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Evaluation of sample pre-treatment procedures for the determination of Cr, Ni and V in biological matrices by ETAAS

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Electrothermal atomic absorption spectrometry (ETAAS) was applied for the determination of chromium, nickel and vanadium in biological materials (earthworms, feather, lucerne). Three different decomposition procedures of biological matrices are presented. The utilization of $NH_4H_2PO_4$, $Mg(NO_3)_2$ and $Pd(NO_3)_2$+ $Mg(NO_3)_2$ as chemical modifiers are investigated and compared. Optimal temperature conditions for the electrothermal determination of Cr, Ni and V are selected. Precision of the determination expressed as relative standard deviations for Cr, Ni and V varied from 6 to 22% . The detection limits are for Cr 8 μg kg^{-1}, Ni 12 μg kg^{-1}, and V 36 μg kg^{-1}. The accuracy of the measured data were verified by determination of the studied elements in certified reference materials. The attained analytical results were in good agreement to each other by all applied methods, as well as to the certified values. Consequently, the method was applied to biological matrices: earthworms, feathers and lucerne which were sampled at the refinery immission site.

Key Words: decomposition, electrothermal atomic absorption spectrometry, Cr, Ni, V determination, biological materials.

Introduction

Analytical procedure optimization is based on the selection of fundamental working conditions (sampling, pretreatment, selectivity, linearity, determination, range, sensitivity, limit of detection, precision and accuracy, etc.) according to which the suitability of a selected method may be evaluated.

One of the most important steps in the analytical procedure for trace element determination in biological matrices is the sample decomposition method. The purpose of such pretreatment is to attain a perfect digestion of organic matrix, decrease in viscosity, increase in homogeneity and release of analytes from various compounds and phases as well as that of component removal which have adverse impacts on the analytical signal.

Requirements for organic matrix removal in analysed samples by particular analytical techniques are rather different. By ETAAS or ICP AES methods with “direct sampling”, or “slurry sampling”, the sample

of biological material is decomposed directly in an apparatus, i.e. "in situ"[1,2].

The total decomposition of samples having biological matrices is possible by performing dry mineralization, i.e. carbonization at temperatures between 200 and 400°C[3], followed by ashing at temperatures between 450 and 550°C, over 10 - 16 h. The decomposition of a sample is, in special cases, performed with the addition of "ashing aid" (HNO_3, $Mg(NO_3)_2$, H_2SO_4, K_2SO_4, or others) in an open, half-closed or closed system and then by leaching with diluted acids HCl and HNO_3[3,4]. The utilization of closed systems is satisfactory for the majority of biological materials. They are rapid, prevent losses of volatile elements (i.e. Pb, As, Se, Cd and others) and have a reduced risk of contamination[5]. The other commonly used decomposition procedure is wet mineralization in various mixtures of concentrated mineral acids and oxidizing agents (HNO_3, $HClO_4$, H_2SO_4, HF and/or H_2O_2)[6,8], by enhanced temperature in an open system using conventional or microwave heating[1,9,10]. Finally, by enhanced temperature and pressure in closed systems (except $HClO_4$) again with conventional[11] or microwave heating[12−14].

Generally, electrothermal atomic absorption spectrometry (ETAAS) for the determination of trace elements in biological samples used to be applied due to the low detection limits found for many elements[15,16]. Chemical modifiers in solution such as $NH_4H_2PO_4$, $Pd(NO_3)_2$ or $Mg(NO_3)_2$ have been demonstrated to be adequate to reduce matrix effects, so that calibration with aqueous standards can be successfully applied[17,18]. Due to the carbide forming processes (V, Cr), some authors used to treat the graphite tubes by impregnating them with metal compounds[15,19,20].

In the present paper, the efficiency of these three decomposition procedures for different kinds of biological matrices were statistically evaluated by Box and Whisker plots. An optimal temperature programme developed for the determination of Cr, Ni and V by ETAAS in model solutions (with Ca, Mg, Na, K content) and 3 modifiers as well as without modifiers was chosen. The sensitivity, precision and limits of detection for studied elements were calculated. The statistical evaluation of the obtained results (t-test, F-test) was made[21]. The verification of the accuracy of the results by ETAAS, and by an independent analytical method (AES-ICP), and by CRMs, was performed. The optimized procedure for Cr, Ni and V determination by ETAAS was applied to soil samples, soil EDTA extracts, lucerne, earthworms and feathers for the study of metal transfer.

Experimental

Apparatus

Microwave Digestion System, Model MDS - 81D (CEM Corporation, USA), was applied for the sample decomposition.

A Varian Techtron model SpectrAA-10 Atomic Absorption Spectrometer, equipped with Graphite Furnace Varian GTA-95 (Mulgrave, Victoria, Australia) and Varian pyrolytic graphite tubes were used for atomic absorption measurements.

Atomic Emission Spectrometer with Inductively Coupled Plasma, sequential, PLASMAKON S 35, KONTRON, (Germany). Working conditions: plasma power -1.5 kW, Ar flow-rates: outer 15 L/min, inner 0.7 L/min, carrier (Ar) 1.2 L/min. Sample uptake rate 1.5 L/min (peristaltic pump), nebulizer – glass, concentric, Meinhard Type B, integration time 5 s, Wavelengths: Cr 267.716 nm, Ni 232.003 nm, V 292.402 nm (all measurements were performed with background correction).

Standards and reagents

Working standard solutions of Cr, Ni and V were prepared by serial dilution of 1000 mg.L^{-1} metal stock solutions (Alfa Ventron, Karlsruhe, Germany). $CaCO_3$, MgO, KCl and NaCl (Specpure, Johnson Matthey, London, UK), were used for the preparation of model hair solution. Analytical-reagent grade nitric (65% v/v) and hydrochloric (35% v/v) acids (Lachema, Brno, Czech Republic) were additionally purified by sub-boiling distillation; perchloric acid (70% v/v); H_2O_2 (30% v/v) and redistilled water were also used. Matrix modifier solutions $NH_4H_2PO_4$ (20 g/L); $Mg(NO_3)_2$ (1 g Mg/L) and $Pd(NO_3)_2$ (1 g Pd/L in 1% HNO_3, v/v) + $Mg(NO_3)_2$ (1 g Mg/L) = 2 + 1 (Slovak Institute of Metrology, Bratislava, Slovakia) were chosen. Certified biological reference materials CRM 07601 (Human hair, Langfang, China), CRM 184 (Bovine muscle, BCR, Brussels, Belgium), CRM 185 (Bovine liver, BCR, Brussels, Belgium) and CRM 12-2-03 (Lucerne P-ALFALFA, SIM, Bratislava, Slovakia) were used in the experiments.

Digestion procedures

1) Acid digestion ($HNO_3 + H_2O_2$) at elevated pressure in microwave oven. The samples in the PTFE vessels were heated by the following program: (1) Power = 100% (600 W), Time = 5 min; (2) Power = 80% (460 W), Time = 30 min; (3) Power = 0, Time = 15 min. After cooling, 1 mL of 30% H_2O_2 was added.

2) Acid decomposition ($HNO_3 + H_2O_2$ + $HClO_4$) at atmospheric pressure. To the biological samples placed in the glass beakers, 5 mL of H_2O_2 were added. This mixture was allowed to stand for 3 h. Then, 7 mL of HNO_3 was added. The samples were left overnight and then evaporated to wet salts. Five mL H_2O_2 and 1 mL conc. $HClO_4$ were added and heated on a hotplate until final evaporation to 1 mL.

3) Ashing and acid decomposition (HNO_3+ HCl) at atmospheric pressure. The samples were dry-ashed in a muffle furnace at 500^{o}C for 10 h and then treated with an acid mixture (8 mL conc. HNO_3 + 2 mL conc. HCl).

A 0.5 g portion of each of the CRM samples were decomposed five times by the selected method. The solutions were transferred into a 50 mL calibrated flask, diluted to volume with doubly distilled water, and filtered through a dry filter. The blank solutions were prepared by the same procedure.

Results and Discussion

Pretreatment and atomization temperatures

Electrothermal Atomic Absorption Spectrometry was used for the direct determination of low concentrations of Cr, Ni and V in the biological samples. The optimal experimental conditions were determined by analyses of model solutions, which contained: Ca (500 mg/L), K (400 mg/L), Na (300 mg/L), Mg (80 mg/L). The solutions were spiked with studied elements 20 μg.L^{-1}Cr, 30 μg.L^{-1}Ni, and 100 μg.L^{-1}V, respectively. These model solutions were treated with the addition of the followed modifiers: $NH_4H_2PO_4$, $Pd(NO_3)_2$+$Mg(NO_3)_2$ and $Mg(NO_3)_2$. The pretreatment and atomisation curves selected for Cr, Ni and V in prepared synthetic solutions are presented in Figure 1. From these curves, the optimum drying, charring and atomization temperatures and the choice of suitable modifier $Pd(NO_3)_2$ + $Mg(NO_3)_2$ for Cr and Ni, though without a modifier for V, were determined. The optimal graphite furnace temperature program and spectrometer working conditions for determined elements are summarized in Table 1.

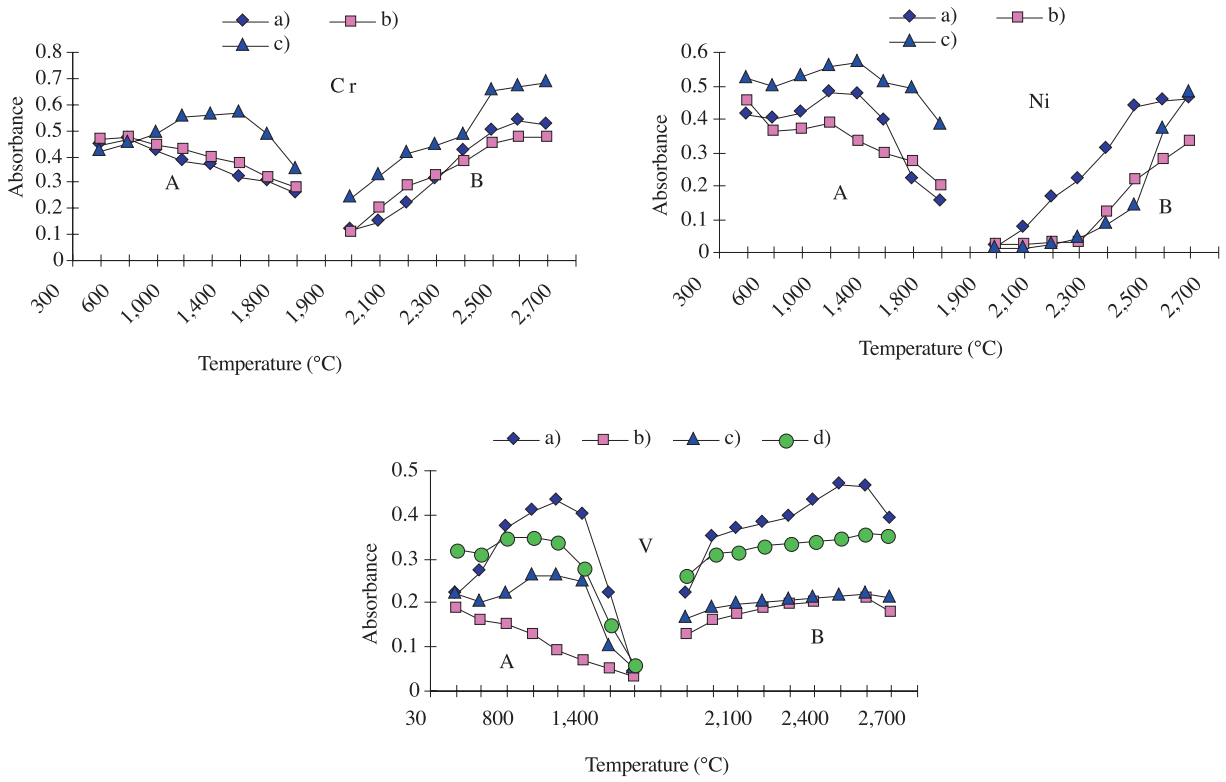

Figure 1. Pretreatment (A) and atomization (B) curves obtained for Cr, Ni and V in model solution (20 μg.L^{-1} Cr; 30 μg.L^{-1} Ni; 100 μg.L^{-1}V) **a)** without modifier; **b)** with modifier $Mg(NO_3)_2$; **c)** with modifier $Pd(NO_3)_2$+$Mg(NO_3)_2$; **d)** with modifier $NH_4H_2PO_4$;

Table 1. Optimized instrumental settings for ETAAS determinations of Cr, Ni and V in biological samples

Element	Cr	Ni	V
Wavelength (nm)	357.9	232.0	318.5
Slit width (nm)	0.2	0.2	0.2
Background correc.	-	D_2	-
Dry (^{o}C) / Time (s)	130 / 60	130 / 60	130 / 60
Char (^{o}C) / Time (s)	1,400 / 20	1,300 / 20	1,300 / 20
Atomize (^{o}C) / Time (s)	2,450 / 3	2,400 / 3	2,550 / 3
Clean (^{o}C) / Time (s)	2,600 /2	2,600 / 2	2,650 / 2

Peak height mode; Internal gas (Ar) flow 300 mL.min^{-1},
Matrix modifier: $Pd(NO_3)_2$ + $Mg(NO_3)_2$ for Cr and Ni
without modifier for V
Batching of volume: 20μl sample + 10 μl matrix modifier

Selection of digestion method

The efficiency of three digestion procedures using CRM GBW 07601 (Human Hair) was tested: 1. procedure - acid digestion (HNO_3 + H_2O_2) at elevated pressure in a microwave oven; 2. procedure - acid decomposition

(HNO_3 + H_2O_2 + $HClO_4$) at atmospheric pressure; 3. procedure - ashing at 500 °C and acid decomposition (HNO_3+ HCl) at atmospheric pressure. Samples were digested five times by the above described methods. The determinations of studied elements in model solutions were performed at optimized ETAAS conditions, and each sample solution was measured five times. The results obtained by the application of different digestion procedures were statistically processed by the graphical plots in a Box and Whisker form (Statgraphic Software Package). Box and Whisker diagrams for the three chosen elements of V, Cr and Ni are illustrated in Figure 2. The central box covers the middle 50% of the data values, between the lower and upper quartiles. The crossed line in each box is the median. The whiskers extend only to those points that are within 1.5 times the box length (interquartile range). The results expressed graphically by Box and

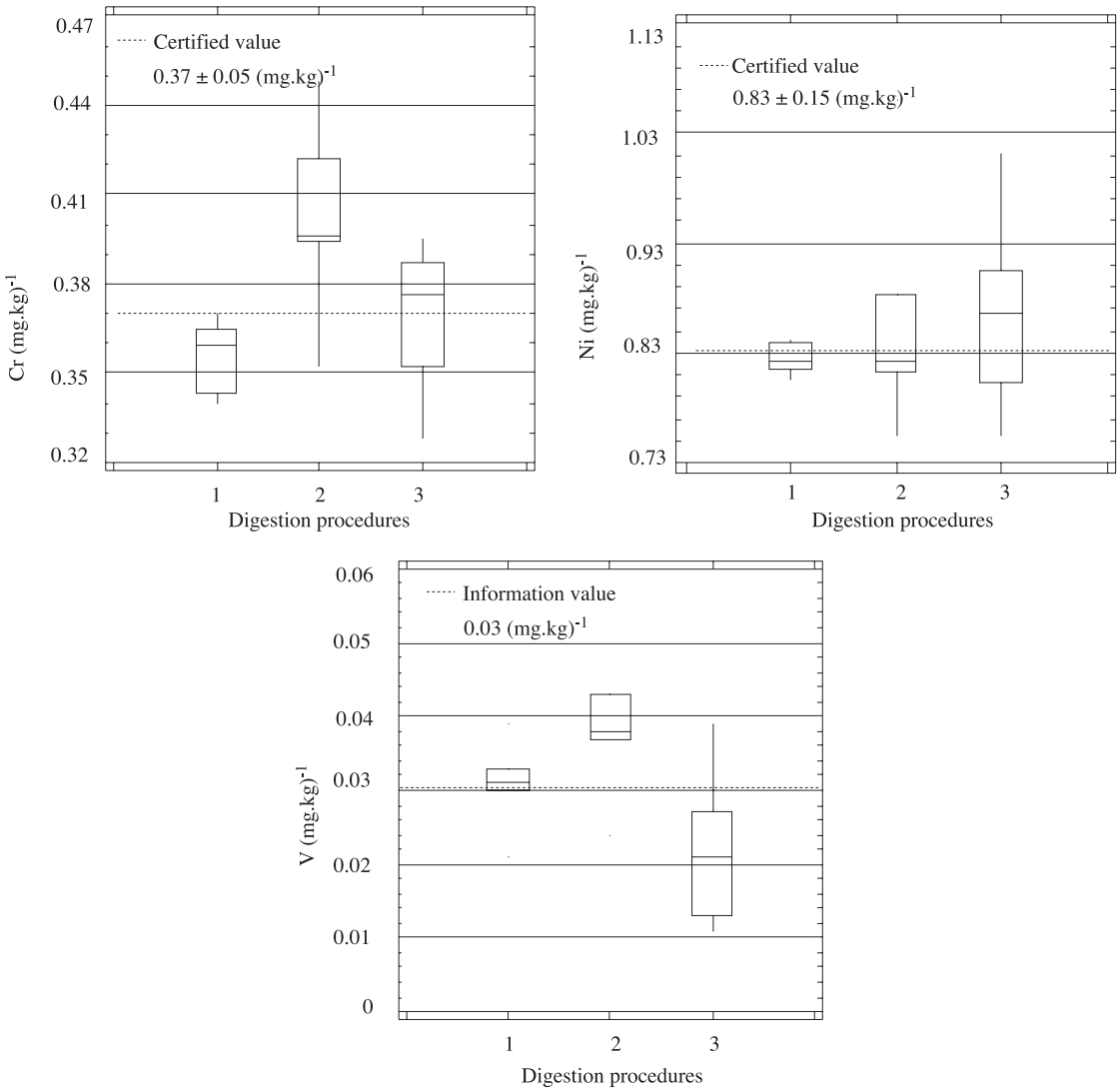

Figure 2. Multiple Box-and-Whisker plots for Cr, Ni and V in CRM 07601 (Human hair) Procedures: **1** - acid digestion (HNO_3 + H_2O_2) at elevated pressure in microwave oven; **2** - acid digestion (HNO_3 + H_2O_2+ $HClO_4$) at atmospheric pressure; **3** - ashing (500 ^{o}C) and acid digestion (HNO_3 + HCl)

Whisker diagrams demonstrate the high efficiency reached by acid decomposition (HNO_3 + H_2O_2) at elevated pressure in a microwave oven for Ni and V (procedure 1). The lowest dispersion of values and the median close

to certified values were obtained by decomposition procedures 1 and 3, for Cr. According to the achieved efficiency of decomposition, the different matrices of biological samples were decomposed by procedure[1]. Furthermore, this procedure is rapid and minimizes a number of losses and contamination errors.

Limit of detection (LOD), sensitivity, accuracy and precision

The detection limits were calculated as the concentration that yields three times the standard deviation of the blank value plus the net blank intensity (3 σ_{blank} criterion). LOD's for Cr are 8 μg. kg^{-1}; Ni 12 μg kg^{-1} and for V 30 μg kg^{-1}.

The absolute sensitivity is defined by the mass of an element which gives a peak absorbance of 0.0044; it was found 3.1 pg for Cr, 10.7 pg for Ni, and 26.9 pg for V, respectively. These values are not significantly different from published results[22].

The precision of the proposed procedure was estimated by the analysis of the CRM hair sample and calculated from 10 repeated ETAAS determinations of individual elements (corrected by the blank value). The precision of the analysis, expressed by relative standard deviation (RSD), ranged from 4% to 22% . The best values of the precision were achieved for Ni from 4% to 12% . The worst precision was attained for V from 8% to 22% , as determined values were at the detection limit for V.

The accuracy of analytical results for Cr, Ni and V in biological samples was checked for by the analysis of CRM samples. For V, only the informative values were found to be published in CRMs 07601 (Human hair), 184 (Bovine muscle) and 12-2-03 (Lucerne P-ALFALFA), therefore the accuracy of determination was verified by the standard addition method. The results obtained were in good agreement with the certified and informative values, respectively. The precision and accuracy of the data are presented in Table 2.

Table 2. Accuracy verification of proposed procedure (95% confidence level, n = 10)

Elements	Value	Human Hair GBW 07601	Bovine Muscle BCR 184	Bovine Liver BCR185	Lucerne 12-2-03
Cr [mg.kg^{-1}]	certif.	0.370 ± 0.050	(0.076 - 0.153)[a]	(0.047 - 0.124)	(0.900)[b]
	found	0.350 ± 0.013	0.080 ± 0.018	0.051 ± 0.006	0.914 ± 0.068
Ni [mg.kg^{-1}]	certif.	0.830 ± 0.150	(0.270)	(1.400)	2.540 ± 0.080
	found	0.820 ± 0.016	0.310 ± 0.011	1.520 ± 0.155	2.611 ± 0.135
V [mg.kg^{-1}]	certif.	(0.030)	(0.076)	n.d.[c]	(0.800)
	found	0.031 ± 0.007	0.079 ± 0.009	n.d.	0.900 ± 0.048

[a]Range of results observed, [b]Indicative value, [c]not determined.

In real biological samples (lucerne, feathers, earthworms) that were collected from an industrial site near a refinery and waste incinerators, the accuracy of Cr, Ni and V determination was verified by a comparison of two independent analytical methods – ETAAS and ICP AES. On the basis of this comparison, (presented in Table 3), the results obtained by ETAAS and ICP AES methods are not significantly different, and thus they are considered to be accurate. The statistical evaluation of the data was made (presented in Table 4) according to Student's test, the calculated values for Cr, Ni and V are smaller than the theoretical ones. The method can be considered to be free from systematic errors. Moreover, we tested the precision of both methods by the F-test. The calculated values were compared with tabulated critical values of the

Fisher-Snedecor distribution. The variance values (squares of the standard devations) of both methods are within the limits of random errors.

Table 3. Determination of Cr, Ni, and V in various biological materials and CRMs by ETAAS and ICP-AES, respectively

Sample	Recommended value	ETAAS	ICP - AES
	Cr ($mg.kg^{-1}$)		
Lucerne		1.31 ± 0.20	1.56 ± 0.08
Earthworms		16.55 ± 0.75	17.38 ± 0.44
Feather		0.523 ± 0.09	n.d.[c]
CRM 12-2-03 Lucerne	(0.900)[a]	0.914 ± 0.068	0.921 ± 0.011
CRM BCR 184 Bovine Muscle	(0.076 - 0.018)[b]	0.080 ± 0.018	0.082 ± 0.009
CRM GBW 07601 Human Hair	0.370 ± 0.050	0.350 ± 0.013	0.314 ± 0.09
	Ni ($mg.kg^{-1}$)		
Lucerne		1.85 ± 0.10	2.04 ± 0.09
Earthworms		3.73 ± 0.33	4.00 ± 0.11
Feather		1.117 ± 0.14	n.d.
CRM 12-2-03 Lucerne	2.540 ± 0.080	2.611 ± 0.135	2.43 ± 0.11
CRM BCR 184 Bovine Muscle	(0.270)	0.310 ± 0.011	0.303 ± 0.012
CRM GBW 07601 Human Hair	0.830 ± 0.150	0.820 ± 0.016	0.819 ± 0.15
	V ($mg.kg^{-1}$)		
Lucerne		2.15 ± 0.25	2.01 ± 0.09
Earthworms		37.37 ± 3.60	34.48 ± 0.75
Feather		0.448 ± 0.04	n.d.
CRM 12-2-03 Lucerne	(0.800)	0.900 ± 0.048	0.840 ± 0.031
CRM BCR 184 Bovine Muscle	(0.076)	0.079 ± 0.009	0.069 ± 0.006
CRM GBW 07601 Human Hair	(0.030)	0.031 ± 0.007	0.049 ± 0.004

[a]Indicative value, [b]Range of results observed, [c]not determined

Moreover, by a comparison of Cr, Ni and V concentrations in soil samples, soil EDTA extracts, lucerne, earthworms and feathers, the selected biota confirmed they had the ability to accumulate these metals. As shown in Table 5, the bioavailable contents of Cr and Ni (soil extracts in 0.05 M EDTA) are approximately the same as the content of these elements in lucerne and feathers, but they are accumulated in earthworms. In the case of V, only a small portion is contained in plant material and feathers, but V content in EDTA extracts of soil samples are comparable with the V concentrations determined in earthworms.

Table 4. Statistical evaluation of results by Student's test and F-test

Element	Student's test	F-test
Cr	1.802	1.611
Ni	0.894	1.296
V	1.084	1.021
t_{theor}	2.365	4.39
ν	7	5; 6
α	0.05	0.05

t_{theor} – tabulated critical value
ν – degree of freedom
α – significance level

Table 5. Mean element contents and concentration range in soil, soil extract, plant and biota

Data	Contents in $mg.kg^{-1}$				
	Total decomp. of soil sampl.	Soil extract 0.05 M EDTA	Lucerne	Earthworms	Feather
		Cr			
Conc. Range	54.90 - 67.28	0.72 - 1.84	0.82 - 1.52	9.18 - 17.71	0.36 – 0.76
Mean ± SD	58.09 ± 3.20	1.25± 0.39	1.09 ± 0.25	14.11 ± 2.73	0.52 ± 0.05
		Ni			
Conc. Range	22.24 - 34.33	1.58 - 3.41	1.24 - 2.50	3.51 - 10.49	1.96 - 5.58
Mean ± SD	29.59 ± 3.40	2.59 ± 0.60	1.63 ± 0.41	7.24 ± 2.50	1.12 ± 0.09
		V			
Conc. Range	59.51 - 90.48	23.33 - 28.42	0.55 - 2.49	21.11 - 34.02	0.02 – 1.27
Mean ± SD	81.40 ± 9.93	25.34 ± 1.70	1.25 ± 0.62	28.80 ± 4.11	0.44 ± 0.08

Conclusions

Three different digestion procedures of biological samples were evaluated for Cr, Ni, and V determination by ETAAS. The efficiency of the digestion procedures was assessed using CRM 07601 (Human hair). The best results, characterized by the lowest dispersion of values and by the median close to certified values, were reached by acid decomposition (HNO_3 + H_2O_2) at elevated pressure in a microwave oven.

The accuracy and precision of the analytical results were checked by the analysis of CRM's 07601 (Human hair), 184 (Bovine muscle), 185 (Bovine liver) and 12-2-03 (Lucerne P-ALFALFA) using the optimized working parameters of the ETAAS method. The attained results were in good agreement with the certified contents of the metals in the above CRM's. On the basis of the evaluation of accuracy, precision and acceptable limits of detection, the selected microwave digestion procedure and optimized ETAAS working conditions offers reliable analytical results for environmental research.

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