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THE EFFECT OF CHEMICAL FORM UPON ABSORPTION - TIME PROFILES OF Pb AND Cd IN BIOLOGICAL SAMPLES EMPLOYING THE »TAPE SANDWICH« SS-ETAAS TECHNIQUE †

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† This paper is dedicated to the late Mr. V. Vrščaj whose excellent work made this research possible.

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Abstract

Pb and Cd were determined in a variety of biological and plant samples by solid sampling electrothermal atomic absorption spectrometry (SS-ETAAS) employing both the slurry and direct solid (»tape sandwich«) introduction techniques. A laboratory assembled spectrometer and graphite cup atomizer with platform were employed for the measurements. The integrated absorbance was used as an analytical parameter. Calibration was made against aqueous standards. Simultaneously the absorption-time profiles were recordered. A reasonable agreement of the results in comparison to the certified or recommended values was obtained for Pb and Cd regardless of sample origin if the slurry sample introduction was employed. A low value was only observed in determination of Pb in a marine plant (Sea Lettuce) which was probably due to partly overlapping processes of Pb atom formation and matrix (NaCl) vaporization. Single peak absorption-time profiles were observed with different times of atom appearance and peak maxima characteristic of the element and sample origin.

Substantially lower results for Pb and possibly for Cd (statistically not significant) associated with the appearance of double peak absorption -time profiles were typical of vegetable samples from heavily polluted soil when the »tape sandwich« sample introduction was used. This feature was presumably the result of partial loss of the analyte in the ashing step and was found specific for the majority of vegetables but not for all plant samples and other biological materials investigated (hair, liver muscle). This phenomenon was obviously caused by the differences in ashing conditions between the sample introduction techniques employed. Namely, ashing of plastic tape disks (»tape sandwich« technique) in an oxygen atmosphere proceeds with considerable smoke formation. Simultaneous liberation of heat raises the temperature of the sample above that of the inner cup-platform (450 °C).

Differences in the shape and/or time of atom appearance in Pb and Cd absorption-time profiles using the »tape sandwich« introduction technique may be related to the various chemical forms of these elements in particular samples and not to the different location of the analytes in the samples. In this respect the environmental conditions and routes of Pb and Cd incorporation play a key role in plant and vegetable samples. Some evidence to support this hypothesis is presented.

Key words: Lead, Cadmium, determination, biological samples, absorption, tape sandwich technique, etass technique.

Introduction

Flame atomic absorption spectrometry (FAAS) and its electrothermal version (ETAAS) are among the most widely used analytical techniques for determination of trace elements in a variety of samples. Measurements are performed rapidly, the technique is fairly specific and highly sensitive. The major problem of the whole analytical procedure often lies in the preparation of the sample solution and frequently associated separations. The latter does not only represent an economic problem but may also be a source of a systematic error due to sample contamination. Therefore, analytical spectroscopists have long been aiming at the development of direct solid sampling techniques. Slurries prepared from finely ground materials were sprayed into flames[1-5] and plasmas[6-10] for atomic absorption and emission measurements, respectively. The convenience of performing such measurements was satisfactory, but the limitations were in the stability of the slurry and inefficient vaporization / atomization due to the extremely short residence time of the analyte in the flame and plasma (10^{-4}s).[10] The advent of ETAAS and its rapid growth in the 1980's (isothermal atomization) was a milestone in further development of solid sampling techniques. In addition to the above mentioned features, the major advantage of ETAAS for use in solid sampling analysis lies in the considerably longer time allowed for sample vaporization (several tenth's of a second) in comparison to flames or plasmas. As a consequence a rapid growth in the number of publications employing either slurry or direct solid sampling ETAAS was observed in the1990's. These included analysis of ultra pure electronic materials[11,12,13] coal, ash, sewage sludge, plastic, metals, metal compounds and alloys, glass, ceramics, graphite powder,[14-24] food and various biological samples.[25-31] In the same period specially designed commercial instruments appeared on the market for slurry (Perkin-Elmer) as well as for solid sampling (Grűn, Analytik -Jena) techniques. A comprehensive description of the theoretical background, instrumentation and applications of solid sampling ETAAS is given in the book edited by Kurfűrst.[32]

Research in direct solid analysis at the J. Stefan Institute was initiated in 1980 and was associated with studies of nebulization and atomization processes of slurries in flames.[33,34] In 1989 a laboratory - assembled ETAA-spectometer with specially designed graphite cups for direct atomization of solid samples was described and its performance evaluated by the analysis of various biological and geological samples[35].

An original, so - called »tape sandwich« sample introduction technique was developed and used for measurements of hair-metal (Pb, Cd, Cr, Zn) longitudinal profiles.[31] It was later demonstrated that this technique could be satisfactorily employed for the analysis of powdered samples.[36] However, the determination of Pb and Cd in biological samples of different origin (plants, vegetables, hair, tissues) employing the »tape sandwich« sample introduction technique revealed several anomalies in the characteristic of the absorption - time profiles. A particularly striking feature (double peaks in the absorption - time profile associated with loss of analyte) was observed from leafy vegetables grown on heavily polluted soil in determination of Pb and possibly Cd. Multiple absorption peaks or delayed absorbance-time profiles have previously been observed in direct atomization of soil,[7,38] glass,[14] gold,[39] plastic[40] and Ni- based alloys[18] employing graphite furnace at atmospheric pressure. Hassel et al.[41] working with a graphite furnace at reduced pressure (0.15 torr) observed a multiple peak Pb absorbance- time profile in direct atomization of a Cu- alloy. However, no significant losses of the analyte were reported in these studies. Although the reasons for these anomalies in absorbance-time profiles observed in atomization of solid samples are rather obscure, some more comprehensive studies indicated that either the location (surface or bulk) of the analyte in the sample[39] or the chemical form[42] may be important parameters.

The main aims of the present research were therefore directed to:

- investigation of the differences in characteristics of ashing conditions employing slurry and »tape-sandwich« sample introduction technique;
- evaluation of the effect of these differences on the shape of absorption- time profiles of Pb and Cd observed in atomization of biological samples of different origin;
- establishment of some association, if any, between the characteristic shape of the absorption- time profile and the specific chemical form or location of the analyte in the sample.

Results and discussion

The results of lead and cadmium determination in various vegetables, the hair sample and certified reference materials are summarized in Tables 1 and 2, respectively. In the atomization of slurries the mass of sample used to prepare the slurry was higher

(~100 mg) than the generally found minimum representative sample masses of these materials (10 – 75 mg).[36] The measured results should therefore represent the true value of the sample bulk. This was found to hold for both lead and cadmium as the results for the CRM's measured along with the samples were very close to the certified values (see Table 1 and 2, first column "c. value"). The precision of the determination is governed by the homogeneity of the slurry, the mass of sample analysed and the precision of the atomic absorption measurement.

Table 1. Lead content (μg/g)* of various plant and biological samples; comparison of slurry and direct sample introduction techniques.

Sample	slurry (n=3) [b]	direct sample introduction (n=10) [c]	
		measured results	% of total
chicory – red [a]	6.75 ± 1.24	3.8 ± 0.83	56
parsley – stems [a]	4.2 ± 0.45	2.7 ± 1.2	64
parsley – roots [a]	18.9 ± 0.9	11.9 ± 1.7	63
cabbage [a]	8.1 ± 0.77	5.0 ± 1.5	62
lettuce [a]	13.8 ± 1.3	10.6 ± 1.2	77
chicory – green	0.78 ± 0.32	0.68 ± 0.21	87
parsley – roots	1.09 ± 0.39	0.65 ± 0.6	60
hair sample [a]	9.3 ± 0.6	9.5 ±1.1	102
Chinese hair reference sample GBW09101	7.5 ± 0.6 c. value 7.2 ± 0.7	7.6 ± 1.0	105
BCR/CRM No. 281 Rye Grass	2.31 ± 0.15 c. value 2.38 ± 0.11	2.32 ± 0.7	97
BCR/CRM No. 278 Mussel Tissue	2.01 ± 0.16 c. value 1.91 ± 0.04	2.07 ± 0.49	108
BCR/CRM No. 279 Sea Lettuce	12.03 ± 0.60 c. value 13.48 ± 0.36	8.1 ± 0.8	60
BCR/CRM No. 185 Bovine Liver	0.52 ± 0.15 c. value 0.501 ± 0.027	0.51 ± 0.53	102

* dry matter: [a] samples from lead smelter area, [b] sample mass 100 mg, water volume 2-5 cm^3. [c] sample mass < 1 mg.

In direct atomization of solid microsamples (generally 0.2 – 1 mg) the heterogeneity of the material was shown to have significant influence and may adversly affect the results.[32,36] It is therefore imperative that the number of measured subsamples is large enough to yield a result representative of the sample bulk. It has been previously shown that generally 25 to 40 subsamples should be measured and the results averaged

if subsamples are taken randomly from the tape.[36] Therefore, in routine analysis of samples a different sampling approach was employed in order to increase the probability of getting a meaningful value of the sample bulk in a reasonable number of measurements. In this case a similar mass of sample (15 – 30 mg) was placed between the tapes but only 8 – 12 segments were punched out for analysis, taken uniformly over the whole area (see Figure 1). This was indeed confirmed by the determination of cadmium in the majority of vegetable samples, hair and the certified reference materials analysed. With one exception the results of the two sample introduction techniques were in good agreement within their standard deviation intervals (see Table 2). Analysis of data using t – test showed that the results are not statistically different at 5% level except for the sample lettuce No.1. The precision of determination was generally not considerably better by slurry sampling than by direct sample introduction. This is quite understandable since the analyte homogeneity in the slurry should not be much better than that in the solid micro-sample if the masses used in measurements are similar. Thus in slurry sampling only appreciable solubility of the analyte can improve the precision.[44]

Table 2. Cadmium content (ng/g)* of various plant and biological samples; comparison of slurry and direct sample introduction techniques.

Sample	slurry[b] (n=3)	direct[c] sample introduction (n=10)
lettuce (No. 1)	246 ± 10	204 ± 15
lettuce (No. 2)	158 ± 20	136 ± 22
chicory-red [a]	865 ± 134	707 ± 259
cabbage [a]	1180 ± 130	1003 ± 88
cabbage [a]	140 ± 28	138 ± 14
parsley [a]	1940 ± 320	1580 ± 820
celery - stems [a]	2550 ± 340	2520 ± 250
hair sample [a]	280 ± 22	275 ± 55
Chinese hair reference sample GBW09101	91 ± 19 c. value 95 ± 12	87 ± 26
BCR/CRM No. 278 Mussel Tissue	328 ± 20 c. value 340 ± 20	317 ± 52
BCR/CRM No. 185 Bovine Liver	285 ± 45 c.value 298 ± 25	257 ± 75
BCR/CRM No. 279 Sea Lettuce	275 ± 32 c.value 274 ± 22	273 ± 84
BCR/CRM No. 281 Rye Grass	113.4 ± 17 c.value 120 ± 3	115 ± 32

* dry matter: [a] samples from lead smelter area, [b] sample mass 100 mg, water volume 2-5 cm^3. [c] sample mass < 1 mg.

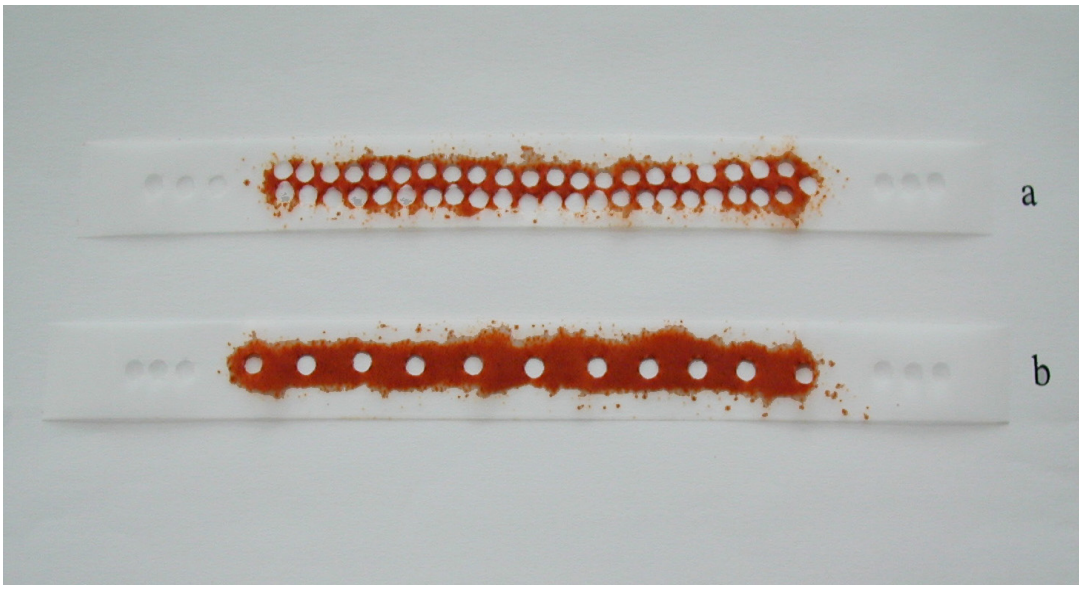

Figure 1. Preparation of samples for direct introduction (»tape sandwich«) electrothermal atomic absorption measurements; estimation of sample homogeneity (a), determination of average sample content (b).

Determination of lead in vegetables by the direct sample introduction technique using the same procedure as described in determination of Cd in general yielded substantially lower results in comparison to slurry sampling (see Table 1). There is obviously a systematic error in the determination caused by the loss of analyte prior to atomization. However, in the atomization of the hair sample measured values for Pb obtained using the two sample introduction techniques did not deviate appreciably. Previously[36] we analysed Chinese Reference Hair GBW09101 (certified value 7.2 ± 0.7 μg Pb/g) employing the " tape sandwich " technique and the value obtained 8.0 ± 2.1 μg Pb/g was within the uncertainty limits of determination. Similar results were also found in determination of Pb in other biological certified reference samples (Mussel Tissue, Bovine liver). Thus the partial loss of lead which apparently occurred in the ashing step was specific for vegetables using the "tape sandwich" sample introduction technique. This loss was not observed when samples were atomized as slurries, the exception being Sea Lettuce, but the reason for the low values was thought to be different. Interestingly, no statistically significant loss of cadmium was observed regardless of the material analysed or the sample introduction technique employed.

In the first instance we identified a major difference in the ashing conditions using the slurry and the "tape sandwich" sample introduction techniques in the oxygen atmosphere. An approximately similar mass of sample was introduced into the graphite cup in both cases. When the slurry was ashed after evaporation of water, the remaining solid material was efficiently oxidized in about 10 – 20 s with moderate formation of smoke but no significant heat generation. The temperature of the sample was therefore approximately equal to that of the graphite cup (< 450 °C). This was not true in ashing of samples when these were contained in a plastic envelope ("tape sandwich" technique). Namely, each sample introduced into the cup consisted of approximately 200 - 800 μg of material to be analysed and 1000 μg of plastic material. Ashing of these plastic segments in the oxygen atmosphere proceeded with considerable smoke formation and heat generation. If the sample (plastic disk) is not properly aligned in the cup[36] the oxidation of the tape organic matter proceeds vigorously resulting in the appearance of a red glow. Since the sample material is in close contact with the plastic tape, the temperature of the sample in this period exceeds that of the inner cup (450 °C), and may even reach 800 °C for a few seconds in the case when a red glow appears. Typical variation of temperature of the inner cup, time intervals of purge gas application and smoke formation in the ashing step are illustrated in Figure 2.

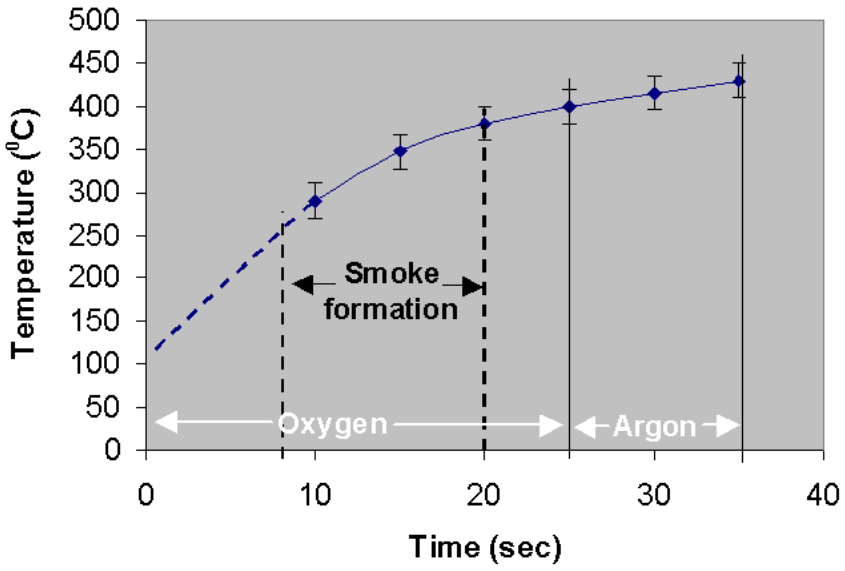

Figure 2. Temperature variation of the inner cup-platform in the ashing step of the atomization cycle.

Absorption – time profiles of Lead and Cadmium.

Absorption – time profiles obtained in the atomization process in ETAAS can give some valuable information about the kinetics of several processes involved in the formation and dissipation of the atomic cloud in the graphite furnace.[45] Additionally, if background absorption is monitored simultaneously, the appearance of molecular species and/or fine particulate matter can also be identified. This is of particular importance in SS-ETAAS where severe interference of the matrix on the time dependent analyte atom concentration may be expected.

The absorption – time profiles of Pb and Cd, and those of nonspecific absorption were recorded in atomization of various biological and plant tissue samples for the following reasons:

- to obtain information about the kinetics of atom formation in atomization of slurries and by direct introduction of solid material employing the "tape sandwich" technique. This may provide an explanation for the systematically lower Pb results observed in the majority of vegetable materials when the latter sample introduction technique was used;
- to evaluate the possible effect of matrix volatility upon the analyte atom concentration – time profile, which should provide a reasonable justification for the use of aqueous standards in calibration of solid biological samples.

However, a prerequisite to using the absorption – time profiles for interpretation of the various phenomena observed in atomization of different biological samples is that these are fairly reproducible. In SS-ETAAS when the "tape sandwich" sample introduction technique was employed, the absorption – time profiles of the same samples obtained on the same day were reasonably reproducible. Even experiments repeated on different days showed the same pattern with very little shift on the time scale. This can be explained by very reproducible sample alignment in the graphite cup (uniform distribution of the powdered sample over the surface of the plastic disk) and constant heating conditions.

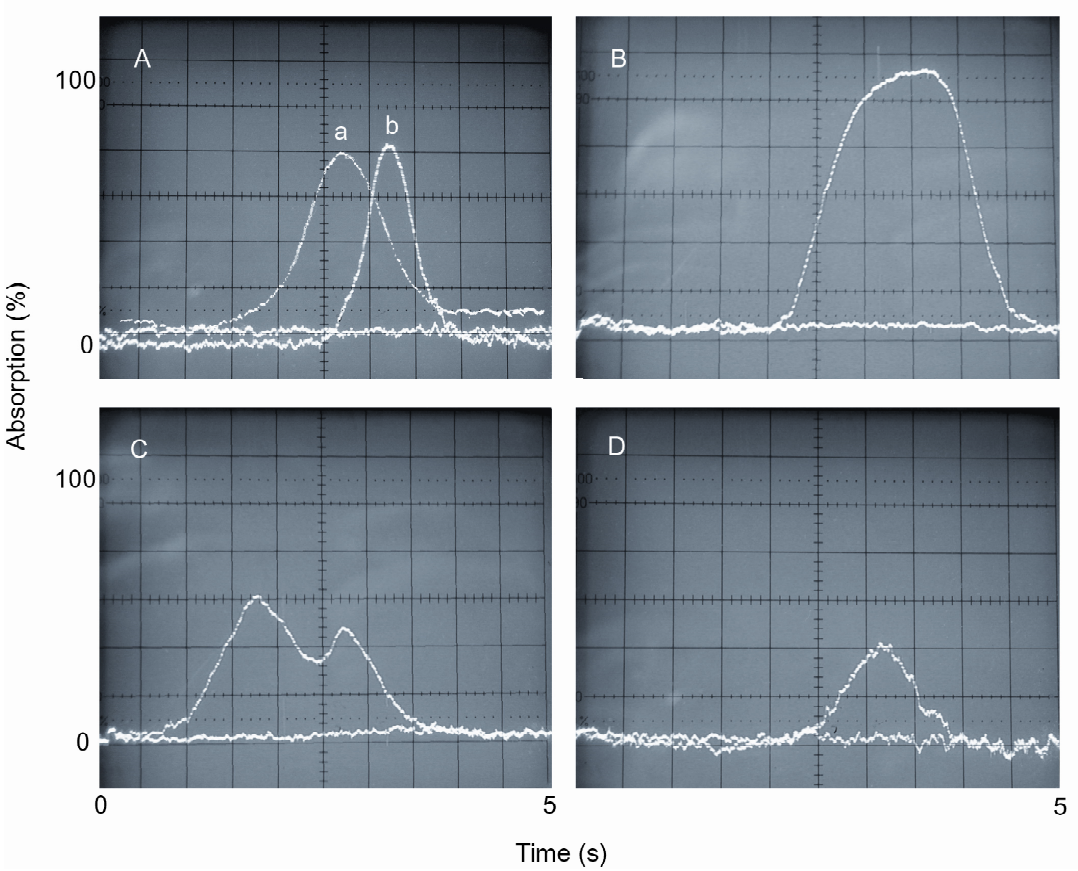

Figure 3. Absorption-time profiles of lead resulting from atomization of aqueous standards and various biological solid samples employing the direct »tape sandwich« introduction technique; A – $PbCl_2$ (a), $PbSO_4$ (b): B – powdered hair: C – green chicory: D – bovine liver.

In Figure 3 the absorption – time profiles of lead resulting from atomization of aqueous standards (chloride and sulphate), and various biological materials are illustrated. The latter were introduced into the cup employing the "tape sandwich" approach. An interesting feature was observed when aqueous solutions of $PbCl_2$ and $PbSO_4$ of approximately equal Pb concentration were atomized under identical heating conditions (see Figure 3A). It is apparent that the kinetics of vaporization of these two Pb species differs considerably. The absorption– time profile of lead in the form of sulphate (Figure 3 Ab) is narrower in comparison to the corresponding profile of lead chloride, (Figure 3 Aa), the atom appearance time and the time of absorption maximum of the former being considerably delayed. This phenomena may be explained by considering the substantial difference in chemical reactions taking part in the formation of free Pb atoms (reactions 1a, 2a, and 5a).

Possible chemical reactions involved in the atomisation of Pb and Cd from aqueous solution and different biological and plant samples are as follows:

$PbCl_2(s) \rightarrow PbCl_2(v) \rightarrow Pb\ (v)$ (1a)

$CdCl_2(s) \rightarrow CdCl_2(v) \rightarrow Cd\ (v\ )$ (1b)

$PbSO_4(s) \xrightarrow{1200\ ^{\circ}C} PbO\ (v) + SO_2(g)$ (2a)

$CdSO_4(s) \xrightarrow{1200^{0}C} CdO\ (v) + SO_2(g)$ (2b)

$PbCO_3(s) \xrightarrow{315^{0}C} PbO(s) + CO_2(g)$ (3a)

$CdCO_3(s) \xrightarrow{500^{0}C} CdO(s) + CO_2(g)$ (3b)

$Pb\text{-}S\ (L) + O_2 \rightarrow PbSO_4\ (PbO) + SO_2(g)$ (4a)

$Cd\text{-}S\ (L) + O_2 \rightarrow CdSO_4\ (CdO) + SO_2(g)$ (4b)

$PbO\ (v) + C(s) \rightarrow Pb(v) + CO$ (5a)

$CdO\ (v) + C(s) \rightarrow Cd(v) + CO$ (5b)

L = ligand

The lead absorption – time profile showing a well resolved double peak presented in Fig. 3 C is typical of vegetable samples analysed in this work but not for CRM Rye Grass and some other plant samples. The absorption – time profiles resulting from atomization of the Rye Grass reference sample showed a single maximum which coincides well with $PbCl_2$ aqueous standard, and the found (2.32 ± 0.7 μg Pb/g) and certified (2.38 ± 0.11 μg Pb/g) values agreed well. The appearance of a double peak absorption – time profile observed in atomization of vegetables was always associated with a lower result for Pb in the measured sample in comparison to the value obtained by slurry sample introduction. Since the second peak maximum in Figure 3C coincides with the absorption peak of $PbCl_2$ solution (Figure 3 Aa), it may be concluded that part of the lead retained in the cup after the ashing phase vaporized at an identical rate. The first maximum which appeared one second earlier corresponds to the amount of Pb vaporized and condensed on the cooler cup walls during oxygen ashing. Some of the lead is obviously removed out of the cup by the smoke before atomization and is lost for absorption.

On the other hand, several biological samples such as hair (Figure 3B) and bovine liver (Figure 3D) exhibit well defined single peak absorption profile in atomization. The

time of atom appearance and the time of peak maxima of these materials resembled closely that of Pb sulphate. There was no systematic error in the results of these materials obtained using the "tape sandwich" introduction approach and an aqueous standard for calibration (see Table 1). It is anticipated that in these tissues lead is strongly bound to various proteins containing SH–groups (for example metallothionein in liver, cystine in hair). It is assumed that the Pb-S bond is strong enough to sustain oxygen ashing. Thus the final species formed in the ashing step is likely to be Pb-sulphate (reaction 4a) which in the atomization step decomposes to PbO, a precursor of Pb atoms following the reaction 5a.

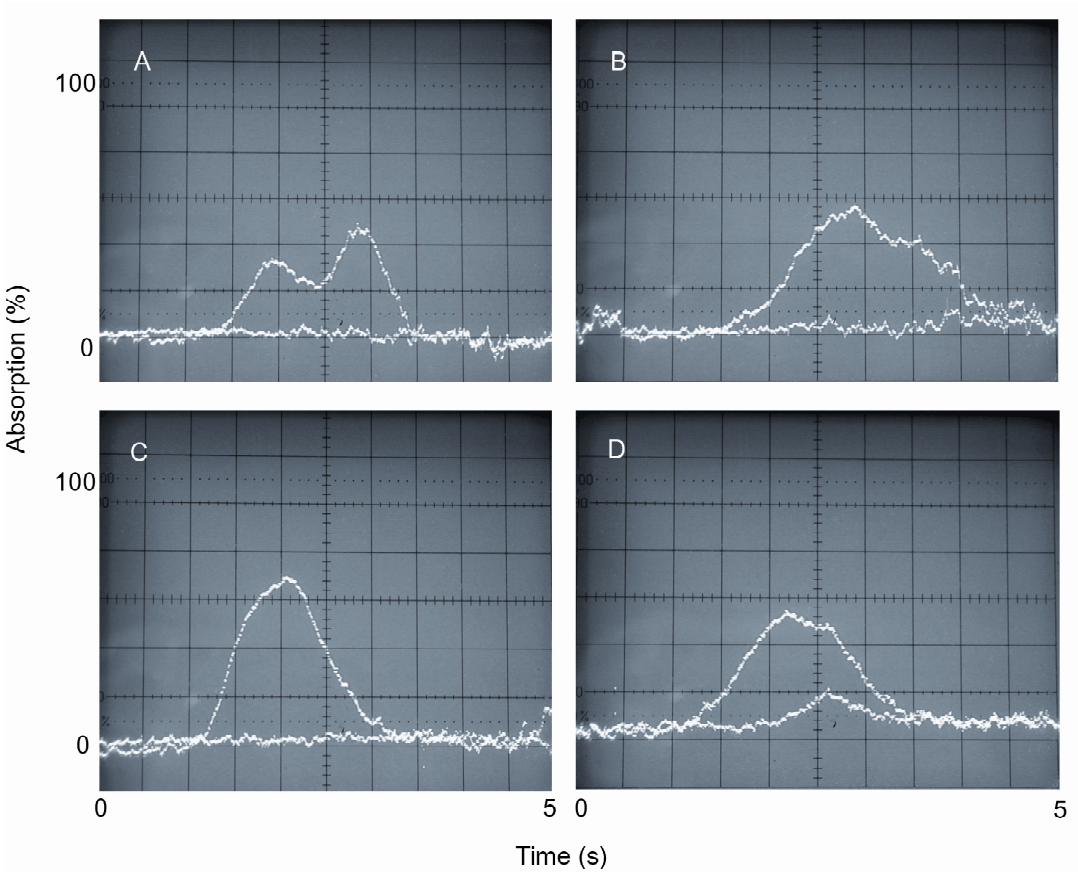

Figure 4. Absorption-time profiles of lead resulting from atomization of different plant materials employing the direct »tape sandwich« and slurry introduction techniques; A – lettuce – direct introduction: B – Sea Lettuce – direct introduction: C – lettuce – slurry introduction: D – Sea Lettuce – slurry introduction.

In another experiment two plant materials (lettuce from Pb polluted soil and CRM - Sea Lettuce) were atomized at identical heating regimes but using different sample

introduction techniques (slurry and "tape sandwich"). The corresponding absorption – time profiles are illustrated in Figure 4. The difference in the absorption – time profiles is particularly well established for the vegetable sample (lettuce). Slurry sampling of this material resulted in a single absorption maximum (Figure 4C) giving an approximately 25% higher average Pb content in comparison to that found when "tape sandwich" introduction was used (Figure 4A). In this instance it should be pointed out that the time of atom appearance and Pb absorption maxima of vegetable samples employing the slurry technique (Figure 4C) appeared earlier in comparison to a Pb chloride solution (Figure 3 Aa). This observation of different vaporization kinetics might be explained by the different chemical form of Pb existing in these samples. Namely, the uptake of Pb by plant species via the root system is limited [46,47] and a substantial proportion of lead in the plant may be absorbed by foliar deposition.[47] This effect is certainly more pronounced for leafy vegetables and lead polluted areas. The dominant part of foliar absorbed Pb remains in the leaf structure in the original inorganic form (mainly as Pb – carbonate[48]), while a certain proportion is transferred to other parts of the plant and is probably bound to amino acids and other ligands present in the xylem. Hence, the major fraction of Pb atoms are produced by the reactions 3a – 5a and 4a –2a –5a. First part of the former reaction scheme proceeds in the ashing step accompanied by liberation of a significant amount of smoke produced by the burning of the plastic tape material which carries part of the Pb-oxide out of the cup.

A somewhat specific vaporization – atomization pattern was exhibited by the marine plant material (CRM – Sea Lettuce) which was characterised by a partly resolved double peak (see Figure 4B). The time of the first absorption maximum corresponds closely to that of $PbCl_2$, and the second maximum to that of $PbSO_4$. It is therefore very likely that these are the major Pb species present in this plant. It should be emphasized that this sea- plant (Utva lactuca) is a kind of algae and has no roots. Thus Pb uptake proceeds directly from the liquid media (sea water). In the case of slurry sample introduction only one absorption maximum was obtained (Figure 4D). In the atomization process of this material nonspecific absorption due to volatilization of the inorganic matrix (NaCl) was observed (see Figure 4D and Figure 6B). The volatilization of the matrix was not completely resolved from the Pb atom removal process and this might be the reason for the interference obtained for both of the sample introduction techniques employed when the calibration was made against a Pb aqueous standard (see Table 1).

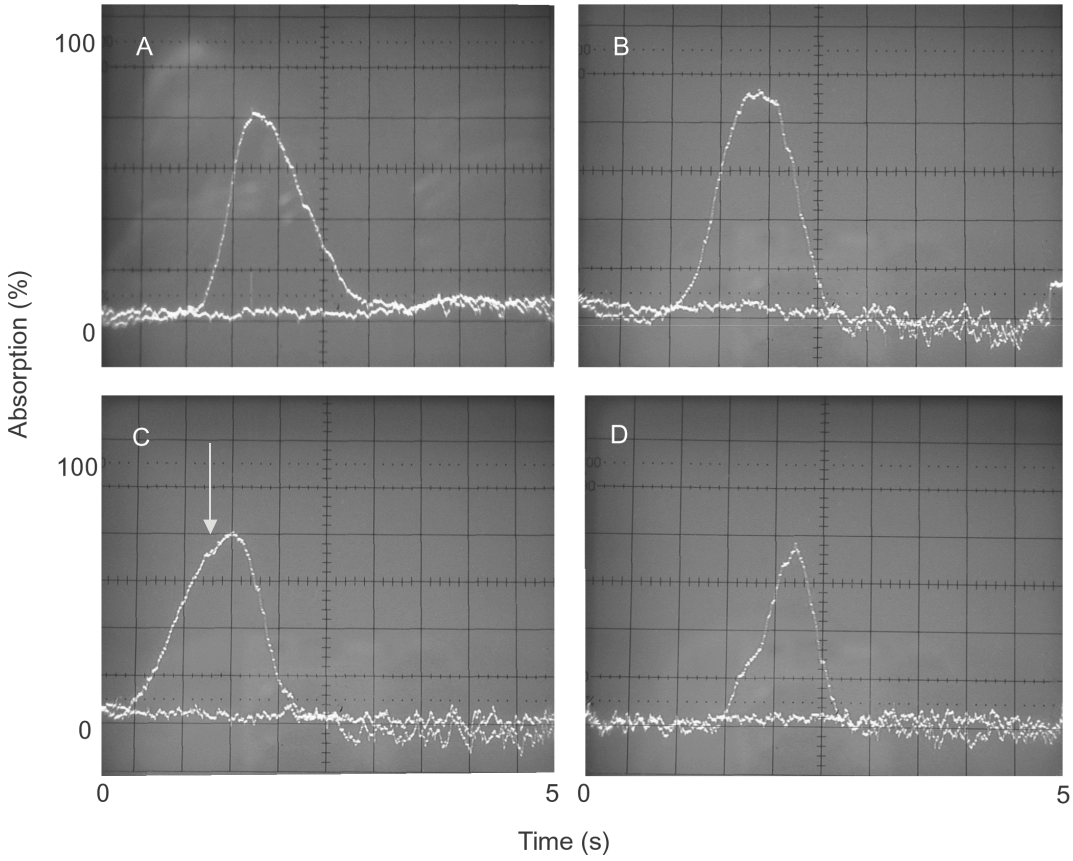

Figure 5. Absorption-time profiles of cadmium resulting from atomization of aqueous standard and various biological solid samples employing the direct »tape sandwich« introduction technique; A – $CdCl_2$: B – powdered hair: C – cabbage: D – bovine liver.

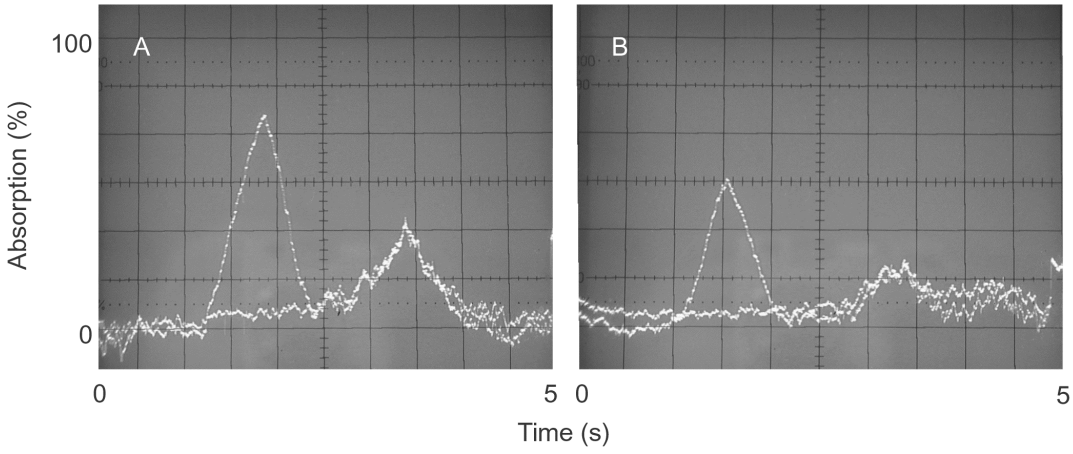

Figure 6. Absorption-time profiles of cadmium resulting from atomization of different biological samples employing the direct »tape sandwich« introduction technique; A – Mussel Tissue: B – Sea Lettuce.

In Figure 5 and 6 absorption – time profiles of Cd obtained by atomization of $CdCl_2$ aqueous solution and various biological tissues are presented. The "tape sandwich" sample introduction technique was used for all the materials with the exception of $CdCl_2$. In neither case were well resolved double absorption maxima observed implying that no substantial vaporization losses of Cd should have occurred. This was indeed true as Cd values for most of these materials (vegetables and CRM´s) obtained by either of the sample introduction techniques were comparable to or within the uncertainty range of the CRMs employed (see Table 2). However, there is some indication (an arrow in Figure 5C denotes the position of the possible first peak maximum) that vaporization of Cd from a vegetable sample taken from polluted soil (20 μg Cd/g) proceeds at two different kinetic rates but due to the rapidity of the processes, the two peaks are not resolved. Therefore the absorption – time profiles of these samples obtained by slurry atomization were not recorded.

Analysis of Cd absorption – time profiles (Figure 5 and Figure 6) leads to the following conclusions:

- vaporization of Cd species from vegetables (cabbage grown on Cd polluted soil – Figure 5C) proceeds much faster than from any other biological tissue examined;
- hair and liver tissue showed the slowest vaporization kinetics (see Figure 5 B and Figure 5 D);
- a similar vaporization – atomization pattern is observed for CRM Mussle Tissue, CRM Sea Lettuce and $CdCl_2$ aqueous standard (see Figure 6A and 6B and Figure 5A);
- a substantial amount of nonspecific absorption was observed in atomization of CRM Sea Lettuce and CRM Mussle Tissue, but the latter was completely resolved from Cd atomic absorption (see Figure 6 A and B).

Similar chemical reactions proposed to explain Pb absorption – time profiles can also be applied for Cd. It is certainly most likely that formation of $CdSO_4$ in atomization of hair and liver tissue is responsible for their slow vaporization kinetics (see Figure 5B and Figure 5D) following reactions 4b and 5b.

In marine organisms such as CRM's Sea Lettuce and Mussel Tissue Cd may be predominantly in the chloride form as they showed atomization profiles similar to $CdCl_2$ aqueous standard. Hence the vaporization kinetics corresponds to that of reaction 1b.

In contrast to Pb, Cd root uptake is very efficient and represents the major route (69-94%) of Cd transport into the plant.[49] In Cd polluted areas, however, foliar uptake should also be considered[47,49,50] but the proportion of this to the total intake is substantially smaller than in the case of Pb. It may therefore be anticipated that the majority of Cd in plants is bound to organic ligands and a smaller part may be present (adsorbed in the leaf structure) in the inorganic forms found in soil or dust particles (carbonate,[48] oxide). Hence, the vaporization-atomization processes involved in the atomization of vegetable sample (Figure 5C) follow two different patterns described by the reactions 3b - 5b (fast kinetics) and 4b-5b (slow kinetics). However, the major proportion of Cd atoms is presumably formed by decomposition of low molecular weight organic Cd- complexes formed in the plant. A minor part of the cadmium in leafy vegetables originates from particulate deposition which is predominantly Cd-carbonate.[48] The former, however, decomposes to CdO (reaction 3b) at the higher temperature than Pb- carbonate. Thus liberation of smoke resulting from the burning of the plastic tape does not coincide exactly with decomposition of Cd-carbonate and does not contribute to significant Cd losses.

So far we have considered the most probable chemical forms of Pb and Cd existing in particular biological samples and chemical reactions involved in vaporization-atomization processes as being responsible for the characteristic pattern or shift of absorption-time profiles. No account, however, has been taken of the role of the location of the analyte in the sample, which could produce a similar effect.[18,39,41] The latter proposition seems indeed feasible for vegetable samples (lettuce, chicory, etc.) grown on polluted soil where part of the analyte is located in the plant (root uptake) and a substantial part on the plant surface (foliar uptake). However, this is only valid for the untreated sample. Grinding of the sample to a particle size of $\leq$30 μm[36] significantly increases the specific surface area and thus practically eliminates the original difference in analyte location.

Conclusions

The so-called “tape sandwich” technique has been developed for direct introduction of solid samples into the graphite furnace for atomic absorption measurements. It was primarily employed for measurements of hair – metal longitudinal

profiles, and its use was later extended to the analysis of powdered samples. The technique enables convenient and rapid sample changing, the drawback being a weighing error of ±1–3% due to variation in the tape blank (±10 μg) which limits the lower acceptable sample weight to 200 μg. The tape material was found to be free of analyte elements (Pb, Cd). A detailed investigation of the ashing step using oxygen (in the first 25 s) for efficient decomposition of organic matter revealed certain differences in the actual experimental conditions between the tape sandwich and slurry sample introduction technique. In the former heat generated by the rapid oxidation of the tape organic matter raises the temperature of the sample above that set by the instrument (450 °C). Simultaneously, a significant amount of smoke is liberated. Several different reactions are possible which might occur in the preatomization period depending on the forms of the analyte in the sample. PbO and CdO species liberated by decomposition of respective carbonates present in majority of vegetable samples are carried away by forced convection, and part of them condenses on the cooler cup walls. As a consequence double peak absorption-time profiles were observed for most vegetable samples in the determination of Pb, associated with systematically lower results. An indication exists of similar phenomena in determination of Cd in vegetable samples, but the effect is less pronounced. Shifts of Pb and Cd absorption–time profiles characteristic of particular types of biological samples were adequately explained by the different chemical forms of these analytes in the sample. The effect of the different location of the analyte which exists in original vegetable samples (root and foliar uptake) could have an identical consequence on the absorption – time profile pattern, but due to the considerable increase in the specific surface area of the ground samples (≤30 μm size) this possibility was assumed unreasonable.

Experimental

Instrumentation. A laboratory assembled atomic absorption spectrometer and graphite-cup furnace were employed, and are described in detail in a previous paper[35] A type B graphite cup with an inner cup acting as a platform was found to be optimal for Pb and Cd measurements. The integrated absorbance was measured as the analytical parameter of interest while simultaneously the absorption-time profiles were recorded on an oscilloscope. The oscilloscope signal was triggered by the signal from the power

unit; zero time on the time scale coincided with the beginning of the atomization step. By this means particular absorption-time profiles could be compared. The instrumental settings and atomization parameters were carefully optimized and were identical for both sample introduction techniques employed. They are summarized in Table 3.

Table 3. Instrumental parameters for direct atomization of biological materials in determination of Pb and Cd.

Parameter	Lead	Cadmium
Absorption line (nm)	283.3	228.8
Atomization cycle		
drying step (s)	5	5
(°C)	90 - 110	90 – 110
ashing step (s)	35	35
(°C)	450	450
atomization step (s)	3	3
(°C)	2000	1700
Argon flow rate (cm^3min^{-1})	3000	
Oxygen flow rate (cm^3min^{-1})	2500	
Background correction	Simultaneous D_2 - lamp	
Measurement mode	Integrated absorbance	

Samples. Various vegetables used in human nutrition were collected from two gardens, one located near a lead mine and smelter, the second one in a small rural settlement far away from traffic and industry. The total Pb and Cd contents of these soils (0-25 cm) were: 3100 μg Pb/g, 20 μg Cd/g and 35 μg Pb/g, 0.69 μg Cd/g respectively. Vegetables were carefully washed to remove any surface contamination (soil, dust), cut into small pieces, and dried in hot air (60 °C). Homogenization of the samples was performed by grinding in an agate ball mill (Fritch Planetary micro mill "Pulverisette 7"). The majority of particles (90%) in the ground samples were estimated to be below 30 μm in diameter.[36] A pulverized hair sample taken from the head of a subject living in the polluted area and various plant and tissue certified reference materials (BCR/CRM No.281 Rye Grass, BCR/CRM No.279 Sea Lettuce, BCR/CRM No.185 Bovine Liver, BCR/CRM No. 278 Mussel Tissue, Chinese hair reference sample GBW09101) were also measured in order to check the accuracy of the results. Slurries were prepared in three parallel batches by mixing ~100 mg of sample and 2 to 10 cm^3 of doubly distilled water using an ultrasonic immersion device. 10 to 20 mm^3 of slurry was atomized for a

single measurement. For direct introduction of powdered samples using the so-called "tape sandwich" technique 8 – 12 subsamples of 0.2 to 1 mg were measured and the average value was taken as the result of each particular determination. The calibration was made against aqueous standards ($PbCl_2$, $CdCl_2$) for both sample introduction techniques since the precision of measurment of aqueous standards was far superior (±2 - 3%) in comparison to the precision of measurements of available solid reference standards (±10 - 30%) due to material heterogeneity. It has been previously shown that the concept of aqueous standards in atomization of solid samples is satisfactory for many elements whose volatility is much different from the matrix, and isothermal atomization has been employed.[18,19,31,36,39,40,43] In the case of biological materials the majority of the matrix constitutes organic matter which is destroyed and removed during the ashing step. The remaining inorganic substances do not influence the atomization of Cd and Pb as they vaporize earlier (see Figures 3 – 6). The exception is constituted by marine organisms (Mussel Tissue and Sea Lettuce) which showed considerable background absorption during atomization which was due to sodium chloride vapour. The appearance of sodium chloride vapour partly interfered with the removal of Pb atoms in atomization of these materials and may influence the accuracy of the results when calibration is made with reference to an aqueous standard. Characteristic masses of 55 pg and 3 pg for lead and cadmium, respectively, were calculated for aqueous solutions.

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Povzetek

Določali smo vsebnost Pb in Cd v različnih bioloških in rastlinskih vzorcih z metodo elektrotermične atomske absorpcijske spektrometrije ter neposredne atomizacije trdnih vzorcev. Vzorce smo vnašali v grafitni atomizator (lonček) v obliki suspenzije oziroma s tehniko »tape sandwich«. Meritve so potekale na laboratorijsko sestavljenem spektrometru, integrirano absorbanco smo uporabili kot analizni parameter. Umeritveni premici smo pripravili z vodnimi standardi. Istočasno smo na osciloskopu spremljali časovne spremembe koncentracije atomov v grafitnem lončku.

Pri atomizaciji vzorcev v obliki suspenzij smo za Pb kot Cd in ne glede na vrsto vzorca ugotovili zadovoljivo skladnost med priporočenimi in izmerjenimi vrednostmi. Edino nižjo izmerjeno vrednost smo opazili pri določanju Pb v morski rastlini (Sea Lettuce) kar je verjetno posledica istočasnosti procesov tvorbe atomov Pb in izparevanja matrice (NaCl). Časovni potek absorpcije je bil v vseh primerih identičen (en sam vrh) vendar z različnimi časi začetka pojava atomov ter njihove največje koncentracije odvisno od elementa in vrste vzorca.

Uporaba tehnike »tape sandwich« za vnos vzorcev v grafitni lonček je vodila do zanimivih opažanj. Bistveno nižje so bile izmerjene vrednosti za Pb in verjetno nekoliko nižje (razlika ni statistično značilna) tudi za Cd v večini vzorcev zelenjave z močno onesnaženih tal. Časovni potek atomizacije pa nakazuje prisotnost dveh kinetično različnih procesov v fazi sežiga organske snovi. Omenjeni fenomen, ki je značilen samo za večino vzorcev zelenjave ne pa za druge preiskovane rastlinske in biološke vzorce (lasje, tkiva) je posledica različnih pogojev v fazi sežiga pri uporabi omenjenih načinov vnosa vzorcev v grafitni lonček. Pri uporabi tehnike »tape sandwich« pride zaradi sežiga plastičnega traku v kisikovi atmosferi do znatnega sproščanja dima in toplote, ki dvigne temperaturo vzorca nad temperaturo lončka – platforme (450 °C), ki je določena z nastavitvijo instrumenta.

Karakteristične razlike v časovnem poteku atomizacije Pb in Cd pri uporabi tehnike vnosa »tape sandwich« so najverjetneje povezane s prisotnostjo različnih kemijskih oblik elementov v posameznih vzorcih, ne pa z različno lokacijo elementa v vzorcu. Stanje okolja ter način privzema elementa sta pri vzorcih zelenjave in rastlin ključnega pomena. V prispevku smo navedli nekaj dokazov, ki podpirajo predloženo hipotezo.