If the concentration of an individual substance exceeds the HBM-I value, but does not reach the value of HBM-II, potential exposure sources should be identified [2,9]. HBM-I and HBM-II values presently exist only for As, Cd and Hg; an earlier HBM-I value of 100 µg/l for Pb in blood was cancelled because this level exceeds the safe level for children. For reference values, it is suggested to use RV95 based on 95 percentile of biomarker values in a population that is not under exposure to the element in question [10].

In the RF, research results are interpreted by comparing elemental content in biological media of exposed groups against regional background values [11,12] or data taken from guides on clinical and laboratory tests [13,14].

Biological liquids are challenging to analyse given the extremely low content of most detectable microelements and also their complex matrix composition. According to WHO recommendations and authors of scientific reviews [2,6], atomic absorption spectrometry with electrothermal atomization (AAS-ETA) and inductively coupled plasma mass spectrometry (ICP-MS) are the most promising techniques for analyzing elements in biological liquids without preliminary decomposition of a sample. The ICP-MS technique is the most efficient for analysis of biological media because many elements are simultaneously measurable; additionally, the technique has low limits of detection, a wide range of determined concentrations, low consumption of analyzed substances, and high production capacity.

We have previously created methodical guidelines for determining 12 chemical elements (As, Cd, Cr, Cu, Mn, Ni, Pb, Se, Sr, Tl, V, Zn) in human biological media using ICP-MS (MG 4.1.3161-14, MG 4.1.3230-14)[15]. The procedure for quantification of these 12 elements in blood is patented (RU No.2585369). Methodical peculiarities in the determination of Sr, V and other elements in blood and urine are detailed elsewhere [16 - 20]. The present study employs ICP-MS to determine the concentration of elements in the blood and urine of a group of West Ural children with no remarkable environmental exposures. We employ an optimal scheme of sample preparation that reduces the "matrix" effect and explains

why it is advisable to use a reaction/collision functioning regime as it enables suppression of interference overlaps.

Our research goal was to present biological monitoring results for a group of unexposed children (n=100), based on distribution analysis and to interpret the obtained data in accordance with contemporary international requirements.

2. Results

We detected 12 toxic and essential elements in blood and urine of children (n=100) in conformity with Methodical Guidelines 4.1.3161-14 and Methodical Guidelines 4.1.3230-14 [15]. Detection limits (LOD) of our technique are given in Tables 1 and 2.

We represented our results in form of basic statistical indicators, namely minimum and maximum values, arithmetic mean (AM), geometric mean (GM), and 5, 50, 95 percentiles (Tables 1 and 2).

Table 1 Elements content in children's blood, $\mu\text{g/l}$

Element	LOD	Min-Max	AM	GM	Percentile		
					P5	P50	P95
^{51}V	<0.008	0.05-0.85	0.16	0.11	0.05	0.10	0.5
^{53}Cr	0.04	0.3-34.2	6.41	5.34	2.60	5.85	13.58
^{55}Mn	0.001	7.3-55.0	13.73	13.10	9.19	13.2	20.04
^{60}Ni	0.001	0.1-59.0	5.86	3.15	0.50	2.95	19.04
^{63}Cu	0.03	551-1215	866.11	854.70	638.70	858.00	1114
^{66}Zn	0.08	3351-7693	4713	4655	3800	4664	5775
^{75}As	<0.07	0.04-2.2	0.34	0.13	0.05	0.5	1.51
^{82}Se	0.12	37-143	87.43	84.97	60.24	84.50	127.10
^{88}Sr	0.003	0.11-115	33.54	28.01	9.50	30.85	66.86
^{111}Cd	<0.003	0.05-3.2	0.39	0.28	0.05	0.32	0.81
^{205}Tl	0.007	0.007-0.19	0.04	0.05	0.007	0.06	0.12
^{208}Pb	0.002	0.25-114	23.65	18.63	7.31	20.75	50.25

Table 2 Elements content in children's urine, $\mu\text{g/l}$

Element	LOD	Min-Max	AM	GM	Percentile		
					P5	P50	P95
^{51}V	0.03	0.05-5	0.68	0.58	0.19	0.62	1.20
^{53}Cr	0.03	0.07-5.3	1.91	1.62	0.67	1.70	4.80
^{55}Mn	0.03	0.1-7	0.96	0.68	0.20	0.70	2.51
^{60}Ni	0.03	0.1—8.1	1.84	2.3	0.40	1.50	4.23
^{63}Cu	0.3	0.6-39.7	13.28	10.39	2.56	12.95	27.72
^{66}Zn	0.7	20.7-1316	270.56	209.02	40.72	223.50	598.85
^{75}As	0.3	0.4-110	18.99	11.63	1.70	12.10	55.81
^{82}Se	0.4	2.9-128	22.55	18.10	5.18	19.25	47.87
^{88}Sr	0.5	192-840	239.09	184.52	38.90	194	564.25
^{111}Cd	0.03	0.015-0.4	0.12	0.11	0.03	0.11	0.23
^{205}Tl	0.03	0.01-0.5	0.16	0.13	0.01	0.15	0.39
^{208}Pb	0.03	0.05-4.9	0.83	0.49	0.17	0.68	2.11

2.1 Blood Studies

The median value in blood approximated the arithmetic mean for Cr, Cu, Mn, Sr, and Zn. The normal distribution of values in samples indicates that the arithmetic mean (AM) can be used to assess the content of these elements. For other elements, the median (50 percentile) or geometric mean (GM) provided a better indicator of content. Applying the 5-95 percentile range allows one to make an adequate assessment of all elements content and to use it as a physiological standard boundary for laboratory indices in an unexposed group for each distribution type.

2.2 Urine Studies

For urine, median values were close to the arithmetical mean for Cd, Cr, Cu, Pb, Tl and V (Table 2) with a normal distribution of values in the sampling. It is necessary to use the median (50 percentile) or geometric mean (GM) for all the other elements detected.

3. Discussion

The results of blood analysis for the present children living in the West Urals rural area are compared with those for children living in Canada [21], rural areas in China [22] and reference levels, including values from ALS Scandinavia diagnostic laboratory [14], from a monograph by N.U. Tietz [13] and reference values for an urban Italian population [23] and an urban French population [24]. There were no coincidences of P5-P95 range for any element among all these reference values.

Blood vanadium content was below the reference range as per Tietz, but P95 was 2 times higher than in Scandinavia and Italy.

The P95 for chromium was 2 times higher than in China and 10 times higher than in Scandinavia and Italy, but it remained within physiological standard boundaries as per Tietz. Blood manganese content as per P95 was 3 times higher than in China and 2 times higher than in Italy. Nickel content as per P95 was 10 times higher than in Canada, 6 times higher than in Italy, 3 times higher than in France, but it remained within physiological standard boundaries as per Tietz. Blood Cu and Zn content was similar to all reference values.

The geometrical mean (GM) for total arsenic content in Europe varied from 0.5 to 1 $\mu\text{g/l}$ in children's blood and P95 varied from 2.12 to 3.69 $\mu\text{g/l}$ [2]; there were no apparent differences between children and adults. We found blood As GM to be 0.13 $\mu\text{g/l}$, with the P95 equal to 1.51 $\mu\text{g/l}$.

Blood Se and Sr content corresponded to all the reference levels. The GM for Sr in children from China was 2 times higher than levels found in the West Ural children.

The reference levels for blood Cd obtained in German research of HBM were $<0,3 \mu\text{g/l}$ for children [2]. Thus, GM amounted to $<0.12 \mu\text{g/l}$ and P95 was equal to $0.23 \mu\text{g/l}$ for German children aged 3-14 [25]. The GM for Italian teenagers aged 13-15 amounted to $0.26 \mu\text{g/l}$ and the P95 was equal to $0.74 \mu\text{g/l}$ [26]. As for our population, the GM amounted to $0.28 \mu\text{g/l}$, the P95 was equal to $0.83 \mu\text{g/l}$, and they corresponded to all the reference ranges.

Reference levels for blood Tl in France and Scandinavia differed from other levels by several decimal orders, which makes it difficult to adequately assess thallium content in blood.

The HBM-I value for Pb, previously set at $100 \mu\text{g/l}$ for children younger than 12, was cancelled in 2010. The German Commission on biomonitoring stated there was no "safe" level of exposure to lead. Even low lead exposures (lower than $100 \mu\text{g/l}$) exert negative impacts on health, and the bottom threshold of negative impact was not detected [27]. Lead content in blood of children aged 3-14 in Germany amounted to $32.3 \mu\text{g/l}$ (GM), and P95 was equal to $62 \mu\text{g/l}$ in 1992; in 2003-06, the level decreased to 16.3 and $29.7 \mu\text{g/l}$ correspondingly [25], and such values are comparable with our present data. In the Czech Republic, the results for children aged 8-10 were $31 \mu\text{g/l}$ Pb (GM) and $54 \mu\text{g/l}$ Pb (P95) [28]. Lead content in blood of children aged 6-11 in Canada amounted to $9 \mu\text{g/l}$ (GM), and the P95 was equal to $19.5 \mu\text{g/l}$ [21], which was 2 times lower than our values.

Table 4 compares the results for urine samples taken from children living in West Ural with data for children living in Canada [21], Germany [29], and reference levels. The latter are taken from ALS Scandinavia diagnostic laboratories data [14], ARUP USA data [30] and the monograph by N.W. Tietz [13]. There were no observed coincidences for half of elements among all reference ranges.

Urine Cr content in our population was higher than in children from Germany but was within the ARUP USA range. The content of Mn in urine corresponded to reference levels but was higher than in children from Canada or Germany. Urine Ni content corresponded to the reference levels and was 2 times lower than in children from Germany. The Sr level was higher in our population than in children from Germany or Scandinavia.

Values for As in urine are often used as a biomarker for assessing exposure to arsenic in the environment. The reference value as per data from German HBM amounts to $15 \mu\text{g/l}$ in the urine of adults and children; the GM amounts to $4.4-6.0 \mu\text{g/l}$, and the P95 is equal to $11-27.5 \mu\text{g/l}$ [2]. Our GM value amounted to $11.6 \mu\text{g/l}$, and the P95 was equal to $55.8 \mu\text{g/l}$, both values being higher than reference ranges in other countries. There are HBM-I and HBM-II values for cadmium in children's urine, and they are equal to $0.5 \mu\text{g/l}$ and $2 \mu\text{g/l}$ respectively [2]. Values in our population did not exceed these levels. Lead content in urine in our population corresponded to reference levels.

Table 3 Criterion estimated levels of elements content in children's blood, µg/l

Element	West Ural, GM/P5-P95, age 3-7	China, GM / P5-P95, age 3-12	Canada, GM/ P5-P95, age 6-11	Reference levels			
				Italy, P5-P95	France, P5-P95	ALS Scandinavia	Tietz N.W.
⁵¹ V	0.11/ 0.05-0.55	0.11/ 0.06-0.28	-	0.03-0.28	-	0.012-0.23	0.06-0.87
⁵³ Cr	5.34/ 2.60-13.58	0.54/ 0.14-6.82	-	0.01-1.2	-	0.4-1.2	0.7-28
⁵⁵ Mn	13.10/ 9.19-20.04	1.77/ 0.77-6.24	9.86 6.94-16.36	7.1-10.5	5-12.8	7-18	4.2-16.5
⁶⁰ Ni	3.15/ 0.50-19.04	-	0.67 0.35-2.13	0.3-3.3	0.1-4.2	0.3-0.77	1.0-28
⁶³ Cu	854.7/ 638.7-1114	972/ 673-1386	973 825-1198	780-1760	-	590-1470	900-1500
⁶⁶ Zn	4655/ 3800-5775	-	5240 4420-6510	4076-7594	-	3500-9100	7000-12000
⁷⁵ As	0.13/ 0.05-1.51	1.51/ 0.56-7.14	0.59 0.23-2.16	1-1.2	3.6-17.8	0.5-4.2	2.0-23
⁸² Se	84.97/ 60.24-127.10	116/ 68.5-173	186.86 159.81-231.76	7-145	89-154	138-277	58-234
⁸⁸ Sr	28.01/ 9.50-66.86	56.6/ 30.3-96	-	7-36	9-41	7-25	н.д.
¹¹¹ Cd	0.28/ 0.05-0.81	0.06/ 0.03-0.18	0.1 0.05-0.23	0.1-1.7	0.15-2.04	0.03-0.54	0-5
²⁰⁵ Tl	0.05/ 0.007-0.12	-	-	0.15-0.63	0.01-0.04	0.021-0.062	<5?
²⁰⁸ Pb	18.63/ 7.31-50.25	0.59/ 0.22-1.99	9 5.3-19.5	4-47	11.4-62.8	4-43	0-99

Table 4 Criterion estimated levels of elements content in children's urine, µg/l

Element	West Ural, P5-P95, age 3-7	Germany, P5-P95, age 2-17	Canada, P5-P95, age 6-11	Reference levels		
				ARUP USA	ALS Scandinavia	Tietz N.W.
⁵¹ V	0.19-1.2	0.056-0.16	<0.1-0.14	-	0.008-0.12	0.08-0.24
⁵³ Cr	0.67-4.8	0.055-0.59	-	0-5	0.04-0.3	0-0.5
⁵⁵ Mn	0.20 -2.5	0.073-0.25	0.05 - 0.45	0-7.9	0.27-2.5	0-2.0
⁶⁰ Ni	0.40-4.23	0.13-7.3	0.38-5.0	0-5.2	0.27-3.7	0-5.2
⁶³ Cu	2.56-27.72	4-26	3.77-27.48	0-80	1.9-15.9	2-80
⁶⁶ Zn	40.72-598.85	60-1026	98.4-860	10-800	170-780	150-1200
⁷⁵ As	1.70-55.81	1-91	2.46-51.31	0-53	5.3-11.7	0-35
⁸² Se	5.18-47.87	4-17	22.4-153.7	0-200	-	0-200
⁸⁸ Sr	38.90-564.25	9-394	-	-	27-220	-
¹¹¹ Cd	0.02-0.23	0.03-0.36	0.09-0.72	0-2.6	0.04-0.36	0-2.6
²⁰⁵ Tl	0.01-0.39	0.01-0.47	-	0-1.4	0.03-0.62	0-10
²⁰⁸ Pb	0.17-2.1	0.1-3.4	0.1-1.31	0-23	0.12-2.9	0-23

4. Materials and Methods

The research was carried out within the Rospotrebnadzor Departmental Program “Hygienic Substantiation for Minimizing the Risks to the Health of the Population of Russia” 2011-2015, clause 3.3: “Development of new highly sensitive selective methods for the determination of pollutants in habitat and biological media”.

4.1 Population

The examined group comprised children constantly living in rural areas of West Ural region of the Russian Federation and attending pre-school children facilities. We chose a region with established values for atmospheric air, drinking water, and soil status. Average concentrations of Cd, Cr, Ni, Pb, As, Sr and Mn oxides air were lower than MPC fixed in the RF (Hygienic standards 2.1.6.1338-03) and reference levels at chronic inhalation exposure RfC (P 2.1.10.1920-04) [31]. We also examined drinking water quality and did not detect any excessive concentrations of As, Cd, Cr, Mn, Ni, Pb or Sr (Sanitary and Hygienic requirements 2.1.4.1074-01). Thus, the RF MPC for arsenic compounds in water amounted to 0.05 mg/l, and the detected level was 0.0005 mg/l; for cadmium compounds, 0.001 mg/l, the detected level was <0.0001 mg/l; for chromium compounds, 0.05 mg/l, the detected level was <0.02 mg/l; for manganese compounds, 0.1 mg/l, the detected level was <0.01 mg/l; for nickel compounds, 0.1 mg/l, the detected level was <0.01 mg/l; for lead compounds, 0.03 mg/l, the detected level was <0.02 mg/l; for strontium compounds, 7.0 mg/l, the detected level was 0.089 mg/l. We also examined the elemental content in soils on the given territory; they did not exceed MPC accepted in the RF (Hygienic Standards 2.1.7.2041-06).

The examined children's age was 3-7 years, 47% were girls and 53% were boys. Children lacked congenital pathology and organic or infectious diseases of the central nervous system (CNS).

All legal representatives of children involved in the sampling gave their written informed consent for voluntary participation in the study. Ethical approval for the research presented was given by the local ethics committee (LEC FSC for Medical and Preventive Health Risk Management Technologies, Perm, Order No. 48, dated March 14, 2016), supervised by Federal Service for Surveillance over Consumer Rights Protection and Human Well-being and operating in full conformity with the RF Federal Law on Citizens' Health Protection passed in 1993 (clauses 30,31,32,33,58) and the Declaration of Helsinki «Ethical Principles for Medical Research Involving Human Subjects» (adopted in June, 1964 (Helsinki, Finland) as revised in October, 2000 (Edinburg, Scotland).

4.2 Analytic Procedures

We performed quantitative determination of elements in blood and urine using an Agilent 7500cx (Agilent Technologies, USA) quadrupole inductively coupled plasma mass spectrometer

with octopole reaction/collision cell (ORS technology). Plasma generator power was equal to 1,550 W. Samples were introduced via a two-channel Scott's spray chamber. Temperature in the spray chamber was 2.0 °C. Samples were introduced into the spray chamber at 0.4 ml/min. The burner was 7.2 mm away from the sampling cone for blood sample analysis, and 9 mm away for urine sample analysis. High purity helium (TC-0271-135-31323949) was used as a gas-reactant for determining all elements. Detector functioning speed was $\geq 100 \mu\text{s}$ per 1 ion. To tune the device, we used ${}^7\text{Li}$, ${}^{59}\text{Co}$, ${}^{89}\text{Y}$ and ${}^{205}\text{Tl}$ solution in 2% HNO_3 with concentration equal to 1 $\mu\text{g/l}$ for each element (Tuning Solution, USA). Highly pure liquid argon 99,99% (TC-2114-005-00204760-99) was used as the carrier gas. Maximum speed of argon flow was 20 l/min, and the pressure in the gas supply channel was equal to $700 \pm 20 \text{ kPa}$, T_{plasma} was 8000-10000 K. Analysis performance process was automated with the use of a G3160B autosampler (Agilent Technologies, Germany).

We used a solution containing 27 elements with concentration equal to 10 mg/l in 5% HNO_3 water solution (Multi-Element Calibration Standard-2A, USA) as a basic standard solution. To prepare internal standard (IS) solutions, we applied complex standard ${}^{209}\text{Bi}$, ${}^{73}\text{Ge}$, ${}^{115}\text{In}$, ${}^6\text{Li}$, ${}^{45}\text{Sc}$, ${}^{159}\text{Tb}$, ${}^{89}\text{Y}$ solution with concentration equal to 10 mg/l in 5% HNO_3 water solution (Internal Standard Mix, USA). We applied ${}^{159}\text{Tb}$ as an internal standard to determine Pb and Tl; ${}^{115}\text{In}$ to determine Cd; ${}^{73}\text{Ge}$ to determine the rest of the elements. The choice of internal standards was based on similarities in ionization potentials and atomic mass.

We applied particularly pure HNO_3 (Sigma – Aldrich, USA) to make calibrating solutions and to prepare samples. Calibrating solution concentrations for determining Mn, Ni, Cr, V, Sr, Se, Tl in blood were equal to 0.0; 0.1; 0.5; 1.0; 5.0 $\mu\text{g/l}$; for Cu, Zn 0.0; 0.1; 0.5; 1.0; 5.0; 10.0, and 50.0 $\mu\text{g/l}$. Calibrating solution concentrations for determining Cd and Tl in urine were equal to 0.0; 0.1; 0.5; 1.0 $\mu\text{g/l}$; for Mn, Ni, Cr, V, Se, Cu, 0.0; 0.1; 0.5; 1.0, and 5.0 $\mu\text{g/l}$; for As, Sr, Zn, 0.0; 1.0; 5.0; 10.0, and 50.0 $\mu\text{g/l}$. We washed laboratory glassware made of glass, Teflon, and polypropylene in ultrasound Elmasonic S 100H washer (Elma, Singen, Germany) prior to analysis. All solutions were diluted with deionized water with specific resistance being equal to 18.2 $\text{M}\Omega \cdot \text{cm}$. Water was purified in Milli-Q Integral system (Millipore SAS, Molsheim, France).

Blood samples were taken from an ulnar vein into a vacuum vial made of propylene and with lithium heparin deposition (PUTH, China). To prepare samples, we applied an acid mineralization technique: 0.1 ml of IS complex solution and 0.2-0.4 ml of concentrated HNO_3 were added to the blood sample with 0.1-0.2 ml volume; the mix was left for 2-3 hours until homogenization. Then, a vial content was diluted to 10 ml with deionized water and centrifuged

for 10 mins at a speed equal to 2,700 - 3,000 turns per min in a ZLMN-P10-01 centrifuge (Elecon, Obninsk, Russia). Such an acid dissolution technique significantly shortens the time required for sample preparation and enables the preparation of 60-70 samples per operator. To check the validity of blood analysis results, we used SERONORM™ Whole Blood L2 standard samples (Sero AS, Billingstad, Norway). All certified control materials underwent the same preparation procedure prior to analysis as our research samples. Sr, Tl and V concentrations for L2 level higher than 100% were found. Deviation did not exceed 10%. Cu, Mn and Ni contents were lower than 100%, and Se content was lower by 20%. Other elements were found at 100%. Thus, 12 microelements were found at certified blood level L2 in concentrations corresponding to the set range.

Urine samples were collected in the morning in 125 ml sterile polypropylene screw-cap containers (F.L.Medical S.r.l., Torreglia, Italy). Urine samples were analyzed immediately after dilution with 1/10(V/V) 1% nitric acid solution: 0.5 ml of urine were added with 4.45 ml of 1% HNO₃ water solution and 0.05 ml internal standard solution. To check the validity of the results, we applied Seronorm™ standard urine samples (LOT 0511545, Sero AS, Billingstad, Norway). All the standard urine samples were prepared for analysis in the same way as our research samples. Control samples were analyzed after each 5th research sample. Cr, Mn, Cu, As, Cd and Pb concentrations were higher than 100%, determination error amounted to 1.5-7%. V, Ni, Zn and Sr concentrations were found lower than 100%. Determination error was within 2-7% range and corresponded to certified values. Se and Tl were found at 100% level.

5. Conclusions

We determined mass concentrations for 12 elements (As, Cd, Cr, Cu, Mn, Ni, Pb, Se, Sr, Tl, V, Zn) in the blood and urine of 100 rural children living in West Ural (Russia) by ICP-MS.

We detected regional peculiarities of elemental state in children living in the West Ural region: Cr, Mn and Ni concentrations in blood are relatively high and Se and Zn levels are relatively low. Other elements are within physiological standard boundaries. Urine concentrations of West Ural children showed relatively high levels of As and Sr. The main health concerns emerging from this study are the high Cr, Mn, Ni in blood, As and Sr in urine of children living in the West Ural region requiring further investigations corrective actions and the implementation of appropriate regulations.

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