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‘IN-HOUSE’ VALIDATION FOR MOBILE NICKEL IN SOLID ENVIRONMENT SAMPLES

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Abstract. In the paper are presented the performance parameters for ‘in-house’ validation regarding the mobile Ni extracted from solid environmental samples polluted with high quantity of metallic elements using AAS technique. The extraction procedure was performed with two different extraction solutions: a mixture of 0.005 M EDTA, 0.1 M TEA and 0.01 M $CaCl_2$, and another mixture with 1 M CH_3COONH_4 and 0.01 M EDTA. The experimental data for repeatability and intermediate precision obtained for both extraction methods applied to a polluted sewage sludge collected from an industrial wastewater treatment plant were situated under the RSD% limit value according to Horwitz and AOAC PVM. The recovery percentages were obtained on three different levels of concentrations and the mean of these values was situated in the accepted range. In the ruggedness tests were performed 8 experiments using full-factorial design for three factors. The factors selected for the extraction procedure were: time of extraction, report solid/solution and the rotative rate of the shaker. An uncertainty budget was developed and the conclusion was that three important factors influence the uncertainty value: the concentration of the sample, all steps repeatability and recovery values.

Keywords: ‘in-house’ validation, sewage sludge, mobile Ni, ruggedness test, recovery.

AIMS AND BACKGROUND

Contamination of the environment by metallic elements is of major concern because of their toxicity. The metals found in soils and sediments are of natural and anthropogenic origin. Examples of potential sources of soil pollution with metals are industrial and traffic dust emitted into the atmosphere, residues of sewage sludge and other materials applied to the soil, mineral, mainly phosphorous, fertilisers and some crop protection chemicals. Elevated levels of metals in soils may lead to their uptake by plants, which depends not only on metals contents in soils but is also determined by soil pH value, organic matter and clay contents, and influences by the fertilisation. The fluctuation of mentioned parameters can not change the total amount of metals in soil but can significantly affect their bioavailability part[1].

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Environmental studies on soil and sediment analysis are often based on the use of leaching or extraction procedures (single and sequential procedures). The development and use of these types of extraction schemes aimed to evaluate the metal fractions available to plants (for estimating the related phytotoxic effects and/or nutritional properties of elements) and the environmentally accessible trace metals (evaluation of the mobility of metals)[2]. The increasing concern to assess the bio-available metal fraction and the mobility of trace metals upon disposal of sediment is reflected by a considerable increase in the frequency of analysis over the last 10 years. Single and sequential extraction schemes have been designed in order to assess the different retention/release of metals in soil and sediment samples[3–7].

The objective of this paper was to validate two methods used for extraction and determination of mobile Ni from environment solid samples, such as: polluted soils, sediments and sewage sludges. In experimental tests were applied two different extraction solutions, one with a mixture of 0.005 M DTPA, 0.1 M TEA and 0.01 M $CaCl_2$ (Ref. 8) (at a modified report solid:solution[6] than in original standard[8]) and another solution with a mixture of 1 M CH_3COONH_4 and 0.01 M EDTA[9]. In the experiments were tested two methods[8,9], usually used for agricultural land. The aim of the study was to verify that the methods can be applied to polluted solid environment samples. The determinations of mobile Ni were performed by flame atomic absorption spectrometry.

EXPERIMENTAL

Apparatus. Atomic absorption spectrometers: ATI UNICAM type 929, Thermo Scientific M6 Dual, Shaker type GFL 3017, Fritsh Analysette 3 Spartan Vibratory Sieve Shaker, Hettich Rotafix 32 Centrifuge, Retsh RM 100 Mill, Analytical Balance Kern ABT 220-50M.

Reagents. Merck solution for calibration (1 g Ni/l, ICP standard, traceable to NIST), hydrochloric acid (37%, Merck quality), DTPA (99%, Merck), TEA (99%, Merck), calcium chloride (Merck), ammonium acetate (98%, Merck), acetic acid (98%, Merck), EDTA (99%, Fluka), extraction solution I (c_{TEA}= 0.1 mol/l, c_{CaCl_2} = 0.01 mol/l, c_{DTPA}= 0.005 mol/l, pH = 7.3 ± 0.2), extraction solution II ($c_{CH_3COONH_4}$= 1 mol/l, c_{EDTA}= 0.01 mol/l, pH= 7 ± 0.02). All reagents used were analytic grade quality.

Extraction procedure. The sample can be soil, sediment or sewage sludge, dried on air condition, mixed, milled and then sieved on a 151-µm sieve (63-µm for sediment). 5 g of pretreated sample were mixed with 50 ml of extraction solution by shaking at 30 rot./min (solution I) or 40 rot./min (solution II) for 2 h. The solid sample was in complete suspension during the extraction procedure. The solution was centrifuged and then analysed with AAS methods in maximum 48 h. For each set of samples was prepared a blank sample (i.e. a container without sample), using the same procedure. The dried matter content was determined on a separate fraction of the sample[7], approximately 1 g, by drying at 105±2ºC until constant weight. For each analytical results was applied a correction to 'dry matter' (d.m., quantity of metal per g dry solid sample).

The experimental data were achieved with two equipments: a Thermo Scientific M6 Dual spectrometer and also, an ATI UNICAM SOLAAR 929 spectrometer, used only in intermediate precision tests. Spectral interference due to line overlap is very rare in atomic absorption spectrometry. AAS is highly specific for a given element. The wavelengths at which individual elements absorb are well defined and the possibility of two elements to absorbing at exactly the same wavelength is so remote, that the technique is generally recognised to be free of spectral interference. In Table 1 are presented the optimum parameters for Thermo equipment, used for the 'in-house' validation of the methods.

Table 1. Optimum parameters for in flame AAS type Thermo Scientific M6 Dual

Spectrometer parameters			
Wavelength	232 nm	bandpass	0.1 nm
Lamp type	hollow cathode lamp	max. lamp current	15 mA
Background correction	D2-HCL	normal use lamp current	11.25 mA
System type	double beam	nebuliser uptake time	4 s
Resample	3 times	delay time	15 s
Flame parameters			
Flame type	air/acetylene	burner height	7 mm
Burner type	50 mm	acetylene flow rate	0.9 l/min
Nebuliser flow rate	8÷10 ml/min		

For the evaluation of recovery, intermediate precision and ruggedness were used 5 different solid matrices: a sewage sludge collected from an urban wastewater treatment plant (N1), a sewage sludge from an industrial wastewater treatment plant (N2), soils S1 and S2 sampled from an industrial site and a sediment sample S3, collected from an artificial lake. The experiment tests were divided in two parts: one part on standard solutions (Table 2), and another part on real samples (Table 3).

The mathematical relations used for the methods performance studies (linearity, precision, repeatability, within laboratory reproducibility, limit of detection, limit of quantification, recovery, ruggedness)[10–12] are presented in Tables 4 and 5. In the ruggedness tests, were selected three important factors for extraction step, which are: time of extraction, report solid/solution and the rotative rate of the shaker. In these tests were performed 8 experiments using full-factorial experimental design for the three factors (Table 6). Minus (–) represents the low level of the factor and plus (+) the high level. These values are lower and higher than the normal value with maximum 10% and for the tested extraction solutions, modified values are presented in Table 7.

Table 2. Experiments on standard solutions used for the characteristics of the methods

Linearity	LOD and LOQ		Test for the homogeneity of the variance	Repeatability precision, recovery
	0.01 M EDTA + 1 M CH_3COONH_4	0.1 M TEA, 0.01 M $CaCl_2$ and 0.005 M DTPA		
10 standard solutions between 1÷10 mg/l 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 ml from 100 mg Ni/l to 50 ml with extraction solution (I or II)	10 independent blank solutions measured each of them 1 time (blank obtained from extraction solution)	10 independent blank solutions fortified on 0.04 mg/l, measured each of them 1 time	10 independent samples of 1 mg Ni/l concentration 10 independent samples of 10 mg Ni/l concentration	10 independent samples of 1 mg Ni/l concentration 10 independent samples of 5 mg Ni/l concentration 10 independent samples of 10 mg Ni/l concentration

Table 3. Experiment tests used for evaluation of recovery, intermediate precision, ruggedness

Method	Recovery	Intermediate precision	Ruggedness
SR ISO 14870/2002 (0.1 M TEA, 0.01 M $CaCl_2$ and 0.005 M DTPA)	– sewage sludge N1, 5 samples for each fortified concentration (2.5, 5 and 7.5 mg/l) – sewage sludge N2, 3 samples for each fortified concentration (2.5, 5 and 7.5 mg/l) – soil S1, 3 samples for each fortified concentration (2.5, 5 and 7.5 mg/l)	sewage sludge N1, 12 experiments with 7 samples each, 3 different days, 2 spectrometers and 2 analysts	sewage sludge N1, 8 experiments with 5 samples each
NF X-31–120/1992 (0.01 M EDTA + 1 M CH_3COONH_4)	– sewage sludge N1, 5 samples for each fortified concentration (2.5, 5 and 7.5 mg/l) – soil S2, 3 samples for each fortified concentration (2.5, 5 and 7.5 mg/l); – sediment S3, 3 samples for each fortified concentration (2.5, 5 and 7.5 mg/l)	sewage sludge N1, 12 experiments with 7 samples each, 3 different days, 2 spectrometers and 2 analysts.	sewage sludge N1, 8 experiments with 5 samples each

In recovery study, usually can be used a certified standard reference material (SRM). In this case, recovery represents the report between the concentrations obtained with the test method and the certified value. For the tested methods and for types of extraction solutions, there is no SRM certified and for this reason it was necessary to fortify samples and to determine the recovery percentage (Table 5).

Table 4. Mathematical equations used for evaluation of linearity and precisison

Linearity		
$a = \bar{y} - b\,\bar{x}$, absorbance units $\bar{y}$ is mean of absorbances, $\bar{x}$ is mean of concentrations	$b = \dfrac{\sum_{i=1}^{N}(x_i - \bar{x})(y_i - \bar{y})}{\sum_{i=1}^{N}(x_i - \bar{x})^2}$, absorbance units × mg^{-1} l, sensitivity, the slope of the calibration curve	$S_{x01} = S_{Y1}/b$, mg/l, method standard deviation
$S_{Y1} = \sqrt{\dfrac{(y_i - \hat{y})^2}{N-2}}$, mg/l, residual standard deviation	$V_{x01} = (S_{x01}/\bar{x}) \times 100$, %, coefficient of variation	$(1 - S_b/b) \times 100$, %, where $S_b = \sqrt{\dfrac{S_{y1}^2}{S_{xx}}}$, $S_{xx} = \sum_{i}^{N}(x_i - \bar{x})^2$ linearity
$s^2_i = \dfrac{\sum_{j=1}^{10}\left(y_{ij} - y_i\right)^2}{n_i - 1}$, where $y_i = \dfrac{\sum_{i}^{10} y_{ij}}{n_i}$; PG = $s^2_{10}/\,s^2_1$ if $s^2_1 < s^2_{10}$ or PG = s^2_1/s^2_{10} if $s^2_{10} < s^2_1$. PG value will be compared with the Fischer–Snedecor F value, which is 5.35 for $F_{9.9;0.99}$. PG value must be lower than F value (test for the homogeneity of the variance)		bias, $((x_f - x_a)/x_a) \times 100$, % bias value < t (99.9) = 2.82

Precision	
Average of the concentrations, mg/l	$\bar{x} = \dfrac{\sum_{i=1}^{N} x_i}{n}$
Standard deviation, mg/l	$s_r = \sqrt{\dfrac{\sum_{i=1}^{N}(x_i - \bar{x})^2}{n-1}}$
Relative standard deviation, RSD %	RSD = $(s_r/\,\bar{x}) \times 100$
Repeatability limit[10,13], mg/l	$r = 2.83 \times s_r$
Repeatability limit (confidence level 95% and different degrees of freedom), mg/l t = 2.45 – 6 degrees of freedom; t = 2.26 – 9; t = 2.16 – 13; t = 2.09 – 20 degrees of freedom; t = 2.02 – 41	$r = t_{5\%}^{\,n-1} \times s$
The Horwitz modified equation, RSD$_r$ %	RSD$_r$% = $0.66 \times 2^{1-0.5\,\lg c}$
The Horrat value r	R = RSD$_r$ % / RSD$_r$% Horwitz
Standard deviation of the mean, mg/l	$s_m = s_r/n^{1/2}$
Mean confidence interval (95% , n – 1 =9), t = 2.2); mg/l	$\bar{x} \pm t \times s_r/n^{1/2}$
Individual confidence interval, (confidence level 95%, 9 degrees of freedom), t = 2.2; mg/l	$\bar{x} \pm t \times s_r$

Table 5. Mathematical equations used for evaluation of LOD, LOQ, recovery and ruggedness

Limit of detection LOD	Limit of quantification LOQ
$X_{LOD} = \bar{x}_{blank} + 3\sigma_{blank,}$ mg/l, used when the blank values are different than zero, σ_{blank} is standard deviation of the concentration blank values	$X_{LOQ} = \bar{x}_{blank} + 10\sigma_{blank,}$ mg/l, used when the blank values are different than zero, σ_{blank} is standard deviation of the concentration blank values
$X_{LOD} = 0 + 3\sigma_{sample,}$ mg/l, used when the blank values are zero	$X_{LOQ} = 0 + 10\sigma_{sample,}$ mg/l, used when the blank values are zero
Recovery	Ruggedness
$\bar{\eta}$ (%) = $((\bar{x}_f - \bar{x}_b)/\bar{x}_a) \times 100$, %, $\bar{x}_f$ = mean value of fortified samples; $\bar{x}_b$ = mean value of unfortified samples; $\bar{x}_a$ = concentration of added standard solution	Absolute effect A = $|(\sum Y_{A+} - \sum Y_{A-})/4|$, mg/l $\sum Y_{A+} = \sum(Y_1+Y_2+Y_3+Y_4)$, $\sum Y_{A-} = \sum(Y_5+Y_6+Y_7+Y_8)$. Relative effect A (%) = $((\sum Y_{A+} - \sum Y_{A-})/\sum Y_{A+}) \times 100$

Table 6. Contrast coefficients for three factors, full-factorial experimental design

Experiment	Factors			Results
	A	B	C	
1	+	+	+	Y_1
2	+	+	–	Y_2
3	+	–	+	Y_3
4	–	+	+	Y_4
5	–	–	–	Y_5
6	–	–	+	Y_6
7	–	+	–	Y_7
8	+	–	–	Y_8

Table 7. Modified values of the factors used in ruggedness tests

Method	Factor A shaker rate (rpm)		Factor B mixing time (min)		Factor C extraction report	
	(+)	(–)	(+)	(–)	(+)	(–)
NF X-31–120/1992 Standard extraction condition: 40 rpm, 120 min, report soil:solution = 1:10 (w/v)	44	36	132	108	1:11	1:9
SR ISO 14870/2002 Standard extraction condition: 30 rpm, 120 min, report soil:solution = 1:10 (w/v)	33	27	132	108	1:11	1:9

RESULTS AND DISCUSSION

The working range is linear between 1 and 10 mg/l Ni for both extraction solutions. In the tests for the homogeneity of the variance, PG values are lower than the Fischer–Snedecor F value and the linearity values are situated in accepted range

(99÷101%). The values of coefficient of variation (1.73% in EDTA and 1.45% in DTPA), less than 2% according to the Horwitz function[10], situate the test methods as good spectrometric method. The detection and quantification limits allow detecting mobile Ni from solid environmental samples (Table 8).

Table 8. Linearity, LOD and LOQ

Mobile Ni with 1 M CH_3COONH_4 and 0.01 M EDTA solution		
$y = 0.0063 + 0.0388x$ curve linear equation	$a = 0.0063$ absorbance units	$b = 0.0388$ absorbance units $\times$ mg^{-1} l
$S_{Y1} = 0.0037$ mg/l	$S_{x01} = 0.095$ mg/l	$V_{x01} = 1.73\%$,
$s^2_1 = 8.96E\text{-}11$,	linearity = 99%,	R=0.9995
$s^2_{10} = 3.29E\text{-}10$, PG = $s^2_{10}/s^2_1 = 3.67 < F_{9.9;0.99} = 5.35$	accepted range: 100 ± 1%	correlation coefficient, accepted values $R \geq 0.997$
the blank values are different than zero: $\overline{x} = 0.0038$ mg/l, $\sigma_{blank} = 0.0016$ mg/l		
LOD = 0.009 mg/l (0.10 mg/kg), LOQ = 0.02 mg/l (0.25 mg/kg)		
Mobile Ni with 0.005 M DTPA, 0.1 M TEA and 0.01 M $CaCl_2$		
$y = 0.0067 + 0.0471x$ curve linear equation	$a = 0.0067$ absorbance units	$b = 0.0471$ absorbance units $\times$ mg^{-1}l
$S_{Y1} = 0.0037$ mg/l	$S_{x01} = 0.080$ mg/l	$V_{x01} = 1.45\%$,
s^2_1=3.25E-10,	linearity = 99.2%, accepted range: 100 ± 1%	R=0.9997 correlation coefficient, accepted values $R \geq 0.997$
s^2_{10}=1.04E-09, PG = $s^2_{10}/s^2_1 = 3.21 < F_{9.9;0.99} = 5.35$		
the blank values are zero, $\sigma_{blank} = 0.0033$ mg/l, fortified concentration was 0.04 mg/l		
LOD = 0.01 mg/l (0.12 mg/kg), LOQ = 0.03 mg/l (0.35 mg/kg)		

In Table 9 are presented the mean, standard deviation, RSD and repeatability for combine experiments (3 days, same analyst and equipment and 6 days, same equipment) for both extraction solutions.

Table 9. Intermediate precision tests

Parameter	1 M CH_3COONH_4 and 0.01 M EDTA solution				0.005 M DTPA, 0.1 M TEA and 0.01 M $CaCl_2$ solution			
	$\overline{x}$ (mg/l)	s_r (mg/l)	RSD (%)	r (mg/l)	$\overline{x}$ (mg/l)	s_r (mg/l)	RSD (%)	r (mg/l)
D1+D2+D3, A1, E1	2.049	0.145	7.07	0.302	1.067	0.096	8.99	0.201
D7+D8+D9, A1, E2	2.133	0.148	6.94	0.309	1.001	0.033	3.32	0.070
D4+D5+D6, A2, E1	2.146	0.227	10.60	0.474	1.068	0.111	10.37	0.231
D10+D11+D12, A2, E2	2.174	0.113	5.19	0.235	1.004	0.058	5.81	0.122
D1÷D6, A1+A2, E1	2.104	0.194	9.24	0.393	1.070	0.097	9.02	0.195
D7÷D12, A1+A2, E2	2.163	0.124	5.73	0.251	0.997	0.057	5.71	0.115

The studies for intermediate precision (inter-run precision), when were used 2 different equipments and determinations were performed by 2 analysts over an extended period of time, show good results, the values are situated under the recommended values for 1–10 mg/l concentration range. Acceptable RSD values according to Horwitz are situated in the range 11.3 –16% and according to AOAC Peer Verified Methods the range is 7.3 –11% (Ref. 10). The data obtained with ATI UNICAM equipment (E1, manufactured in 1996) has higher values of relative standard deviation (9.24% for all results) than RSD values reported with Thermo Scientific spectrometer (5.73% for all results), E2 equipment, manufactured in 2008. The differences between results obtained with E1 and E2 equipment are given by the performance of the equipments not by the influence of extraction procedure.

Table 10. Recovery and precision data: standard Ni solution extracted with EDTA solution

Parameter	x_a =1 mg/l	x_a =5 mg/l	x_a =10 mg/l
$\overline{x}_f$, mg/l	1.002	5.065	10.019
$\overline{\eta}$, %	99.76	101.24	100.15
s_{xf}, mg/l	0.019	0.065	0.093
x_{blanc}, mg/l	0.004	0.003	0.004
s_η, %	1.89	1.31	0.95
$\overline{x}_f \pm s_{xf}$, mg/l	1.00 ± 0.02	5.07 ± 0.07	10.02 ± 0.09
$\eta_m \pm s_\eta$, %	99.76 ± 1.89	101.24 ± 1.31	100.15 ± 0.95
Bias, %	0.20	1.30	0.19
RSD_r, %	1.86	1.28	0.93
s_m, mg/l	0.006	0.021	0.029
Mean confidence interval, mg/l	0.013	0.045	0.065
Repeatability limit $r = 2.26s$, mg/l	0.043	0.147	0.21
The Horwitz value, RSD_r %	7.47	5.86	5.28
The Horrat value r	0.25	0.22	0.18

The value of the Horrat index must be situated in the range 0.5–2 or lower than 0.5. All the Horrat values obtained for standard solutions and real samples are in admissible range. Also, the bias values are situated under the limit value, which is 2.82% for 99% confidence level and 9 freedom degrees.

For 1 to 10 mg/l level of concentration, the recovery percentage must be situated in the 80 to 110% range[10]. In Tables 10–13, all the obtained values of recovery percentage for different types of samples and standard solutions with respect to requirement are presented.

Table 11. Recovery and precision data: real sample extracted with EDTA solution

Fortified sewage sludge N1	2.5 mg/l	5 mg/l	7.5 mg/l
$\overline{x}_f \pm s_{xf}$, mg/l	4.29±0.03	6.37±0.03	8.39±0.10
$\eta_m \pm s_\eta$, %	93.86±1.38	88.53±0,54	91.99±1.42
Fortified soil S2			
$\overline{x}_f \pm s_{xf}$, mg/l	4.96±0.10	7.54±0.05	9.66±0.08
$\eta_m \pm s_\eta$, %	89.60±4.11	96.53±1.08	92.59±1.10
Fortified sediment S3			
$\overline{x}_f \pm s_{xf}$, mg/l	4.28±0.03	6.76±0.05	9.26±0.05
$\eta_m \pm s_\eta$, %	93.24±1.74	96.16±0.98	97.46±0.71
Parameter	sewage sludge N1	soil S2	sediment S3
$\overline{x}$, mg/l (10 determinations)	2.027	2.724	1.945
RSD_r, %	4.88	4.31	4.12
s_r, mg/l	0.099	0.117	0.080
s_m, mg/l	0.031	0.037	0.025
Mean confidence interval, mg/l	0.068	0.081	0.055
Individual confidence interval, mg/l	0.218	0.26	0.176
Repeatability limit, mg/l	0.22	0.26	0.18
The Horwitz value, RSD_r %	7.26	9.07	9.58
The Horrat value r	0.67	0.40	0.43
Range values for $\overline{x}$, mg/l	1.959 ÷ 2.095	2.643 ÷ 2.805	1.89 ÷ 2.00

Table 12. Recovery and precision data: standard solution extracted with DTPA solution

Parameter	$x_a = 1$ mg/l	$x_a = 5$ mg/l	$x_a = 10$ mg/l
$\overline{x}_f$, mg/l	0.986	5.061	9.99
$\overline{\eta}$, %	98.64	101.22	99.91
s_{xf}, mg/l	0.011	0.051	0.12
s_η, %	1.08	1.01	1.17
$\overline{x}_f \pm s_{xf}$, mg/l	0.99±0.01	5.06±0.05	9.99±0.12
$\eta_m \pm \mathrm{s}_\eta$, %	98.64±1.08	101.22±1.01	99.91±1.17
Bias, %	1.40	1.22	0.1
RSD_r, %	1.10	1.01	1.17
s_m, mg/l	0.0034	0.016	0.037
Mean confidence interval, mg/l	0.007	0.035	0.030
Repeatability limit $r = 2.26\ s$, mg/l	0.024	0.115	0.264
The Horwitz value, RSD_r %	7.47	5.86	5.28
The Horrat value r	0.15	0.17	0.22

Table 13. Recovery and precision data: real samples extracted with DTPA solution

Fortified sewage sludge N1	2.5 mg/l	5 mg/l	7.5 mg/l
$\bar{x}_f \pm s_{xf,}$ mg/l	4.07±0.05	6.29±0.09	8.15±0.09
$\eta_m \pm s_\eta$, %	95.91±1.85	92.36±1.74	92.53±1.23
Fortified sewage sludge N2			
$\bar{x}_f \pm s_{xf}$, mg/l	6.84±0.07	9.24±0.05	11.44±0.08
$\eta_m \pm s_\eta$, %	88.27±2.75	92.20±1.07	90.41±2.82
Fortified soil S1			
$\bar{x}_f \pm s_{xf}$, mg/l	3.05±0.06	5.54±0.04	9.66±0.08
$\eta_m \pm s_\eta$, %	91.87±2.41	95.87±0.86	93.55±1.56
Parameter	sewage sludge N1	sewage sludge N2	soil S1
$\bar{x}$, mg/l (10 determinations)	1.675	4.630	0.755
RSD_r, %	0.84	2.55	1.99
s_r, mg/l	0.014	0.118	0.015
s_m, mg/l	0.044	0.037	0.005
Mean confidence interval, mg/l	0.010	0.081	0.011
Individual confidence interval, mg/l	0.031	0.26	0.033
Repeatability limit, mg/l	0.032	0.27	0.03
The Horwitz value, RSD_r %	6.91	5.93	7.79
The Horrat value r	0.12	0.43	0.26
Range values for $\bar{x}$, mg/l	1.665 ÷ 1.685	2.469 ÷ 2.631	0.744 ÷ 0.766

If the residue obtained after extraction step was washed with distillated water and then analysed, the total recovery percentages increased and the values were situated in the range 98–102% for all types of matrices. In recovery tests it was used only filtration procedure, not the centrifugation followed by filtration. The conclusion of the recovery tests was that in the extraction procedure the centrifugation step can be changed with a common filtration and (also) the extraction procedure became easier. In Table 14 are presented (final) results for ruggedness, data obtained from all 8 experiments. The extraction report has higher influence in the extraction step for both methods, but the effects for all factors are lower than comparative value.

In order to estimate the values of uncertainty for each concentration of mobile metallic elements, an uncertainty budget was developed[14]. The equation used for evaluation of the final results of mobil Ni from solid environment samples, relation used in the uncertainty budget, was the following:

$$C_{sample} = C_{bc} \times V_{bc}/m_{sample} \times 100/\mathrm{dm} \times f_{rep} \times f_{time} \times f_{report} \times f_{rate} \times 1/\bar{\eta}^{1/2},$$

where C_{bc} is the concentration of the extracted solution, mg/l; V_{bc} – volumetric flask volume, ml; m_{sample} – mass of the sample, g; dm – dry matter, %; f_{time} – the influence of time extraction; f_{rep} – the influence of analytical process repeatability; f_{report} – the influence of extraction report; f_{rate} – the influence of shaker rate; $\bar{\eta}$ – recovery.

Table 14. Ruggedness tests

Mobile Ni with 1 M CH_3COONH_4 and 0.01 M EDTA solution								
Parameter	MU	Comparative value (mg/l)	Factor values					
			factor A rate of shaker		factor B extraction time		factor C extraction report	
			∑A+	∑A–	∑B+	∑B–	∑C+	∑C–
∑F+ ∑F–	mg/l	–	7.285	7.500	7.333	7.452	6.966	7.819
Absolute effect	mg/l	1.4×0.194= 0.272	0.054		0.029		0.213	
Relative effect	%	–	2.95		1.61		12.25	
Mobile Ni with 0.005 M DTPA, 0.1 M TEA and 0.01 M $CaCl_2$ solution								
∑F+ ∑F–	mg/l	–	2.902	2.914	2.909	2.907	2.744	3.072
Absolute effect	mg/l	1.4×0.097= 0.136	0.003		0.0005		0.082	
Relative effect	%	–	0.39		0.07		11.95	

In the equation above, the value of the influence factors, which are: extraction time, repeatability, extraction report and shaker rate is one, their contribution is important only in uncertainty budget measurement not for evaluation of concentration. The uncertainty budget was estimated using data obtained in precisison, repeatability and recovery studies. The uncertainty values of mobile Ni were affected only by three important factors: the concentration of the sample, all steps repeatability and recovery values. The factors tested in ruggedness tests are insignificants reported with the other one (Figs 1–4). The value of uncertainty is different if the concentration is situated near the lowest value of the linearity range (1 mg/l) or near the highest value (10 mg/l). For this reason, is important to evaluate the uncertainty on the inferior part, middle and superior part of the calibration curve. The uncertainty must be reported in RSU % (relative standard uncertainty) and applied to each value of mobile Ni (Table 15).

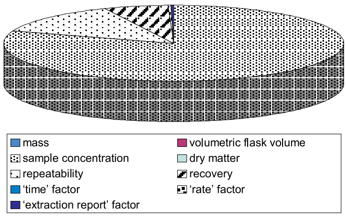

Fig. 1. Influence of factors on uncertainty budget for mobile Ni extracted with DTPA, TEA and $CaCl_2$ (inferior part of the calibration curve)

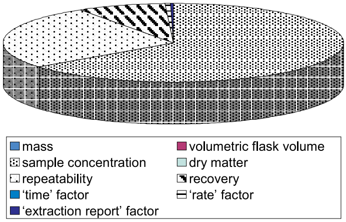

Fig. 2. Influence of factors on uncertainty budget for mobile Ni extracted with DTPA, TEA and $CaCl_2$ (superior part of the calibration curve)

Table 15. Relative standard uncertainty for mobile Ni

Matrix type	Type of extraction solution	RSU % value		
		inferior	middle	superior
Sewage sludge	0.005 M DTPA+0.1 M TEA+0.01 M $CaCl_2$	12.1	10.5	8.5
Sewage sludge	1 M CH_3COONH_4+0.01 M EDTA	12.4	11.7	9.9

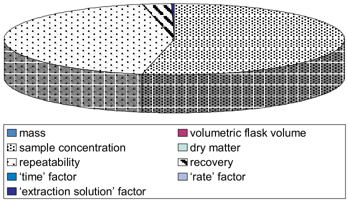

Fig. 3. Influence of factors on uncertainty budget for mobile Ni extracted with EDTA and CH_3COONH_4 (inferior part of the curve)

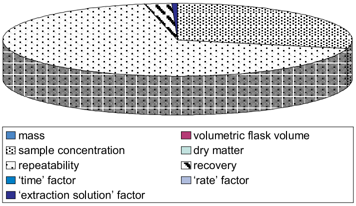

Fig. 4. Influence of factors on uncertainty budget for mobile Ni extracted with EDTA and CH_3COONH_4 (superior part of the curve)

CONCLUSIONS

The statistical interpretation of the experimental results proved that the proposed methods can be successfully used for the analytical control of the mobile Ni from polluted environment solid samples. The extraction procedure can be simplified, using a common filtration and entire process became easier. The ruggedness tests applied to extraction step show that the variation with 10% for operating condition parameters can not affect significantly the extraction procedure. An uncertainty budget was developed; three important factors influence uncertainty value: the concentration of the sample, all steps repeatability and recovery values.

REFERENCES

1. A. MOCKO, W. WACLAWEK: Three Step Extraction Procedure for Determination of Heavy Metals Availability to Vegetables. Anal. Bioanal. Chem., **380**, 813 (2004).
2. G. RAURET, J. F. LOPEZ-SAUCHEZ, A. SAHUQUILLO, R. RUBIO, C. DAVIDSON, A. URE, Ph. QUEVAUVILLER: Improvement of the BCR Three Steps Sequential Extraction Procedure Prior to the Certification of New Sediment and Soil Reference Materials. J. Environ. Monit., **1**, 57 (1999).
3. G. VASILE, L. CRUCERU, J. PETRE, M. NICOLAU, V. IANCU: Measurement of Mobile Trace Metals from Polluted Soils in Industrial Sites. J. of Environm. Protection and Ecology, **9** (2), 278 (2008).
4. G. VASILE, L. CRUCERU, J. PETRE, V. IANCU, R. MITRAN: Analytical Investigation Concerning the Methodology for Extraction of Exchangeable Forms of Heavy Metals from Different Types of Soils. J. of Environm. Protection and Ecology, **7** (3), 562 (2006).
5. G. VASILE, L. CRUCERU, J. PETRE, I. Gh. TANASE: Performance Analytical Method for Extraction and Determination of Mobile Cd from Polluted Soil. Rev. Chim. (Bucharest), **58** (12), 1332 (2007).
6. G. VASILE, L. CRUCERU: Optimised Method for Extraction of Mobile Species of Heavy Metals from Polluted Soils. J. of Environm. Protection and Ecology, **8** (4), 801 (2007).
7. G. VASILE, I. Gh. TANASE: Comparative Study of Different Extraction Methods for Evaluation of Bioavailability of Terrestrial Sediment-bound Metallic Elements. Revue Roumaine de Chimie, **53** (11), 1041 (2008).
8. SR ISO 14870-1999: Soil Quality: Extraction of Microelements in Buffer Solution of DTPA.
9. NF X 31 – 120/1992: Soil Quality – Measurement of Heavy Metals from Soil after Extraction with a Mixture of Ammonium Acetate and EDTA.
10. I. TAVERNIERS, M. DE LOOSE, E. V. BOCKSTAELE: Trends in Quality in the Analytical Laboratory. II. Analytical Method Validation and Quality Assurance. Trends in Analytical Chemistry, **23** (8), 535 (2004).
11. D. B. HIBBERT: Quality Assurance for the Analytical Chemistry Laboratory. University Press, Oxford, 2007, 80–89.
12. SR ISO 11465: Soil Quality: Determination of Dry Matter Content – Gravimetric Method.
13. L. P. van REEUWIJK: Guidelines for Quality Management in Soil and Plant Laboratories. FAO Soils Bulletin, ISRIC 74, 1998, 92–96.
14. G. VASILE: Traceability of Mobile Metallic Elements from Polluted Soils and Sediments. Ph. D. Thesis, University of Bucharest, 2009.

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