

# Analysis of Trace Elements in the Hair of Farm Animals by Atomic Emission Spectrometry with Dc Arc Excitation Sources

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## Abstract

To determine the essential, potentially dangerous and toxic elements in farm animal's hair the atomic emission spectral (AES) methods with dc arc and two-jet arc plasmatron were developed. Two approaches – carbonization and ashing of hair were compared. The developed techniques allowed us to determine up to 16 trace elements at concentration from 0.01 to 1000 ppm wet in bristle and hair from several farm animal species.

**Keywords:** AES analysis, determination of microelements, farm animal's hair, two-jet arc plasmatron.

## INTRODUCTION

There are many articles devoted to the description of techniques for determination of essential, potentially dangerous and toxic elements in a human hair and animal's organs and tissues [1-5]. Most of published articles are devoted to a human hair analysis. A wealth of information about composition of a human hair were accumulated; a standard level of microelements in the hair of healthy people, in the hair of people suffering from various diseases or living in ecologically safe area and in area with high levels of pollution was defined [6-19]. There is also a series of articles devoted to the analysis of animal hair, such as cows, rats, dogs and others [6, 20]. Fragmentary data are focused on the content of Cd, Cu, Pb, Zn and certain radionuclides – <sup>137</sup>Cs, <sup>90</sup>Sr [21-25]. It should be noted that control of the content of essential, potentially dangerous and toxic microelements in tissues of the farm animals is an important task, as the meat and milk of these animals are consumed [26]. Therefore the necessity of enhance knowledge in this field determines the relevance of the development of an express multi-element techniques.

Analysis of animal hair is one of the most fast and effective way to obtain adequate information about deficiency or excess of elements in the organism, availability or degree of anthropogenic pollution. This approach is used for the diagnosis of disease caused by a violation of mineral metabolism. In literature many methods are described, the most of them are based on pre-dissolved of experimental samples in a mixture of acids at high temperature. This pre-treatment makes longer the analysis; increases the likelihood of contamination of samples and increases the loss by decomposing impurities. In a few studies, X-ray techniques are described [27, 28]. Technique of AES analysis for biological samples (including bristle of rats) by two-jet arc plasmatron has

been proposed in our lab earlier [29]. Recently "VMK-Optoelectronic" Ltd. developed a new version of a two-jet arc plasmatron "Fakel" for the AES spectrometer "Grand". The simplicity of sample preparation is a significant advantage of the developed analytical instrumentation [30].

## MATERIALS AND METHODS

The AES complex based on the spectrometer "Grand" [31] with new two-jet arc plasmatron (TJAP) "Fakel" (VMK-Optoelectronic, Russia) [32] was used. The two-jet arc plasmatron "Fakel" has an output of 10 ÷ 15 kW and provides an opportunity to carry out elemental analysis of powder samples. The analysis was performed under the following operation conditions: current – 85 A; plasma and carrier argon flow rates – 4.0 and 0.75 L/min respectively; detection zone – 25 mm from the base of the plasma torch. The zone ahead the merging of jets of plasmatron was focused on the slit of a Grand spectrometer with a 2400 lines/mm grating.

The AES complex based on the spectrometer "PGS-2" (Carl Zeiss Jena, Germany) with direct current arc (DCA) was used. Spectra were registered using multichannel analyzer of emission spectra MAES and generator "Fireball" (VMK-Optoelectronics, Russia). The analysis was performed under the following conditions: current – 13 A, the dispersion of the spectrograph – 0.74 hatch/mm, total exposition – 20 s.

For both methods, determination of impurities was carried out using calibration samples (CSs) based on graphite powder. CSs were prepared from the certified reference material such as GSO 2820-83 SPG-27 [33].

ICP-AES method was used as the reference method. The measurements were performed with the use of a high-resolution spectrometer iCAP-6500 (Thermo Scientific, USA). The plasma was observed axially to

obtain the best possible sensitivity (in a wavelength range of 166–847 nm). The spray system consisted of the concentric pneumatic nebulizer “SeaSpray” and the spray chamber of cyclone type “Tracey”. Measurements were carried out under conditions recommended by the manufacturer.

For the ICP-AES analysis deionized water (18.2 M $\Omega$ , Direct-Q3, Millipore) and high-purity nitric acid distilled by subboiling distillation (HNO<sub>3</sub> ~ 14 M, DuoPUR, Milestone) were used. For preparation of CSs the multi-element standard solutions (MES, Scat Company, Russia) were used. Argon of 99.996 purity was used.

Sample preparation procedure included following steps: sort of contaminants (straw, leather); washing with liquid soap, deionized water, and acetone. Then heat pretreatment in the quartz furnace or muffle under air atmosphere was performed.

The procedure of the sample carbonization was as follows: the hair was weighed in a quartz cup after washing (m~100 mg). Carbonization was carried out in two steps: (1) 250°C, 15 minutes; (2) 450°C, 15 minutes. Further, the furnace was turned off and cooled to the room temperature without removing cup with sample. Additionally after carbonization sample was powdered in agate mortar. Then spectrographic buffer (high purity graphite containing 4% NaCl for DCA-AES analysis and 15% NaCl for TJAP-AES analysis) was added to resulting sample.

The procedure of the sample ashing: the hair was weighed in a quartz cup after washing (m~200 mg). The ashing was carried out in two steps: (1) 250°C, 15 minutes; (2) 450-500°C, 2-2.5 hours. Further, the furnace was cooled to the room temperature. The resulting samples were homogeneous. Samples were diluted with spectrographic buffer (high purity graphite with NaCl). The dilution factor was 2, 10, 100, 500, 1000 and 5000.

## RESULTS AND DISCUSSION

### *Temperature pretreatment*

The heat treatment may be carried out in two variants – carbonization and ashing. After carbonization procedure, the samples look as a black powder containing the most of the organic components. In this case, the mass of each sample was slightly decreased. After ashing, the samples represent a light-toned powder and their mass was significantly reduced. In the first case, temperature of the process was notably higher than that of the second case, for the reason the probability of microelement’s loss is increased.

The Table 1 summarizes the results of pig bristles analysis by DCA-AES method. The contents of Ag, Bi, Cd, Cr, Mo, Ni, Ti are below the LODs (<0.01-1  $\mu$ g/g). Results for Al, Ca, Cu, Mg and Mn are in a good agreement within the confidence limits for different heat treatment. For Fe and Si the results are limited by the control experiment that leads to inability of correct determination of their content. For Zn in the case of carbonization the relative standard deviation (RSD, %) was 20 % (like a typical RSD for DCA-AES method) but this element in the case of ashing was lost. We have partial and uncontrolled losses of this element. In addition, attention was paid to the fact that

RSD, % of determination after carbonization procedure is higher than after ashing procedure. The reason of this fact is in the presence of an organic component in the bristle, which partially remains after a short heat treatment.

Thus, RSD, % and LODs for ashing are low due to the complete removal of organics. However, this pretreatment procedure leads to loss of important element – Zn. Unlike ashing, the carbonization procedure allows determining of Zn, but LODs for all elements are higher because of significant dilution of the samples (100-1000 times).

### *Analysis by Two-Jet Arc Plasmatron*

It was earlier shown that the effect of organic matrices on the analytical signals in TJAP is lower than in DCA [29]. For this reason, in TJAP-AES the carbonization procedure with minimum dilution factor (2-10-25) can be used. Table 2 shows the results of hair analysis from Hereford steers for different dilution factor.

The contents of Bi, Cd, Co, Cr, Mo, Nb, Ti, W are lower LODs. The results of Al, Fe, Mg, Mn, Si and Zn analysis are in a good agreement for different dilution factor. It should be noted, that RSDs were not more than 15 %, thus new two-jet arc plasmatron “Fakel” in combination with the carbonization procedure pretreatment provides satisfactory metrological characteristics.

### *LODs*

For calculating of LODs the blank sample spectra was used. A graphite powdered sample containing NaCl was used as a blank sample. The LODs were defined as the concentration of analyte that produced a signal equal to 3 times standard deviation of the background fluctuation ( $n \geq 10$ ) [34]. LODs for DCA-AES analysis (dilution coefficient of 10 was taken into account) are show in the Table 3. LODs for AES technique with TJAP (dilution coefficient of 2 was taken into account) are shown in the Table 4. The LODs of Ca, Fe and Si were limited by the blank experiment, which does not affect their determination in hair samples due to the relatively high contents.

It should be noted that Ag and Cu can be determined by DCA-AES technique only (plasma touch of TJAP is made of copper and high blank signal of Ag and Cu was observed), whereas Co, Nb and W can be determined by TJAP-AES technique. LODs for two techniques are comparable.

### *Validation of analysis*

Table 5 shows results for bull hair analysis by DCA-AES, TJAP-AES and ICP-AES. ICP-AES analysis was carried out after chemical decomposition of samples in high purity HNO<sub>3</sub> in microwave system MARS-5. The results of different techniques are in a satisfactory agreement within the confidence level, that fact confirms accuracy of the results.

### *Correlation analysis*

Interactions between microelement’s contents in bull bristles were investigated by the correlation analysis. The statistical analysis of the data was carried out using the package “STATISTICA 6.0”. The correlations between

each pair of elements in the samples are shown in Table 6. Because the parameters were not normally distributed, a Spearman's correlation test was used ( $R_s$ ).

As it can be seen from Table 6 the positive correlation is in the pairs of Al/Fe ( $R_s=0.75$ ) and Mn/Zn ( $R_s=0.66$ ). Scatterplots on Fig. 1 shows the relationships between concentrations of Al/Fe and Mn/Zn in the hair of Hereford steers. It is known that Al inhibits assimilation of

Ca, Mg, Fe, B<sub>6</sub>, ascorbic acid. Ascorbic acid, in turn, promotes the absorption of Fe, i.e. the more Al enters into the body, and the less digestible is Fe. Zn promotes the absorption of vitamin E. Vitamin E, in turn, promotes absorption of Mn [35]. Perhaps, the obtained correlations reflect these processes.

**Table 1. The effect of different sample preparation techniques (carbonization and ashing) on the element's evaluation in the pig bristle,  $\mu\text{g/g}$  ( $P=0.95$ ,  $n=4-6$ )<sup>a</sup>**

Element $\lambda$ , nm		Carbonization		Ashing	
		Found	RSD <sup>b</sup> , %	Found	RSD, %
Ag	328.068	n/d <sup>c</sup>	-	n/d	-
Al	308.215	$(1.1\pm 0.9)\cdot 10^1$	33	$7.4\pm 1.7$	22
Bi	306.772	n/d	-	n/d	-
Ca	317.933	$(8\pm 3)\cdot 10^1$	29	$(1.3\pm 0.1)\cdot 10^2$	8
Cd	228.874	n/d	-	n/d	-
Cr	284.324	n/d	-	n/d	-
Cu	324.754	$7.2\pm 2.6$	23	$7.2\pm 2.1$	27
Fe	302.064	Blank	-	Blank	-
Mg	277.983	$(5.5\pm 1.3)\cdot 10^1$	19	$(7.8\pm 1.0)\cdot 10^1$	12
Mn	280.108	$1.0\pm 0.3$	28	$1.3\pm 0.2$	18
Mo	317.035	n/d	-	n/d	-
Ni	300.249	n/d	-	n/d	-
Si	288.157	Blank	-	Blank	-
Ti	308.803	n/d	-	n/d	-
Zn	213.856	$(1.3\pm 0.4)\cdot 10^2$	20	losses	-

a – the results of analysis are shown as the average between different dilution factors;

b – relative standard deviation;

c – not detected.

**Table 2. The results of the element's evaluation with different dilution factor in the Hereford steer's hair,  $\mu\text{g/g}$ .**

Element	Dilution factor		Element	Dilution factor	
	K=2.9	K=29.4		K=2.9	K=29.4
Al	$4\cdot 10^1$	$4\cdot 10^1$	Mn	$5\cdot 10^1$	$4\cdot 10^1$
Bi	n/d <sup>a</sup>	n/d	Mo	n/d	n/d
Ca	-	-	Nb	n/d	n/d
Cd	n/d	n/d	Ni	n/d	n/d
Co	n/d	n/d	Si	$1\cdot 10^2$	$1\cdot 10^2$
Cr	n/d	n/d	Ti	n/d	n/d
Fe	$1\cdot 10^2$	$1\cdot 10^2$	W	n/d	n/d
Mg	$5\cdot 10^2$	$4\cdot 10^2$	Zn	$3\cdot 10^1$	$2\cdot 10^1$

a – not detected.

**Table 3. The values of LODs for DCA-AES,  $\mu\text{g/g}$ .**

Element	Carbonization	Ashing	Element	Carbonization	Ashing
Ag	0.05	0.01	Mg	0.4	0.07
Al	0.5	0.08	Mn	0.1	0.03
Bi	0.2	0.08	Mo	0.4	0.07
Ca	4	0.80	Ni	0.4	0.09
Cd	0.1	0.02	Si	4.0	0.80
Cr	0.4	0.09	Ti	0.5	0.09
Cu	0.1	0.03	Zn	0.4	-
Fe	0.4	0.09			

**Table 4. The values of LODs for TJAP-AES, µg/g.**

Element	Carbonization	Element	Carbonization
Al	0.09	Mn	0.06
Bi	0.3	Mo	0.1
Ca	0.2 (blank)	Nb	0.9
Cd	0.08	Ni	0.3
Co	2	Si	3 (blank)
Cr	0.3	Ti	0.2
Fe	0.3 (blank)	W	0.7
Mg	0.1	Zn	0.3

**Table 5. The comparison of the results on element's evaluation of the Hereford steer's hair by different methods, µg/g.**

Element	DCA-AES (P=0.95, n=3)	Two-Jet Arc AES (P=0.95, n=3)	ICP-AES (P=0.95, n=3)
Al	n/d <sup>a</sup>	4.0±0.3	n/d
Ca	(3.1±1.1) 10 <sup>2</sup>	(2.2±0.7) 10 <sup>2</sup>	(2.2±0.2) 10 <sup>2</sup>
Cu	8.8±1.1	b	7.8±0.4
Fe	(2.2±0.4) 10 <sup>1</sup>	(2.2±0.1)·10 <sup>1</sup>	(2.1±0.8)·10 <sup>1</sup>
Mg	(5.9±1.5) 10 <sup>1</sup>	(4.3±0.3)·10 <sup>1</sup>	(4.3±0.4)·10 <sup>1</sup>
Mn	1.1±0.1	1.1±0.6	(9.5±0.5) 10 <sup>-1</sup>
Zn	(2,0±0,6) 10 <sup>2</sup>	c	(1,5±0,6) 10 <sup>2</sup>

a – not detected;

b – plasma heads is made by copper;

c – no data.

**Table 6. The relationship between the content of different elements in the Hereford steer's hair (Spearman Rank correlation coefficients, n=17).**

Element	Al	Cu	Fe	Mg	Mn
Al	-	-	-	-	-
Cu	0,29	-	-	-	-
Fe	<b>0,75<sup>a</sup></b>	0,28	-	-	-
Mg	0,06	-0,49	-0,06	-	-
Mn	-0,43	0,01	-0,42	-0,11	-
Zn	-0,31	0,24	-0,20	-0,31	<b>0,66<sup>a</sup></b>

a - correlation coefficients are significant at p &lt; 0.05

### CONCLUSION

In this work, the capabilities of the AES methods with DCA and Two-Jet for analysis of farm animal's hair were studied. The effect of heat pretreatment was investigated. Graphite powder and NaCl were used as spectrographic buffers. The interactions between microelements in bull hair were investigated by the correlation analysis. Positive correlations in the pairs Al/Fe ( $R_s=0.75$ ,  $p<0.05$ ) and Mn/Zn ( $R_s=0.66$ ,  $p<0.05$ ) were found.

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