

IMPROVEMENT OF THE PROCEDURE OF CATTLE HAIR SAMPLE PREPARATION FOR CHEMICAL ANALYSIS

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Abstract. The aim of the study was to determine an optimal procedure of terminal cattle hair preparation for chemical analysis. The object of the study was cattle hair and the level of its purity for trace element analysis. In the present work, methods of spectrophotometric and microscopic analysis were applied along with ICP-MS testing including “wet” mineralization of the sample. A large number of exogenous substances on the cattle hair surface, microscopically detected damage of its structure, as well as high dispersion of sampling on the tested elements content, expressed by a coefficient of variation, indicate a poor sample preparation quality of traditional approach. A combined application of both ethanol rectification in the concentration of 40% or above and ultrasound with the frequency of 35 kHz are proved to be an optimum for the qualitative sample preparation for analysis. The method proposed gives us an opportunity to achieve the necessary purity of the sample with minimal time, energy and labour costs.

Keywords: metabolism, trace elements, laboratory diagnostics, sample preparation, cattle hair.

GALVIJŲ PLAUKŲ MĖGINIŲ CHEMINIO TYRIMO TOBULINIMAS

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Santrauka. Šio darbo tikslas – optimaliai paruošti galvijų terminalinius plaukus cheminių elementų tyrimui atlikti. Darbo objektas buvo galvijų plaukai ir jų švarumo laipsnis. Atliekant tyrimą taikyti spektrofotometriniai ir mikroskopiniai metodai, taip pat mėginio „šlapios“ mineralizacijos metodas ICP-MS. Daugybė egzogeninių dalelių plauko paviršiuje, pažeista plauko struktūra mikroskopuojant, taip pat aukštas tiriamų mikroelementų dispersijos lygis, išreikštas variacijos koeficientu, rodo, kad, taikant tradicinius tyrimo metodus, mėginiai tyrimui paruošti nekokybiškai. Nustatyta, kad 40 proc. ir didesnės koncentracijos etilo spirito rektifikatas naudojant ultragarsą, kurio dažnis 35 kHz, yra optimalus būdas kokybiškai paruošti medžiagą tyrimui. Siūlomas metodas leidžia pasiekti reikalaujamą plaukų švarumo laipsnį su minimaliomis laiko, energijos ir darbo sąnaudomis.

Raktažodžiai: metabolizmas, mikroelementozės, laboratorinė diagnostika, mėginių paruošimas, plaukai.

Introduction. The clinical manifestation of trace elements metabolism pathology is highly variable and is more often presented by the symptoms that are unlikely to state a peculiar type of deficiency or excess of the element in the organism. Therefore, assessment of the essential trace elements content via biomarkers system representing certain biochemical and physiological parameters should lie at the basis for diagnosing such diseases.

The most frequently used biomarkers for assessing the status of trace elements are the concentration of trace elements in the following biological material: blood, blood components, hair, liver (including biopsy), bones, urine, saliva, milk, eggs and brain.

Regarding the supply of the organism, the detection of trace elements in each of the biological samples mentioned above has its advantages and disadvantages. Consequently, comparative diagnostic value of such detections is the subject of much academic writing.

In this respect hair as an object of testing has attracted the attention of both native (Kalnitski, 1985; Erukashvili, 1992; Anohin, Sazonova, 1997; Skalnaja et al., 2002, Zamana, 2006) and foreign researchers (Trupa et al., 2000; Koutzenogii et al., 2002; Krejpcio et al., 2002; Ulvi

et al., 2002; Wojciak et al., 2002; Sin, Tsang, 2003) for several decades.

Nowadays hair is of a particular value when it comes to the detection of toxic effect of heavy metals on the organism (Anke et al., 1981; Szentmihalyi, 1981; Walger et al., 1981; Gumz, 1990, Smith, 1993; Ashraf et al., 1995; Schiegel-Zawadska et al., 1997; Nowak, 1999; Merlin et al., 1999; Tsai et al., 2000; Koutzenogii et al., 2002; Krejpcio et al., 2002; Skalnaja et al., 2002; Ulvi et al., 2002; Wojciak et al., 2002).

One of the key techniques in testing hair for elements detection is a preliminary preparation including the clearance from exogenous substances. Literary data on the preparation of animal hair samples for analysis demonstrate the absence of a common approach among researches. A wide range of opinions on the issue varies from water usage with or without soap to spirit ether and acetone at different temperatures and time periods.

In the modern human medicine, the method (IAEA) of consecutive hair washing according to the pattern: acetone – water – acetone (Report on Second ..., 1985) is most widely used. The control of purification level represents complex and continuous technology detecting

the elements content in the initial (dirty) hair, washing solutions and rinsed hair. The downside of this method is doing harm to hair lipid and keratin structure when processed with acetone, which leads to the loss of endogenous (inside the hair) macro and trace elements. This significantly decreases the reliability of further analysis (Zamana, 2006).

Preliminary purification is not carried out to determine biologic system changes of macro- and trace elements homeostasis of a human during various diseases (Podunova et al., 2003). As far as animals are concerned, the application of the method described is insufficient due to a high pollution level, which leads to the results of the research being incorrect.

Similar to the above is the method of cattle hair sample preparation for macro- and trace elements content analysis, including soaking for 10–12 hours and further exposure to washing environment (water), along with the degree of purification control of exogenous pollution substances. The exposure is conducted mechanically, providing high speed vertical movement of a sample in washing environment for 1 hour, using a mixer with 2 nozzles of circular rotations at 1000 rotations per minute. The level of purification is examined by evaluating the turbidity of washing solution (Usenko et al., 2007).

The disadvantages of the stated method are a long period of sample preparation, mechanic damage to hair structure together with the loss of endogenous trace elements, a loss of milled hair, the use of electric appliances and a poor adaptability to laboratory practice.

In this respect the search of more advanced methods of cattle hair sample preparations for trace elements analysis proves to be scientifically and practically significant, which determined the objective of the present work. Our study is the first to achieve an optimal level of animal dirty hair purification for trace elements metabolism pathology research in their organism. Both combined and originally consecutive applications of the suggested washing environments along with sample processing by an ultrasound acoustic wave of the designed frequency make it possible to obtain the necessary level of sample purity with minimal time, energy and labour costs.

Object and methods. The research was carried out in 2007–2010 on the basis of the Vitebsk State Academy of Veterinary Medicine and the laboratory of State Service of Medical and Forensic Expertise in Vitebsk Region of the Republic of Belarus.

During the working out of a hair sample preparation method of testing for trace elements, the total of 216 samples was used. The results obtained were checked on the basis of the industrial sector of the country, including over 800 fattening bulls of black and white breed and of a different age.

The hair was tested for the content of such mineral substances as Li7, Be9, Na23, Mg24, Al27, K39, Ca44, V51, Cr52, Mn55, Fe56, Co59, Ni58, Cu63, Zn65, Ga71, As75, Se79, Rb85, Sr88, Ag107, Cd112, In115, Cs133, Ba137, Hg200, Tl204, Pb207 and Bi209. Quantitative analysis of the elements was conducted by means of a

mass-spectrometry method with inductive combined plasma (ICP-MS) using the spectrometer Varian ICP-810-MS. While preparing biomaterial for analysis, the method of “wet” mineralization was used up to the total decomposition of the sample with the help of the microwave Mars Xpress, «CEM Corporation», USA.

A series of tests conducted was based on the comparison of mineral substances concentration for the research, using the five variants offered, with the known and the applied method (Usenko et al., 2007).

The practical realization of the methods described below was carried out with the help of sample-analogues, the sample of each tested animal being divided into 5 parts of the same size. Each sample-analogue had the same weight, length and visual level of pollution.

Conceptually, we took into consideration the fact that the subject of scientific research in the sample preparation context, regardless of any method details, is a range of consecutive steps. The latter include preliminary soak and/or washing in water (other fluids), mechanical or physical exposure to the washed sample and the step of repeated sophisticated washing (rinsing), drying and the analysis itself.

Therefore we applied methodically different approaches so as to obtain a maximum degree of cattle hair clearness from exogenous substances. The evaluation criteria of the obtained sample pureness were: 1) turbidity of the water (liquid) after washing detected by spectrophotometer (spectrophotometer *SOLAR PV 1251C*) with the wave length of 589 nm; 2) microscopy (magnification 200x) of the washed hair (microscope *LOMO-MIKMED 6*); 3) trace element content analysis of the sample by means of ICP-MS.

Mathematical evaluation of the achieved results was performed by the program «STATISTICA 8». At the first stage, the group data of the total statistics was calculated: the average arithmetic quantity (M) and the average error (m). Furthermore the visualization of the data sharing with the help of frequency bar chart was carried out and the variation coefficient by means of frequency histogram was determined (V). In order to choose the evaluation criterion of concernment of the paired differences, we checked the conformity of the form of dispersion to the normal using the criterion χ^2 , and we also controlled the equality of the general dispersions by using the F-criterion of Fisher. Taking into consideration that only a part of empiric sharing of the features was in compliance with such conditions, the checking of the hypothesis for the equality of the general averages in all cases was undertaken by means of the U-criterion of Mann-Whitney for independent variables. A zero hypothesis was rejected in case of $p < 0.05$. The value of the difference between the general parts (frequencies) was told according to the difference between random parts (frequencies) by means of the corresponding the Student t criteria.

Results. During the first experiment, a 2 g sample of hair was put into a laboratory glass of warm water ($t=40-60^{\circ}\text{C}$). One minute later, the water was discharged and the sample was put into clean warm water of the same temperature. The procedure was repeated until the water

in the glass became visually clear. The process described took 5.56 ± 0.153 minutes. After that, we put the hair samples into a sieve with a cell diameter of 2 mm, placed it under strong flowing water, destroying the sever dirtiness by a glass stick. The following procedure took on the whole 6.08 ± 0.244 minutes. Then we put the samples into distilled water and left them for 1 minute. The water was discharged and measured by means of spectrophotometer with the wave length of 589 nm. The procedure was carried on until we achieved sustainable extinction of the agent of about a zero. Thus to gain the desirable results the procedure had to be done 4–6 times.

After that, we took 10 hairs from each sample and studied them with a microscope (magnification 200*). Each hair was analyzed throughout the whole length, which was measured and the average amount of the exogenous substances was calculated per 1 cm. The amount was equal to 6.86 ± 0.230 substances per 1 cm of the hair. As a result, the level of pollution did not have statistically reliable differences (Mann-Whitney criterion). Then each sample was put into a glass with 96% ethanol for 1 minute and we studied the hair again with a microscope. The number of the exogenous substances per 1 cm of the hair at this stage of preparation for analysis was on average 0.22 ± 0.013 in all samples, varying from 0.15 to 0.44 substances. After that, the sample was dried in the drying device and tested by means of spectrometry in inductive combined plasma. Consequently, the whole preparation process of a single sample for analysis took 18–20 minutes. Herewith, a substantially higher degree of cleaning from exogenous substances was provided.

Comparison of the results obtained by analysis of the content of macro and trace elements in the hair sample prepared with the use of the tested and controlled methods showed a significant difference according to such elements as Na and Bi (42 – 43 %); Cr, Cu, Cd and Ba (32 – 36 %); Mg, Al, Fe, Ni and Zn (20 – 25 %). As for K and Co, no significant difference was observed. It has to be especially emphasized that there were significant variations in macro and trace elements content of the samples taken from the same animal. For statistical characteristics of the differences between the received data from the groups, we used the variation coefficient. The analysis of the received results showed that the index varied within a wide range (from 34 to 172 %), which demonstrates heterogeneity of the received index within the group. We assume it can be explained by non sufficient and non uniform level of hair cleaning prepared by the suggested method.

The second tested method was different from the previous one in the following way: after washing with distilled water and obtaining a washing agent of about zero extinction, the hair sample was put into a tube of water and centrifuged for 15 min at the speed of 3000 rotations per min. After the centrifugation, 10 hair samples were studied with a microscope. As a result, 5.64 ± 0.370 exogenous substances were detected per 1 cm of the hair. The statistical research showed that the purification of the hair using the second method is more

significant, since the level of pollution of the hair at this stage is substantially lower ($U=2$, $p<0.05$) than without centrifugation. To continue analysis, the samples were processed with 96% ethanol, in the same way as in the first variant. Further microscopic studies of the prepared hair detected 0.23 ± 0.016 exogenous substances per 1 cm, which can be interpreted as the absence of significant differences between the former and the latter methods. Such interpretations were confirmed after ICP-MS of the hair prepared in the way stated above. Biometrical evaluation of the results by means of mono factor dispersion analysis as well as calculating of criteria by Fisher, Kruskal-Wallis and Van der Waerden showed the absence of a major difference in data between the tested and controlled samples. Moreover, the same conclusion was made after conducting a comparative analysis of the first and the second test. Meanwhile, the dispersion of the sample features expressed by a coefficient of variation ranged from 35 to 173 %, which can be explained as a non uniform and non sufficient level of hair washing.

Our next method of hair preparation for analysis differs from the above by putting the sample into the magnet mixer instead of centrifugation. Previously the sample was cut into 1.5–2 cm long fragments and distilled water ($t=20-25^{\circ}\text{C}$) was used as a washing agent. The samples were left in the magnet mixer for 60 minutes. On studying the washed hair with a microscope, we detected 6.87 ± 0.115 exogenous substances per 1 cm. During the microscopic visualization of the samples on the matter of exogenous substance existence we noticed that the following procedure led to the damage of the hair structure.

The hair damage was suggested to contribute to the loss of endogenous macro and trace elements. The suggestion was confirmed during further ICP-MS of the hair when we detected that most of the tested samples prepared by such a method had significant differences both within group and among groups. Besides, the average amounts of the data received were by 16–25% lower than in the previous tests. Therefore the previously described method of sample preparation proved unacceptable to the laboratory practice.

Taking into consideration all the above results we continued our research in finding out an optimal method of cattle terminal hair preparation that could be in compliance with the suggested requirements of the most effective cleaning of the hair from exogenous pollution without losing endogenous mineral substances. As a result, we decided to affect the hair by using ultrasound in various types of washing fluid.

The first series of tests using ultrasound were based on putting a long hair sample weighing 2 grams and above into a glass of water. The glass was placed into a laboratory ultrasound tank with the frequency of 35 kHz. 60 minutes later, the water was changed and the procedure was repeated 6 times. The following manipulations proved to be ineffective because even the slightest visual changes of the pollution rate were not observed.

Furthermore, another method of hair sample

preparation suggested its 2–3 hour soaking in warm water including further irrigation under the water stream with simultaneous destroying of the dirt by a glass stick. After that the lab glass with the sample was filled with water and left in an ultrasound tank with the frequency of 35 kHz. 60 minutes later, the water was discharged and the hair was put into 96% ethanol and left again in an ultrasound tank for 60 minutes. After discharging ethanol, we placed the hair into bidistilled water and left it for 60 minutes into an ultrasound tank. When the procedure was over, we discharged the water and studied 10 hairs of each sample with a microscope. 0.059 ± 0.0058 exogenous substances per 1 cm of the hair were finally detected. ICP-MS of the hair did not show substantial difference between the stated and the known methods of hair preparation. However, the level of Mg, Al, Cu and Zn in the controlled group turned out to be lower (to a different extent) than in the tested groups. As a result, the described method of hair sample preparation proved very effective and required less efforts. Nevertheless, we decided to

make certain improvements and reduce the costs required.

After the initial processing of the hair with ultrasound, each sample was divided into 6 parts. Then the samples were put into laboratory glasses filled with ethanol and left in an ultrasound tank for 60 minutes. 6 different rates of ethanol concentration were applied respectively: 96%, 70%, 50%, 40%, 30% and 20%.

It should be stated that at this stage we observed substantial and contradictory differences in the adequacy of methodical approaches to sample preparation of the suggested and already known methods. Thus, at the stage of extinction of washing agent (double distilled water) the level of received numbers in the tested and controlled groups was comparable and varied from 0.001 to 0.003, indicating the proper level of hair cleaning.

However at the second stage of the hair cleaning analysis by studying it with a microscope, significant differences were detected both in the number of exogenous substances and in the structural hair integrity.

Table 1. **The level of pollution of the tested material** (the number of exogenous substances per 1 cm of hair) **depending on the concentration of C₂H₅OH**

Biometric Index	Concentration C ₂ H ₅ OH, %						Controlled
	96	70	50	40	30	20	
Mean	0.075	0.065	0.042	0.041	0.613**	0.914**	0.701**
Std Error	0.013	0.015	0.010	0.005	0.160	0.064	0.170
Std Deviation	0.020	0.019	0.013	0.011	0.358	0.442	0.321
Coefficient of Variation	26	30	31	27	49	48	44
Lower 95% CL for Mean	0.036	0.027	0.016	0.019	0.018	0.029	0.079
Upper 95% CL for Mean	0.114	0.104	0.069	0.063	1.209	1.798	1.341

Annex: ** - the level of importance of the reliable criterion $p < 0.01$

Mathematical calculations showed (Table 1) that 1 cm of the hair prepared by means of the already known method contained 0.7 exogenous substances (95% CL = 0.08 – 1.34), whereas the same sample in the tested groups with the concentration of ethanol from 96 to 40 % was 0.04 – 0.07; and in groups with the concentration of a washing agent (C₂H₅OH) from 20 to 30 % the level of exogenous substances was the same as in the controlled group and varied from 0.6 to 0.9 (95% CL = 0.02 – 1.79). Besides we should take into account the distinction between the variation coefficient in the groups exposed to ethanol processing with the concentration range 96–40% on the one hand and all the remaining groups on the other hand, which can be interpreted as a principal difference of sample preparation in these groups.

Moreover, during microscopy the damages of the structural integrity of the hair prepared by controlled method were detected, while in the suggested variant the structure of the hair stayed invariable.

The stated factors were represented in the results of ICP-MS of the hair content of mineral substances (Table 2). The samples prepared by means of 20 and 30% ethanol have statistically reliably (different degree of reliability) higher numbers, statistically confirmed, in comparison with all the other methods regarding the

content of such elements as Na (68–107 %), Mg (28–47 %), K (42–62 %), Fe (28–54 %), Cu (32–73 %) and Zn (33–48 %).

It should be stated that a variation coefficient in the stated groups was substantially higher than the determined level of 33 % (see Table 2), which points out the dissimilarity of the individual meanings within the sampling.

Discussion and conclusions

Comparative analysis of the suggested procedure of cattle hair sample preparation and prototypes shows that significant technological and qualitative distinctions were achieved during the testing. Thus the time of preliminary soaking of the samples is 2–3 hours as compared with 10–12 hours of the control method. Alongside with distilled water we recommend to use 40 % ethanol as a washing agent. The most important distinctions regard the damaging mechanical exposure to the hair structure of mixer nozzles (the loss of endogenous trace elements demonstrated by us experimentally); besides significant external hair pollutions are left when we use the control method while the terminal degree of the washing agent extinction remains the same. The experimentally received mentioned distinctions are sure to affect the objectivity of the result.

Table 2. The content of mineral elements in the terminal hair samples depending on the concentration of C₂H₅OH

Element	Groups of Samples						
	Tested						Controlled
	96 %	70 %	50 %	40 %	30 %	20 %	
M±m, V							
Li, ng/kg	<1	<1	<1	<1	<1	<1	<1
Be, ng/kg	<3	<3	<3	<3	<3	<3	<3
Na, mg/kg	17.48±2.481; 31.7	21.51±3.090; 32.1	31.96±4.505; 31.5	19.87±2.973; 32.4	38.19±12.559; 73.5	47.18±14.972; 71.0	17.70±2.664; 35.9
Mg, mg/kg	360.7±54.87; 28.5	421.9±63.07; 33.4	317.4±47.831; 32.1	255.3±38.35; 32.9	434.8±149.33; 76.7	496.3±142.71; 64.3	256.4±34.39; 35.1
Al, mg/kg	1.30±0.240; 33.5	1.630±0.237; 32.4	1.250±0.203; 31.5	0.971±0.132; 30.5	1.515±0.239; 41.8	1.748±0.238; 36.2	0.992±0.152; 39.3
K, mg/kg	32.9±5.298; 35.9	29.9±4.418; 33.0	36.6±5.043; 30.8	40.7±5.960; 32.7	50.5±14.811; 65.6	56.7±17.220; 67.9	30.5±4.919; 42.7
V, ng/kg	<3	<3	<3	<3	<3	<3	<3
Cr, mg/kg	0.382±0.050; 29.2	0.520±0.085; 28.9	0.884±0.152; 35.0	1.068±0.123; 25.7	0.912±0.134; 43.8	1.141±0.233; 45.7	0.381±0.064; 37.5
Mn, mg/kg	5.628±0.892; 33.7	6.075±0.832; 30.6	7.052±1.200; 33.8	7.622±1.197; 31.1	8.144±1.166; 44.3	8.846±1.262; 36.4	5.637±0.870; 36.3
Fe, mg/kg	31.65±4.449; 31.4	35.15±5.437; 31.7	40.26±5.466; 30.4	34.05±5.524; 30.4	45.12±13.582; 67.3	54.30±16.849; 69.4	32.06±7.636; 53.2
Co, mg/kg	0.077±0.014; 32.8	0.109±0.023; 34.1	0.116±0.022; 34.6	0.123±0.016; 29.8	0.132±0.024; 40.0	0.139±0.026; 41.5	0.078±0.017; 49.5
Ni, mg/kg	0.299±0.040; 29.6	0.326±0.056; 34.6	0.385±0.056; 32.4	0.416±0.062; 28.5	0.457±0.096; 46.9	0.503±0.108; 48.2	0.310±0.050; 48.7
Cu, mg/kg	2.925±0.421; 32.2	3.584±0.598; 31.7	4.072±0.684; 32.6	4.732±0.650; 30.7	5.022±1.826; 81.3	6.611±1.903; 64.4	3.012±0.534; 39.6
Zn, mg/kg	58.32±8.834; 33.7	65.32±9.630; 33.0	76.39±11.870; 32.1	88.79±13.48; 33.9	96.03±24.356; 56.7	106.83±27.67; 57.9	58.29±11.370; 43.6
Sr, mg/kg	3.842±0.553; 32.2	4.364±0.654; 33.5	5.007±0.703; 31.4	5.827±0.869; 33.4	6.397±1.312; 45.9	6.917±1.385; 44.8	3.915±0.760; 43.4
Ga, ng/kg	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
As, ng/kg	<20	<20	<20	<20	<20	<20	<20
Se, ng/kg	<400	<400	<400	<400	<400	<400	<400
Ag, ng/kg	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6
Cd, mg/kg	0.042±0.014; 76.5	0.063±0.016; 57.7	0.073±0.013; 39.8	0.095±0.027; 41.5	0.109±0.022; 45.1	0.111±0.021; 42.3	0.048±0.018; 84.5
In, ng/kg	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Cs, ng/kg	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Ba, mg/kg	1.754±0.246; 31.3	2.514±0.366; 32.6	3.366±0.580; 38.5	2.905±0.466; 35.8	3.368±0.699; 46.4	4.038±0.858; 47.2	1.846±0.364; 44.1
Hg, ng/kg	<1	<1	<1	<1	<1	<1	<1
Tl, ng/kg	<1	<1	<1	<1	<1	<1	<1
Pb, ng/kg	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
Bi, mg/kg	0.013±0.008; 139.2	0.022±0.009; 95.7	0.027±0.011; 95.9	0.031±0.010; 68.6	0.042±0.011; 58.6	0.045±0.011; 54.2	0.015±0.009; 136.0

Annex: 1) Symbol «□» – the real threshold of the sensitiveness of the device, detected during testing;
2) Elements sequenced according to increasing atomic weight.

A series of the conducted researches demonstrates that native cattle hair has a high level of endogenous pollution, which can be detected both visually and by means of ICP-MS. The following phenomenon is expressed by a very high variation of a single index (element) both within the same group and individual. Meanwhile a coefficient of

variation reaches 150–200 % which contributes to the heterogeneity of the achieved results and the importance of the received variations; this demonstrates the impossibility of putting methodical approaches described in tests 1–3 into routine laboratory practice. The analysis of Table 2 also shows a significant variability of the

element content within hair samples prepared by the control method: the coefficient of variability was 36–76 %. Within groups 1–4, this index generally varied from 28 to 32 %. Due to biometry laws such a result can be interpreted as a considerable degree of mutability of the variation row, supposing its uniformity. It is essential to point out that despite general appropriateness of the uniformity of the values obtained in the groups regarding such elements as Cd and Bi there was no uniformity achieved during our research, and the coefficient of variation for the elements above was 41–139 %.

The concentration of such elements as Na, Fe, Co, Ni, Sr, Cd, Ba and Bi in the control group was at the average level, typical of the qualitatively prepared testing material, while the quantity of Mg, Al, K, Mn, Cu and Zn was lower (to a various extent). The stated differences indicate the loss of endogenous trace elements pool in the hair samples prepared by the control method due to their mechanical damages done by the mixer parts and aggravated by distorting the result because of the admixture left after sample preparation.

The effectiveness of hair sample preparation using 96, 70, 50 and 40% ethanol do not have significant differences and thus should be interpreted as equivalent positions to achieve the desirable results.

Thus, our study is the first to represent the possibility of achievement of technical results in polluted animal hair purification for macro and trace elements content analysis, as long as consecutive application of the following washing fluids take place: bidistilled water – 40% ethanol – bidistilled water processed with an acoustic wave at a frequency of 35 kHz in an ultrasound tank. The suggested procedure makes it possible to obtain the necessary level of sample purity with minimal time, energy and working costs.

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