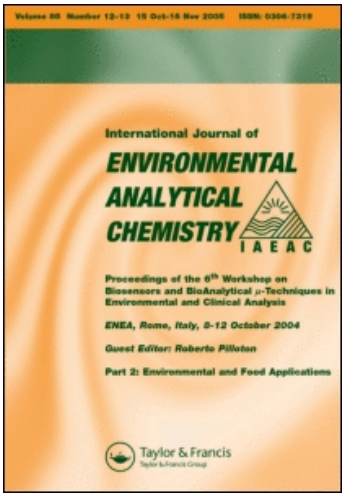

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Determination of lead in plants in controlling phytoremediation processes using slurry sampling electrothermal atomic absorption spectrometry

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DETERMINATION OF LEAD IN PLANTS IN CONTROLLING PHYTOREMEDIATION PROCESSES USING SLURRY SAMPLING ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

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The comparative determination of lead in plant samples by two atomic spectrometric techniques is reported. At first, slurry sampling electrothermal atomisation atomic absorption spectrometry (ETAAS) was applied. The results obtained were compared with those found after a wet digestion procedure by flame atomic absorption spectrometry (FAAS) or ETAAS. The accuracy of the studied methods was checked using a certified reference material (CL – 1 CRM, Cabbage Leaves). The recovery of lead was 90% for slurry sampling ETAAS, and 86.6% for liquid sampling ETAAS. The advantages of the slurry sampling ETAAS method are the simplicity of sample preparation and very good sensitivity.

Keywords: Lead in plants; Slurry sampling atomic absorption spectrometry

INTRODUCTION

Exhaust gas from car engines contains products of tetraethyl lead decomposition, which are emitted to the atmosphere, surface waters and soil thus posing a serious hazard not only to the functioning of the ecosystem but to human health as well. Therefore, research has been carried out aimed at finding new and effective methods of cleaning lead-contaminated environments. One of these methods is the use of plants for environmental cleaning in the process called phytoremediation [1–6].

In our experiments we have used rape plants, which absorb lead compounds through their root system and then transport them to the green tops, which can be mechanically

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TABLE I Instrumental parameters

Wavelength (nm)	283.3		
Spectral bandpass (nm)	0.5		
Background correction	D_2 lamp		
Lamp current (mA)	10		
Atomizer type	Pyrolytic	Graphite-coated tube	+ L'vov platform

TABLE II Thermal programme

Step	*Temperature (°C)*	*Ramp time (s)*	*Hold time (s)*
Drying	300	5	40
Pyrolysis	1000	10	6
Atomisation	2300	2	1
Cleaning	2400	2	2

The pure gas flow rate was 300 mL min^{-1} in all steps except for the atomisation step for which stop–flow was used. Read was set up in the atomisation step.

removed from a polluted area. Therefore, it is very important to understand the mechanisms of lead accumulation in different parts of plants.

The aim of the study was to develop an efficient procedure for the determination of lead accumulated in roots, stems and leaves of plants during the phytoremediation process. For that purpose, the slurry sampling electrothermal atomic absorption spectroscopy (SS ETAAS) technique was used. The above technique offers the best conditions for the direct determination of lead because the sample introduction into a graphite tube in the form of slurry allows the mineralisation process to be performed *in situ*. Since 1992, many papers dealing with metal determinations in biological and environmental solid samples have reported good results from this method [7–15].

In our study, the most important parameters of the analytical procedure, i.e. analyte homogeneity, number of particles present in an injected volume, suspension medium, furnace heating conditions and evaluation of the method, were optimised.

EXPERIMENTAL

Apparatus and Operating Conditions

A Varian SpectrAA plus atomic absorption spectrometer equipped with deuterium background correction and a GTA-96 graphite furnace with autosampler were used in this study. Argon gas provided an inert atmosphere inside the furnace. Pyrolytic graphite-coated graphite tubes with preinserted pyrolytic L'vov platforms were employed. A Pb HCL (hollow cathode lamp) was used as a radiation source. An ultrasonic processor, Sonopuls, Germany, with a 3-mm titanium probe provided automatic agitation of the slurry. The instrumentation parameters and operating conditions are listed in Tables I and II.

Reagents

Commercial stock standard solution, 1000 g l^{-1} (Merck, Germany); working standards were obtained by diluting with 2% (v/v) nitric acid. Nitric acid of spectral purity

(Merck, Germany), palladium modifier for graphite-furnace AAS (containing nitric acid) (Merck, Germany), magnesium nitrate hexahydrate (Merck, Germany). Solutions were prepared with Milli-Q water (Millipore Corporation).

Plant Material

Seeds of plants were soaked in water for 4 h and then germinated in the dark at 24°C for three days. Seedlings were cultivated hydroponically in a Hoagland medium supplemented with 1 mM $Pb(NO_3)_2$. Roots and leaves of the tested plants were sampled after 1, 2, 3, 4 and 14 h after the application of lead ions, and were rinsed and then frozen in liquid nitrogen. A certified reference plant CL – 1 CRM, Cabbage Leaves (University of Mining and Metallurgy, Poland) was used.

WET DIGESTION

In order to determine the total amount of lead taken up by particular plant organs, 0.25 g of the plant material (particle size $< 60\,\mu m$) frozen at $-80°C$ was homogenised with a mortar and pestle. Then 5 mL of 40% HNO_3 were added and the mixture was warmed until a clear solution formed. Then 1 mL of H_2O_2 was added and the extract was heated again. After cooling the whole amount was diluted to 20 mL with ultrapure water.

Slurry Sample Preparation

Homogenised plant samples were weighed and then transferred to acid-cleaned polypropylene autosampler cups. All operations were carried out on a clean bench. Three slurries were prepared for each sample and all measurements were carried out with at least five replicates. The slurry was homogenised using an ultrasonic processor. Then 20 μL of the slurry were collected and delivered to the atomiser. Before that, 20 μL of a chemical modifier were deposited on the atomiser platform. The heating program presented in Table I was employed throughout. Calibration was performed using aqueous reference solutions.

RESULTS AND DISCUSSION

Slurry Optimisation

In order to optimise the slurry concentration, preliminary experiments were performed with 0.2–6.0% m/v of the plant slurry, employing the heating program (Table I) and using a mixture of 3.5 μg Pd + 1.1 μg Mg as a modifier. For slurries containing < 0.4 or $> 2\%$ m/v, the precision of measurements ($n = 10$) was impaired (Fig. 1). The lower the slurry concentration, the greater was the influence of sample inhomogeneity on the reproducibility of results, because a smaller number of particles was introduced into the atomizer [15,16]. Higher sample concentrations caused an increase in the background absorption due to a greater amount of introduced organic material. For plant slurries with concentrations up to 2%, there is a linear relationship between the

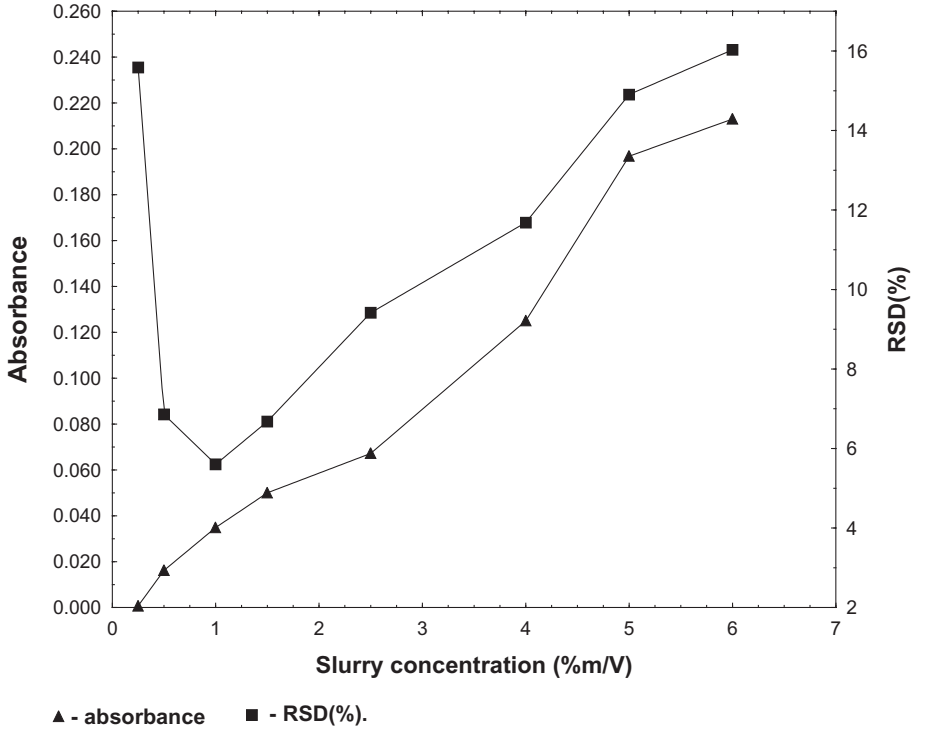

FIGURE 1 Effect of plant slurry concentration (Pb content estimated at $0.30 \pm 0.021\,\mu g\,g^{-1}$) on the absorbance and the measurement precision ($n = 6$).

integrated absorbance and the amount of sample introduced into the atomiser (Fig. 1). Above 2% m/v slurry (400 µg of sample), the amount of interferents introduced increases and systematic errors are expected [17]. Moreover, the introduction of large amounts of biological material into the graphite tube results in the formation of a carbonaceous residue, which can encrust the optical path, causing a drastic deterioration in the signal-to-noise ratio [9]. Air ashing [18] or hydrogen peroxide treatment [19,20] are usually employed to destroy the organic sample matrix, but the tube lifetime is then shortened [21]. As a compromise between the atomiser lifetime, sensitivity and precision, we have decided to work with the plant slurry within the 0.5–1.5% m/v range.

When using the slurry technique, sampling errors are usually associated with the difference in analyte sampled mass (sampling efficiency), which results from differences in the particle size, number of particles present in the injected volume, analyte homogeneity, suspension medium, slurry concentration, stirring method and sampling depth [17,22–24]. The success of the slurry technique depends on the strict control of these variables [22]. Therefore, additional experiments were performed in order to confirm the stability of the slurry. The diameter of particles of a plant sample (CL – 1 CRM, Cabbage Leaves, five rape leaf samples and five rape root samples) was examined several times by scanning electron microscopy and optical microscopy, and it was found that the average particle diameter was $< 60\,\mu m$. The average plant density was $1.1\,g\,cm^{-3}$, so a large number of particles was introduced into the atomiser, which ensures a low sampling error. Next, a study on the slurry sample stability was carried out with 0.5 and 5.0% v/v HNO_3 as a liquid medium for the slurry preparation.

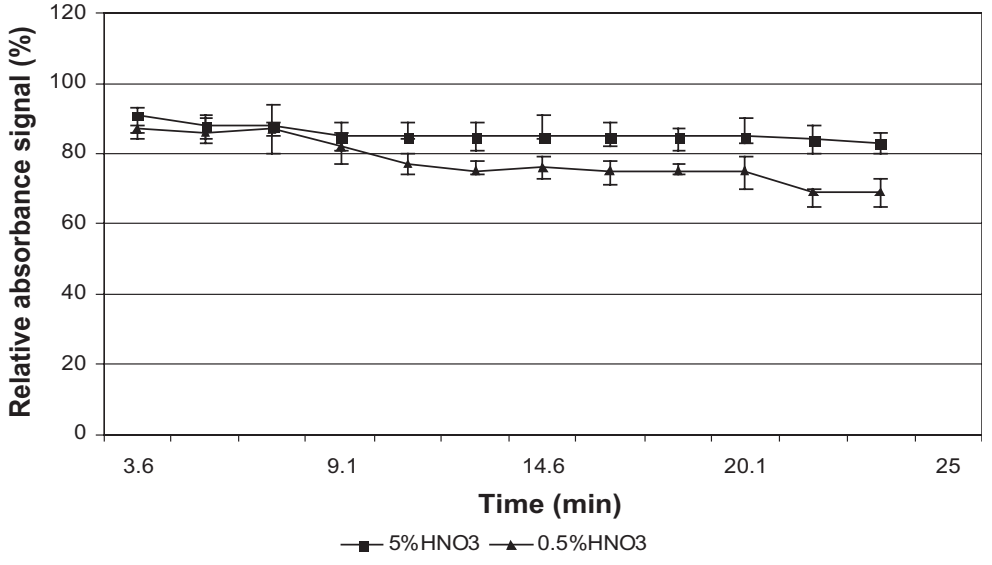

FIGURE 2 Relative absorbance signal as a function of time for Pb in slurry. The slurry samples were agitated with an ultrasonic probe only before the injection of the first aliquot.

Ultrasonic mixing was performed for 25 s before sampling. Results of this study are shown in Fig. 2 as variation of the relative absorbance signal of lead as a function of time. Under these conditions, the sampling efficiency varied from 95% to 82% ($n = 6$) for slurries in 5% HNO_3 during 60 min of determination. When slurries were prepared in 0.5% HNO_3, sampling efficiencies were lower, from 87% to 68% ($n = 6$). The agitation of slurries prior to pipetting prevents the settlement of particles and sample agglomeration, thus minimising sampling errors. At the next stage of the study, we have used 5% HNO_3 as a liquid medium to prepare slurries and ultrasonic mixing was performed for 25 s before sampling.

Furnace Heating Conditions

Lead is quite a volatile element, therefore it escapes from the graphite atomiser at temperatures above 400°C in the absence of a chemical modifier (Fig. 1). Systematic experiments were carried out to optimise temperature and time for drying, pyrolysis and atomisation steps in the presence of 3.5 μg Pd + 1.1 μg Mg as a chemical modifier. The conditions were optimised for a slurry soil sample (10 mg of a certified reference plant CL − 1 CRM, Cabbage Leaves in 1 mL of 5% HNO_3) and Pb standard aqueous solution (30 ng mL^{-1}). When a slurry sample is atomised, a high background influences the deuterium background correction system, which results in overcorrection of the atomic signal. Because of this, negative integrated absorbance values are sometimes obtained. Therefore, only peak height absorbance was taken into account in this work. Since interference in the vapour phase and high lead volatility can be encountered in the determination of lead, the use of chemical modifiers becomes an imperative to circumvent these problems. An experiment was carried out in which $Pd(NO_3)_2$ and $Mg(NO_3)_2$ at levels corresponding to 3.5 μg Pd and 1.1 μg Mg were used. Other

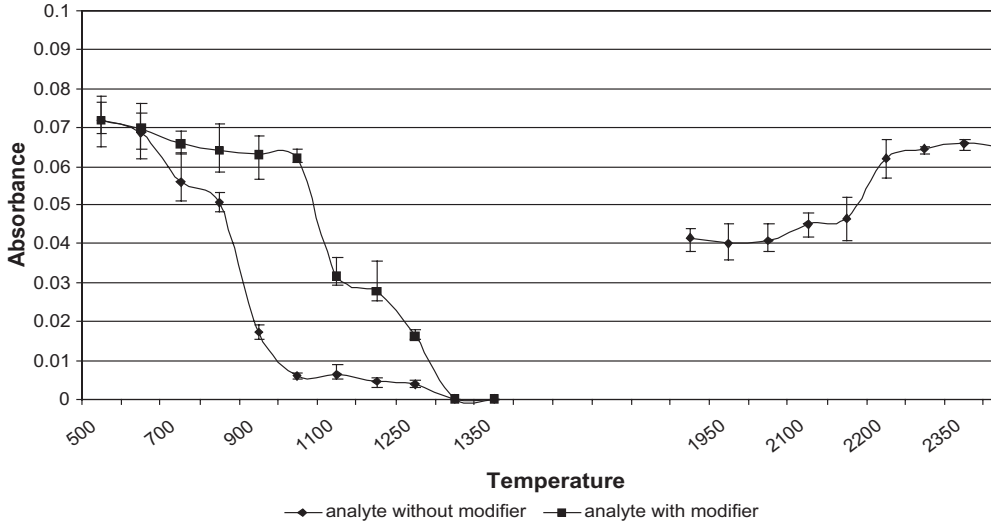

FIGURE 3 Pyrolysis (500–1350°C) and atomisation (1950–2350°C) curves for the slurry prepared in the presence of 5% HNO_3 as a liquid medium and 3.5 μg Pd + 1.1 μg Mg as a chemical modifier.

concentrations tested did not produce good stabilization of lead and were ignored for simplification. In this experiment, the atomisation temperature was fixed at 2300°C and pyrolysis temperature varied from 400 to 1350°C. A sample containing 2.3 μg L^{-1} (Fig. 3) was used. In the absence of the modifier, a loss of lead, resulting from its high volatility, was detected at 400°C for the sample. With Pd + Mg as the modifier, lead was stabilized at 1000°C (Fig. 3). After considering three parameters, namely the stabilisation of the analyte at elevated temperatures, production of similar results for the standards and samples and precision of results, the pyrolysis temperature was fixed at 1000°C and Pd + Mg was used as the most suitable chemical modifier for this method.

Calibration

The simplest calibration method, i.e., measurements against aqueous standard solutions, can be applied to the whole analysis. The fact that the signal form and the pyrolysis/atomisation curves for lead were very similar for the slurries of plant material (CL − 1 CRM, Cabbage Leaves) and the aqueous standards, indicates the absence of matrix interference. The standard addition and the aqueous standard methods were compared for the plant material.
The equations obtained were:

$$\text{Calibration graph: } Q_A = 0.002 + 4.74 \times 10^{-3}\, c \quad r = 0.998$$

$$\text{Standard additions: } Q_A = 0.058 + 4.55 \times 10^{-3}\, c \quad r = 0.997$$

where Q_A is the integrated absorbance, r is the regression coefficient and c is the lead concentration. The slopes of the calibration graphs for the two calibration methods

TABLE III Lead content in different samples and certified reference materials

CRM/Sample	*Day*[a]	*Lead Content* ($\mu g\, g^{-1}$)[b]		*Certified value*	*Recovery*
		Suspension	*Wet digestion ETAAS*		
CL – 1 CRM, Cabbage Leaves	–	0.27 ± 0.021	0.26 ± 0.022	0.30 ± 0.021	90[c]/86.6[d]
Rape leaves	1	4.69 ± 0.15	4.43 ± 0.18	–	–
	2	4.80 ± 0.20	4.95 ± 0.21	–	–
	3	3.18 ± 0.14	3.24 ± 0.18	–	–
	4	4.09 ± 0.20	4.15 ± 0.22	–	–
	14	5.06 ± 0.24	4.95 ± 0.28	–	–
Rape roots		Wet digestion FAAS	Wet digestion ICPOES		
	1	68.6 ± 6.6	71.2 ± 5.7	–	–
	2	52.9 ± 4.5	50.1 ± 5.6	–	–
	3	60.9 ± 5.6	63.7 ± 6.4	–	–
	4	45.8 ± 3.8	43.8 ± 5.8	–	–
	14	833.8 ± 36.6	814.8 ± 31.2	–	–

[a] Results expressed in $\mu g \cdot g^{-1}$ dry matter (average values ± standard deviation, $n = 6$; $P = 95\%$).
[b] Denotes the day after the application of lead ions.
[c] For slurry sampling ETAAS.
[d] For wet digestion and determination by ETAAS.

did not differ significantly (*t*-test at 95% level). For lead, the standard curve was linear with $r > 0.998$ in the range tested from 80 to 400 $\mu g\, L^{-1}$.

Applications

The results obtained for the different plants analysed using the procedure described above, as well as the reference method based on wet mineralisation, are summarised in Table III. The reliability of the method was further proved by using the certified reference material CL – 1 CRM, Cabbage Leaves. The results obtained are also shown in Table II together with the certified value. A comparison of the results obtained by both procedures was carried out using the paired *t*-test because the data follow a normal distribution. The value of the statistical $t = -0.210$ ($P = 0.812$) indicated that there were no significant differences between the results obtained by the two methods or, in the case of reference materials, between the results obtained by the developed method and the certified values (confidence level 0.05).

Analytical Characteristics

According to the definition recommended by IUPAC, the detection limit (3σ) of the analytical results for CL – 1 CRM, Cabbage Leaves for the determination of lead was 0.058 $mg\, kg^{-1}$; the relative standard deviation (RSD) was 7.3% ($n = 6$; $c = 0.5\%$ m/v), 6.0% ($n = 6$; $c = 1.0\%$ m/v) and 8% ($n = 6$; $c = 2\%$ m/v) and the characteristic mass was 8.5 pg/0.0044A.

Conclusion

The method developed for the determination of lead, based on the slurry technique, allows rapid plant analysis mainly because there is no necessity for sample pretreatment. In this respect, problems with contamination, losses of lead during digestion

procedures and operator errors are avoided. The slurry is sufficiently stable in 5% HNO_3 medium to carry out the full set of measurements required for quantification. The results obtained were in good agreement with the certified values. Two most important limiting factors in the slurry analysis are as follows: a homogenisation system is mandatory and the slurry concentration range intended for analysis is limited. Therefore we can use this method only to determine lead in rape leaves ($3.18–5.06\,\mu g\,g^{-1}$). The amounts of lead in rape roots, which we have determined ($45.8–833.8\,\mu g\,g^{-1}$), were higher than the concentration range that can be analysed ETAAS.

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