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Determination of chromium, manganese, lead and cadmium in biological samples including hair using direct electrothermal atomic absorption spectrometry

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Abstract

Direct analysis of solid samples employing a laboratory assembled electrothermal atomic absorption spectrometer is demonstrated to be a feasible approach for determination of trace elements in plant tissue and hair samples for special applications in plant physiology and biomedical research. As an example, the kinetics of Cr uptake by cabbage and its distribution have been measured as a function of chromium speciation in the nutrient solution. Further, longitudinal concentration gradients of Cr, Pb and Cd have been measured in hair of various population groups exposed to different levels of these elements in ambient and/or occupational environments. The techniques are validated for the determination of these trace elements by neutron activation analysis, dissolution atomic absorption spectrometry and by analysis of certified reference materials. Slurry sample introduction is found appropriate for routine trace element determination and in homogeneity testing. Direct sample introduction is indispensable in the analysis of very small (< 1 mg) tissue biopsy samples in the determination of trace element distributions.

Keywords: Botanical samples; ETAAS; Hair samples; Slurry sampling; Solid sampling

1. Introduction

The unique properties of electrothermal atomic absorption spectrometry (ETAAS), such as the long residence time of the analyte in the furnace, high sensitivity, selectivity, and the ability to handle small samples make this technique one of the most promising and appropriate for direct elemental analysis of solid samples. Therefore it is not surprising that recent developments in background correction and furnace technology, arising from work of L'vov, Slavin and co-workers [1–3] have stimulated research into and the applications of ETAAS in direct solid analysis.

In addition to the reduced analysis time there are several other even more important features of solid sampling in ETAAS such as substantially minimized risk of contamination and/or loss of analyte, the lower zero blank, the improved detection limits and the submilligram sample size offering the possibility of micro heterogeneity measurements. A survey of the published literature on direct determination of lead, cadmium, manganese, chromium and other elements by ETAAS indicates an

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extreme popularity of this technique in the analysis of biological, food and related samples [4–18]. Two approaches of sample introduction (slurry sampling and direct sample introduction) have been employed; however, the former has proved more popular due to the simplicity of operation. For slurry preparation a number of diluents have been proposed by different authors. Some preferred pure water [4,5,14], others preferred water with the addition of a surface active agent and/or matrix modifier [7,9,10,15,16], whilst another group of researchers favoured dilute nitric acid [8,11,12,17,18] in order to increase the fraction of the analyte in the liquid phase. Miller-Ihli [18] who produced excellent research on slurry sampling in ETAAS [18–20] investigated the effect of analyte partitioning in the slurries on the precision of measurements. A new term "representative sample mass' was introduced to define the portion of the sample represented by the analysis. Partial calcination of the sample material before slurry preparation by ashing in an electrical oven [11,14,16] or sulphuric acid digestion has been suggested to reduce the particle size and to prevent deterioration of the pyrolitic coating in the graphite tube. A variety of techniques such as magnetic stirring [15,16], vortex mixing [6], sonification [12,15,17,18,19,20], or the application of thickening agents [8], have been successfully employed for slurry stabilization. Ultrasonic homogenization is no doubt the most efficient approach applicable not only for biological samples but one must be cautious of contamination from the ultrasonic probe or the beaker material [12]. A certain extent of disagreement can be noticed in the literature about the influence of particle size of slurried biological sample upon the precision of measurement and accuracy of determination. Ebdon et al. [7,9] claimed that 90% of the particles in the suspension should be smaller than 20–25 μm in diameter for optimal slurry electrothermal atomization. Working with such fine powders, excellent precision and accuracy was reported for a number of biological certified reference materials (CRMs). Only manual shaking of the suspensions before pipetting for sample introduction was adequate. On the contrary, Miller-Ihli pointed out [19] that uniformity of particle size may be more important than the absolute size in determining precision of biological slurry measurements. In general simple aqueous standard calibration was found satisfactory for quantitation of biological slurries for most analytes [7,8,9,12, 15,18,20]. However, certain authors [5,16,17] reported interferences arising from the matrix and recommend calibration by the method of standard additions. Introduction of oxygen [5] or air [14] during the ashing step which facilitates an efficient destruction of organic matter at low temperature has been proposed to reduce the non-specific absorbance and prevent formation of carbonaceous residues in the graphite tube.

Apart from showing an acceptable precision and accuracy, the current literature survey on direct determination of trace elements in botanical samples failed to demonstrate some specific features of this technique that can be used in plant physiology studies. Indeed only a few papers deal with real problems [10,11,16]. The feasibility of using ETAAS for direct analysis of a single hair segment was first realized by Renshaw et al. [21,22]. In their study, longitudinal concentration gradients of lead and copper were measured in a population in order to evaluate the possibility of discriminating between hair samples from different sources. Alder et al. [23] measured the distribution of 13 trace elements along the length of individual hairs by direct furnace atomization of 1 cm segments and measurement of atomic absorption.

The present study was initiated with the objective of demonstrating the potentialities of direct ETAAS in trace element determination associated with specific problems in biomedical and environmental research. Plant tissue biopsy samples were collected for chromium and manganese determination to study the kinetics of metal uptake and translocation. The distribution of these elements in different parts of the plant was also measured.

Chromium, lead and cadmium were measured in scalp hair of tannery workers and children. The slurry sample introduction technique was employed for determination of bulk hair trace element levels, and direct introduction of small hair segments for measurement of longitudinal concentration gradients. The advantages of the latter approach are discussed and data are presented to illustrate its use in monitoring trace

element nutritional status and occupational or environmental exposure.

2. Experimental

2.1. Apparatus and instrumental parameters

A laboratory assembled atomic absorption spectrophotometer employing a graphite cup atomizer was used in this work. A detailed description of the instrumental arrangement was given in a previous paper [15]. A graphite cup of type A without the inner cup was used for chromium and manganese determination. The instrumental parameters summarized in Table 1 were carefully optimized for each particular element and mode of sample introduction. Argon was replaced by oxygen for the first 12–15 s of the ashing step lasting in total 35 s which facilitates an efficient destruction of organic matter. This resulted in a substantial reduction of the background signal during atomization which was particularly serious in the measurement of cadmium. The use of chemical modifiers was found to be unnecessary except in the atomization of cadmium. All calibrations were performed using aqueous standard solutions.

2.2. Sample collection and preparation procedures

2.2.1. Botanical samples

Depending on the nature of the study, collection of botanical samples was performed either by biopsy or the whole mass of a particular plant tissue was taken for analysis. In the latter case the plant material was dried at 105°C, pulverized by grinding and analysed by slurry sample introduction. Biopsy sampling of botanical material was performed using special tools which are illustrated in Fig. 1. Discs 6 mm in diameter were cut from each plant leaf using a punch made of aluminium. Cuttings from the root tissue were obtained in a similar way. A teflon plate served as a support. In metal distribution studies the biopsy samples (leaf discs or root cuttings) were analysed individually by direct sample introduction. A quartz rod with a sharp end (see Fig. 1) was used to assist sample transfer. The mass of each particular sample

Table 1
Instrumental parameters for direct atomization of botanical and hair samples

Parameter	Element			
	Lead	Cadmium[a]	Chromium	Manganese
Absorption line/nm	283.3	228.8	357.9	279.5
Atomization cycle				
drying step/s	5[b]–20[c]	5[b]–20[c]	5[b]–20[c]	5[b]–20[c]
/°C	110	110	110	110
Ashing step/s	35	35	35	35
/°C	450	550	620	620
Atomization step/s	3	3	10	5
/°C	2000	1700	2500	2400
Argon flow rate/cm^3 min^{-1}	3000			
Oxygen flow rate[d]/cm^3 min^{-1}	2500			
Background correction	Simultaneous D_2 lamp			
Measurement mode	Integrated absorbance			
Sample introduction mode				
Direct	0.2–4 mg of moist plant tissue or hair			
Slurry	10–20 mm^{-3} of 0.2–10% m/v aqueous or 1 M HNO_3 suspension			

[a] $(NH_4)_2HPO_4$ or $Pd(NO_3)_2$ modifier.
[b] Direct sample introduction.
[c] Slurry sample introduction.
[d] Replaces argon only for the first 12–15 s in the ashing step.

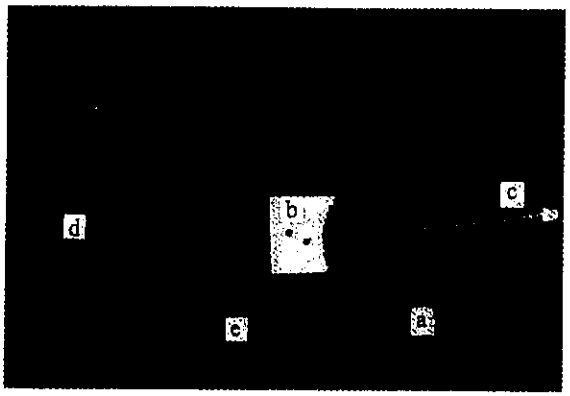

Fig. 1. Sampling of botanical material for direct sample introduction: a, plant leaf; b, teflon plate; c, punch and teflon piston; d, quartz rod with sharp end; e, graphite cup.

(range 1–2 mg) was determined using a Mettler MU3 electronic microbalance. The dry mass of the atomized sample was calculated assuming that the moisture content did not vary appreciably within each particular plant. The latter was determined on a separate sample. In plant metal-uptake studies a large number of tissue biopsy samples were taken in vivo from the same plant species. Identical tissue samples were combined, dried and pulverized. Generally 10–100 mg of dried tissue material was obtained for analysis which was performed by the slurry introduction technique.

Efficient pulverization of dried plant tissue was obtained by grinding samples in a vibration mill (Retsch, Model MM-2) using agate beads and capsule. A typical particle size distribution of powdered plant tissue material obtained after approximately 30 min grinding is illustrated in Fig. 2. It is evident from the cumulative frequency distribution function that median particle diameters are around 10 μm, and 90% of the particles are smaller than 30 μm. No measurable contamination (chromium, manganese) resulted from grinding in agate material, in contrast to that resulting from the more efficient zirconia. Similar contamination problems due to grinding in zirconia have been reported by others [19].

Generally twice-distilled water without any

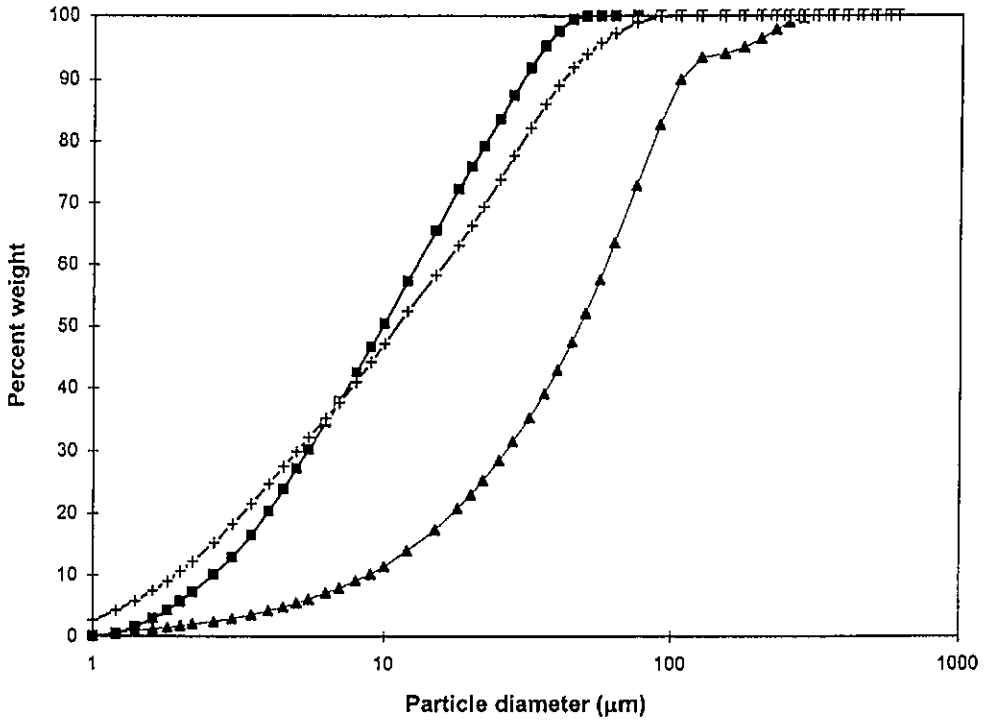

Fig. 2. Typical particle size distribution of powdered plant tissue and hair samples: –■–, Rye Grass No. 281 (ground); –▲–, Rye Grass No. 281 (original); –×–, Scalp Hair (ground).

additives was employed for preparation of slurries. The mass-to-volume ratio of the suspension varied depending on the element being measured. In determination of chromium, 2 ml of solvent was mixed with 50–200 mg of pulverized plant tissue, while for determination of manganese only 2.5–10 mg of plant material was slurried with the same volume of solvent. Prior to manual sampling using micropipettes, the slurries were mixed three times for 12 s using a Cole–Palmer Model 4710 ultrasonic homogenizer. The original stainless steel microtip was replaced by a similar one machined from pure aluminium to avoid contamination of the slurry. Because the density of plant tissue material is close to unity, settling of fine particles was slow. Additional improvement of slurry stability was produced by surface active water soluble proteins released from the plant tissue during sonification. After initial ultrasonic homogenization, shaking of the suspension before each particular sampling was found to be adequate to regenerate the suspension.

2.2.2. Hair samples

In the present paper two possible approaches to direct trace element hair analysis were investigated: (a) determination of bulk trace element content using slurry sample introduction; (b) measurement of the longitudinal concentration gradient by direct atomization of small hair segments. In either case scalp hair was sampled from the nape of the neck. Usually two bundles, each containing 50–90 single hairs were cut as close as possible to the skin. One hair bundle was analysed unwashed; the other was subjected to the pre-analysis washing described by Kumpulainen et al. [24]. This consisted of four successive 20 min washings in a mixture of *n*-hexane–ethanol (1 + 1), followed by three successive 5 min washings in distilled water. After that hairs were dried at 60–80°C for 4 h.

(a) A 25 mm long section of the proximal part of hair was cut from the bundle for determination of bulk trace element contents. In this slurry sample introduction approach either the prewashed or unwashed dried hairs were first ground. Hair grinding was performed the same way as described for botanical samples (Section 2.2.1). Similar contamination problems arose from using zirconia material for grinding. A typical particle size distribution of a powdered hair sample (illustrated in Fig. 2) shows an identical pattern to that of a similarly ground botanical sample. Slurries were prepared by weighing 20–200 mg of hair powder into cleaned glass vials and adding 2 ml of twice-distilled water. Homogeneity of the slurries was achieved by the sonification procedure described in Section 2.2.1. The favourable density of hair material (1.074) and water soluble proteins facilitate an excellent slurry stability.

(b) Despite the obvious benefits outlined earlier by Renshaw et al. [22] and others, measurements of trace element concentration gradients along the hair length has not received much attention, possibly due to technical problems. Our first attempt to measure longitudinal concentration gradients of chromium in a bundle rather than in a single hair by analysing 4 mm segments failed due to the propensity of hair to static charging. After a number of different trials the only reasonable way of reaching a practical solution was to seal an undisturbed hair bundle between two strips of Scotch tape (Scotch Magic Tape, France). This material was chosen because of its negligible blank values for lead, cadmium and chromium. Segments 2–4 mm in diameter were punched out at selected distances along the hair length and introduced individually into the graphite cup for atomization. The exact mass of the hair material in the segment was obtained by weighing the total mass of the segment

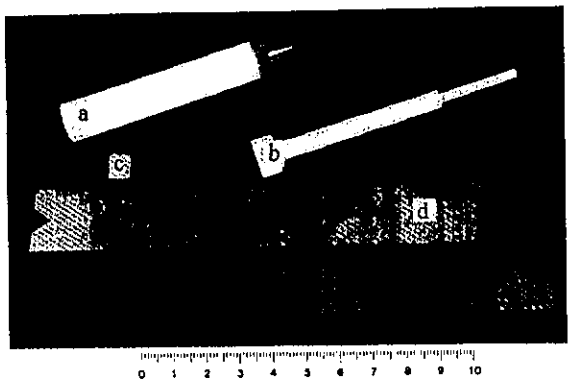

Fig. 3. Hair sampling tools for direct segmental analysis: a, punch; b, teflon piston for extruding disc; c, quartz rod with sharp end for handling disc; d, hair bundle fixed between two tapes.

and subtracting the mass of the tape. The latter was determined separately and was found to be reasonably constant (±0.01 mg). Two typical sealed hair bundles with 4 mm diameter segments cut out and the tools required for punching and handling hair segments are illustrated in Fig. 3. The punch was made of high quality steel containing only traces of chromium and no measurable contamination resulted from this operation.

3. Results and Discussion

3.1. Sensitivity, accuracy and precision of measurements

Characteristic masses of 15 pg, 8 pg, 55 pg, and 3 pg for chromium, manganese, lead and cadmium, respectively, were calculated for aqueous solutions using a cup shaped atomization device. These were considerably higher in comparison to those reported for commercial tube-like atomizers [3,12]. The poorer sensitivity is primarily a consequence of the shorter residence time of analyte atoms in the cup in relation to the pyrolitically coated tube. It is important, however, to note that the above characteristic masses remained reasonably constant (variation of ±5–10%) over a two year period. This enabled fast semi-quantitative screening of biological samples without calibration.

Reasonably good accuracy and precision of measurements are a prerequisite of any analytical technique and procedure employed in studies in the life sciences. Artifacts in analytical data may lead to totally erroneous assumptions and/or conclusions. Therefore the majority of papers quoted in the Introduction deal with the optimization of parameters influencing the accuracy and precision of slurry measurements. A number of different biological reference materials were used in those studies.

Two major sources of error may be encountered in direct analysis of solid samples; the first is associated with the representativeness of sampling, and the second is due to error in the atomic absorption measurements. The first source of error might become dominant when the total mass of material to be analysed is substantially larger than the mass actually measured. In this case the homogeneity of the material with respect to the analyte is critical and can be improved by reducing the particle size. However, in a variety of biomedical applications the analytical information is required on a very small mass of the material (less than 1 mg), as for example a short hair segment or a small tissue biopsy sample. In such situations analyte inhomogeneity in the sample, even if it is present, has little significance for the analyst and the entire analytical error is due to the atomic absorption measurement.

The intention of this research was not to favour a particular sample introduction technique but to demonstrate their best use in certain applications. In this respect our main concern was to achieve acceptable accuracy and precision of measurements using simple aqueous standard calibration. For this purpose a number of international biological standard or certified reference materials (SRMs/CRMs) and internal hair reference samples were analysed in triplicate by the slurry sample introduction technique. The data were averaged, and the standard deviation calculated. Reference

Table 2
Analyte partitioning[a] in biological reference materials and hair slurries

Material	Analyte element			
	Cd	Pb	Mn	Cr
Rye Grass[b]	6	13	40–50	–[d]
BRC-CRM No. 281	97[c]	100[c]	89[c]	
Olea europaea	2	14	–[d]	–[d]
BRC-CRM No. 62	80[c]	97[c]		
Citrus Leaves[b]	–[d]	–[d]	40–65	30–40
NBS-SRM No. 1572			83[c]	83–97[c]
Mussel Tissue				8
BRC-CRM No. 278	85[c]	–[d]	–[d]	45–65[c]
Hair Internal Reference	5	0.5	–[d]	22
Sample No. 4	84[c]	80[c]		47[c]

[a] Percentage analyte in aqueous phase.
[b] Additionally ground material.
[c] 1 M HNO_3 slurry.
[d] Not determined.

Table 3
Determination of lead, cadmium, chromium and manganese in biological reference materials and internal hair reference samples ($n = 3$)

Reference material	Element concentration			
	Pb; $\mu g\ g^{-1}$	Cd; $ng\ g^{-1}$	Cr; $ng\ g^{-1}$	Mn; $\mu g\ g^{-1}$
Rye Grass[a]	2.26 ± 0.14[b]	143 ± 4[b]	–[g]	85.8 ± 1.5[b]
BRC/CRM No. 281	2.38 ± 0.11[c]	120 ± 3[c]		86.1[d]
				81.6 ± 2.6[c]
Olea europaea	27.30 ± 0.87[b]	–[g]	–[g]	–[g]
BRC-CRM No. 62	25.00 ± 1.50[c]			
Mussel Tissue	–[g]	363 ± 7[b]	788 ± 76[b]	7.46 ± 0.04[b]
BRC/CRM No. 278		328[d]	800 ± 80[c]	7.30 ± 0.20[c]
		340 ± 20[c]		
Milk Powder	0.974 ± 0.051[b]	–[g]	–[g]	–[g]
BRC-CRM No. 150	1.000 ± 0.040[c]			
Citrus Leaves[a]	–[g]	36 ± 0.8[b]	637 ± 26[b]	24.4 ± 1.0[b]
NBS/SRM No. 1572		30 ± 10[c]	800 ± 200[c]	23 ± 2[c]
Internal Hair Reference Sample				
No. 1	–[g]	–[g]	148[b]	0.146[b]
			161[e]	0.141[e]
No. 2	0.62 ± 0.04[b]	38 ± 3[b]	380 ± 19[b]	–[g]
	0.61 ± 0.05[e]	34 ± 4[f]	480[f]	
No. 3	6.47 ± 0.48[b]	275 ± 14[b]	4280 ± 278[b]	–[g]
	6.70 ± 0.40[e]	262 ± 12[f]	3720[f]	

[a] Ground in agate ball mill.
[b] Slurry electrothermal atomic absorption spectrometry.
[c] Certified values.
[d] Slurry in 1 M HNO_3.
[e] HNO_3 dissolution electrothermal atomic absorption spectrometry.
[f] Neutron activation analysis.
[g] Not determined.

materials were suspended in twice-distilled water and 1 M HNO_3. The mass-to-volume ratio of the suspensions varied depending on the analyte and its concentration in the reference material.

The following masses of SRMs/CRMs were suspended in 1 ml of the diluent: 25–100 mg, 2.5–20 mg, 10–50 mg and 10–40 mg for the determination of chromium, manganese, lead and cadmium, respectively. A 10 mm^3 sampling volume was employed in manganese measurements and 20 mm^3 for the other elements. Analyte partitioning was determined in these slurries by centrifugation (10000 rev min^{-1} for 20 min) of a homogenized suspension and filtration of the supernatant through a 0.2 μm millipore membrane. Data on analyte partitioning in aqueous and 1 M HNO_3 suspensions are summarized in Table 2. It may be concluded from the data in Table 2 that lead and cadmium compounds present in these materials are much less soluble in water in comparison with chromium and manganese compounds. However, the solubility of all four elements is substantially enhanced in the presence of 1 M HNO_3. Data for manganese partitioning in an HNO_3 acid slurry of SRM Citrus Leaves 1572 agree well with those obtained by Jordan et al. [12], considering our more rigorous separation of the liquid phase. The data for other elements

Table 4
Reproducibility[a] (RSD ± %) of measurement ($n = 5$) of biological reference materials and hair slurries, affected by analyte partitioning and particle size distribution

Material	Analyte element			
	Cd	Pb	Mn	Cr
Rye Grass BRC/CRM No. 281	7.0[b] 6.0[b,c]	4.2[b], 14 3.0[b,c], 6.8[c]	6.0[b] 8.1[b,c]	–[d]
Olea europaea BRC/CRM No. 62	–[d]	9.2 2.0[c]	–[d] –[d]	–[d] –[d]
Citrus Leaves NBS/SRM No. 1572	7.4[b]	–[d]	4.7[b]	50[b] 33[b,c]
Mussel Tissue BRC-CRM No. 278	4.5 4.5[c]	–[d]	4.5 5.1[c]	23 22[c]
Hair Internal Reference Sample No. 4	11 9[c]	6.5 8.9[c]	–[d]	6.0 10[c]

[a] An average precision of manual sampling of ± 2% was estimated by measurements of aqueous standards.
[b] Additionally ground material.
[c] 1 M HNO_3 slurry.
[d] Not determined.

investigated are generally in the same range as those reported in the literature [17–19]. The exception is lead partitioning in botanical HNO_3 acidic slurries, where high values (80–100%) were reported by Miller-Ihli [18] and in this work, and very low values (1.5–2.5%) by Dobrowolsky and Mierzwa [17].

The results for manganese, chromium, lead and cadmium determination in various international biological SRMs/CRMs and laboratory-prepared hair samples are summarized in Table 3. Because no reference values were available for hair samples, these were also analysed by neutron activation analysis and a conventional dissolution — AAS procedure, and the results were compared. Two of the botanical CRMs (Rye Grass, Citrus Leaves) were additionally ground by the procedure described in Section 2.1.1. The primary reason for so doing was the poor homogeneity of these materials. Additional grinding increased the number of particles in the sampling volume by the factor of 64. In the slurries involved in evaluation of the accuracy of determination (Table 3) and precision of measurement (Table 4) the minimum mass of solid material per unit volume (see the data in Ref. [25]) required to produce 50 particles in the sampling volume (10–20 mm^3) was considerably exceeded. However, it should be pointed out that the real number of particles in the sample volume may be substantially different from that assuming perfect spherical shape of the particles. The latter is particularly well demonstrated in Fig. 4 for ground botanical and hair materials. In poorly ground material (Citrus Leaves, Fig. 4(a)) one can distinguish between particles of two different structures (fibrous and planar). It is very likely that the analyte contents of these two particle groups vary considerably. The apparent differences in particle structure disappear on additional grinding of this material, but a fairly irregular particle shape can still be observed (Fig. 4(b, c)). An irregular shape with some fibrous structure is also characteristic of ground hair material (Fig. 4(d)).

With the exception of the cadmium value measured in CRM (Rye Grass No. 281), all other data obtained for elements in botanical reference materials are reasonably within the uncertainty limits of the certified values. These intervals were in certain cases rather broad, as for example for chromium and cadmium in Citrus Leaves. In contrast, some of the reference materials were found to be homogeneous even in the submilligram range, for example Mussel Tissue where no significant difference in cadmium values was found between aqueous and 1 M HNO_3 slurries, despite the considerable difference in representative sample mass. The same applies for manganese determination in the ground Rye Grass sample. No feasible explanation could be proposed for the apparent difference in the measured and certified cadmium value in this material.

Excellent agreement with neutron activation data in the measurement of cadmium in hair implies good accuracy for the direct ETAAS determination for this element. Slurry and conventional solution ETAAS yielded essentially the same results for chromium and manganese in hair sample 1 but neutron activation data was either not applicable or less precise for determining these elements. Because neutron activation is not a suitable technique for measurement of lead, the

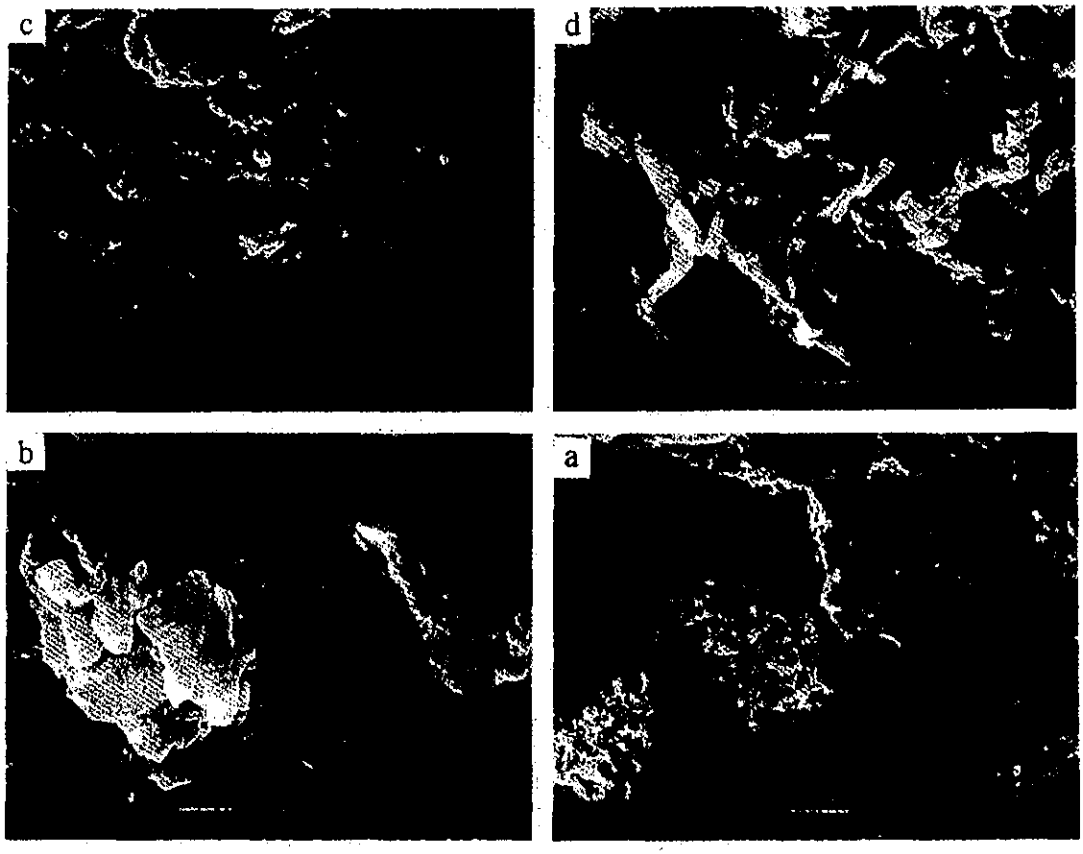

Fig. 4. Scanning electron microscopy photographs of hair and plant material particles: (a) original NBS SRM 1572 Citrus Leaves; (b), (c) ground NBS SRM 1572 Citrus Leaves; (d) ground hair.

accuracy of direct determination of this element in hair was examined by comparing the results with those obtained by conventional solution ETTAS. A reasonable agreement was obtained for both hair samples.

3.1.1. Effect of particle size and analyte partitioning on the precision of measurement

Theoretically, particle size and analyte partitioning in the slurry may influence the precision and/or accuracy of measurement [26,27]. The beneficial effect of analyte extraction on these values has already been demonstrated [18–20] in measurements of slurries of different nature and homogeneity. Part of this study was therefore directed towards evaluation of the effects of particle size and analyte partitioning on the precision and/or accuracy of measurements of biological slurry preparations. Table 4 summarizes reproducibility data of cadmium, lead, manganese and chromium measurements in biological SRM and hair slurries. Each value in the table represents the standard deviation of five consecutive measurements of a single slurry preparation.

The poor homogeneity of SRM Citrus Leaves used in the validation of slurry results was established by electron microscopy (Fig. 4(a)). A similar degree of inhomogeneity might also be expected for the other two botanical SRMs having approximately equal particle size distribution. Corresponding values of D_{90} are 100 μm, 158 μm and 265 μm for the Rye Grass, *Olea eropaea* and Citrus Leaves [15] respectively. This might explain the large uncertainty intervals for some certified elements (lead, cadmium, chromium) in these materials even at 500 mg sample mass. Additional,

Table 5
Distribution of chromium and manganese in cabbage secondary roots[a]

Sample no.	Chromium/ ($\mu g\ g^{-1}$)		Manganese/ ($\mu g\ g^{-1}$)	
1[b]	2990		250	
2[b]	3260		520	
3[b]	2550,	3020 ± 294	160,	332 ± 170
4[b]	3020		220	
5[b]	3280		510	
6[c]	3110		600	
7[d]	1390		220	

[a] Mass of sample 20–30 mg, aqueous 0.3% m/v slurry.
[b] Samples taken randomly over the whole length of secondary roots.
[c] Upper part of secondary roots.
[d] Lower part of secondary roots.

grinding which substantially reduced the particle size ($D_{90} \approx 20$–$40\ \mu m$) should therefore improve the precision of slurry measurements. This was indeed observed for the measurement of lead in SRM Rye Grass (Table 4). It should be emphasized that reduction in particle size affected the precision of measurement more strongly than the enhanced solubility of lead in the slurry at the same total sample mass (35 mg ml^{-1}).

It may generally be expected that large enhancements in analyte extraction such as experienced in the case of lead and cadmium may result in an improved precision of measurement providing the sample material consists of relatively coarse particles ($D_{90} \geqslant 150\ \mu m$). Additionally, no further improvement in the precision of measurement can be obtained with slurries of finely ground material ($D_{90} \leqslant 35\ \mu m$) by more complete extraction of the analyte. An exception to these generalizations was found in measurement of chromium in Citrus Leaves.

3.2. The use of the proposed method in plant mineral nutrition studies

Several major, minor and trace elements are essential for the normal growth and reproduction of higher plants. In contrast, a number of elements, even at trace levels, will inhibit plant growth or may cause plant death. Mineral nutrition and toxicology are therefore important areas of research both in basic and applied science. Significant progress has been made during the last few decades in understanding the mechanisms of mineral uptake, translocation and their function in plant metabolism. This progress was largely affected by the development of analytical chemistry. Solid sampling — ETAAS has some unique features which can be utilized in studies of plant mineral nutrition and toxicology.

The studies performed in the scope of this communication can be divided into two categories:

(1) measurement of chromium distribution in plant tissues (leaf and root);

(2) measurements of the kinetics of chromium uptake and translocation.

Short-term laboratory experiments (up to 7 days) were performed in nutrient solutions on cabbage plants (*Brassica oleracea* L. var. conica cr. Raket).

3.2.1. Preparation of plant material

Cabbage plants used in laboratory experiments

Table 6
Homogeneity of chromium distribution in plant tissue

Sample	Cabbage leaves/($\mu g\ g^{-1}$)			Cabbage roots/($\mu g\ g^{-1}$)		
	Biopsy	Bulk[a]	Δ/%	Biopsy	Bulk[a]	Δ/%
Blank	0.23	0.19	+21	1.40	1.90	−26
Cr(VI) (0.5 $\mu g\ cm^{-3}$)	0.92	0.97	−5	873	556	+57
Cr(VI) (5 $\mu g\ cm^{-3}$)	2.38	2.11	+13	2144	1337	+60
Cr(III) (0.5 $\mu g\ cm^{-3}$)	0.28	0.32	−13	843	543	+55
Cr-oxalate (0.5 $\mu g\ cm^{-3}$)	0.66	0.74	−11	210	242	−13

[a] Analysed by dissolution of 2 g of homogenized sample.

were grown in a modified Arnon-Hoagland solution [28] for three months. The ambient air temperature in the greenhouse was 15–22°C, illumination 11 hours per day.

3.2.2. Homogeneity of chromium distribution in plant tissue

One of the most important reasons for studying the homogeneity of metal distribution in plant tissues is the question of how accurately the biopsy sampling of the plant tissue would resemble the average content of the metal in that tissue (bulk content). The distribution of chromium was measured in the leaf and root tissue of cabbage plants placed for seven days in a nutrient solution of very low (about 50 ng Cr cm^{-3}) and high (500 ng Cr cm^{-3}) chromate concentration.

Fifteen discs of 6 mm diameter were punched randomly from a particular leaf using the tools presented in Fig. 1. Each of the discs was weighed and measured for chromium content. The variation in the mass of discs within one leaf was found to be ±2%, but variations between different leaves of the same plant were up to 100%. Variations in the chromium content of individual leaves was ±35% for the plants grown in the low chromium solution (average chromium content 0.16 $\mu g\ g^{-1}$) and ±11% for those grown in high chromium solution (average chromium content 0.54 $\mu g\ g^{-1}$). Similarly, leaves of different age from the plant fed high chromium(VI) showed remarkably different chromium contents (variation of up to 300%).

Additionally, the distribution of chromium and manganese was measured in secondary roots of the high chromium(VI) fed plant. Five samples of the whole length were collected randomly from the root system. Samples No. 6 and No. 7 consisted only of the upper and lower part of secondary roots, respectively. The samples were washed, dried and pulverized. The analysis was performed by slurry sample introduction. The results illustrated in Table 5 showed definite variation in the concentration of both elements with the root length. Between the whole length root sub-samples

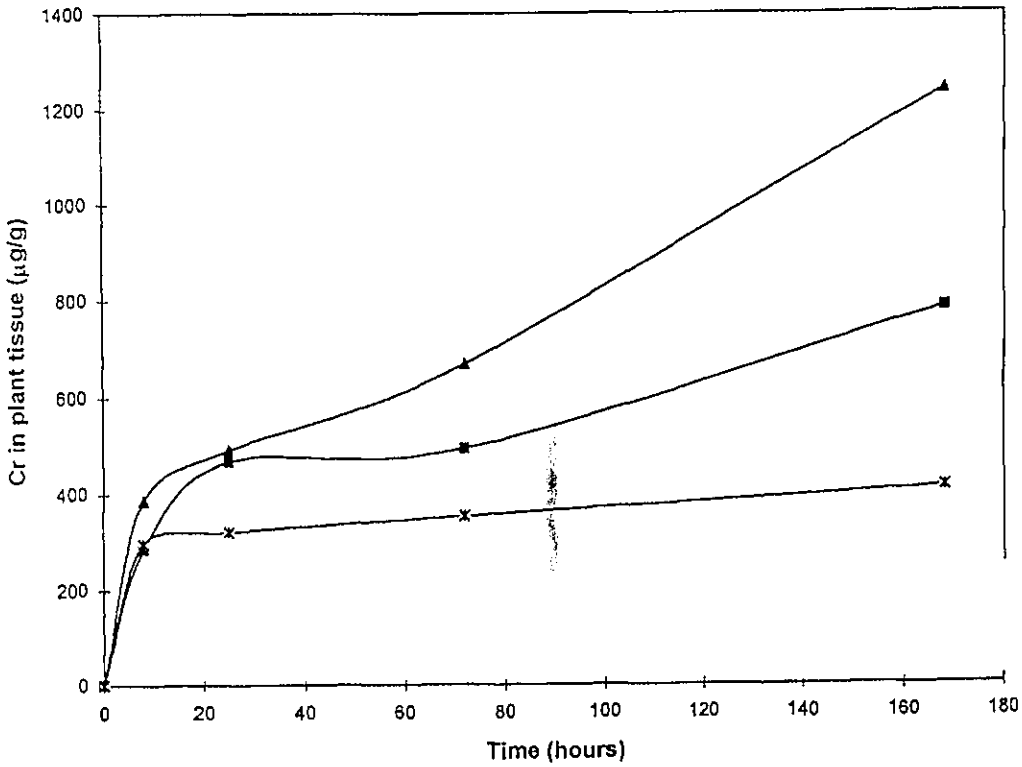

Fig. 5. Kinetics of chromium uptake by cabbage plant (plant roots): –■–, chromate; –▲–, Cr(III); –*–, Cr(III) oxalate complex.

there is also significant variation for manganese (± 50%) and less remarkable variation for chromium (± 10%).

Another more elaborate homogeneity test was performed in which five approximately equal cabbage plants (with 14 developed leaves) exposed for 7 days to different chromium species at various concentration levels were sampled for chromium determination. Two different sampling strategies were used.

(1) Biopsy sampling. Seven discs of 6 mm diameter were punched from each leaf of a plant and the samples were combined. Similarly, three whole lengths of secondary roots were cut from each plant. Samples were dried, pulverized and analysed by the slurry sample introduction technique.

(2) Bulk sampling. The rest of the plant tissue (root and leaves separately) were analysed by the conventional dissolution procedure.

The results of this experiment are summarized in Table 6 and are in general agreement with the expected homogeneity of chromium distribution in these tissues obtained by previous measurements. The accuracy of the proposed biopsy sampling should be satisfactory for different plant physiology studies such as metal uptake and translocation. The "root" values tend to be positively biased, particularly when large quantities of the metal are deposited in the root cells by adsorption. This might be explained by the fact that a larger proportion of the finer roots with larger specific surface area are sampled by the biopsy technique in comparison to bulk sampling.

3.2.3. Kinetics of chromium uptake and translocation in plants

In studies of the kinetics of metal uptake and translocation by different plant species, it is essential that temporal changes in the metal content of particular tissues are monitored in the same plant. Namely, the metabolic rates of individual plants of

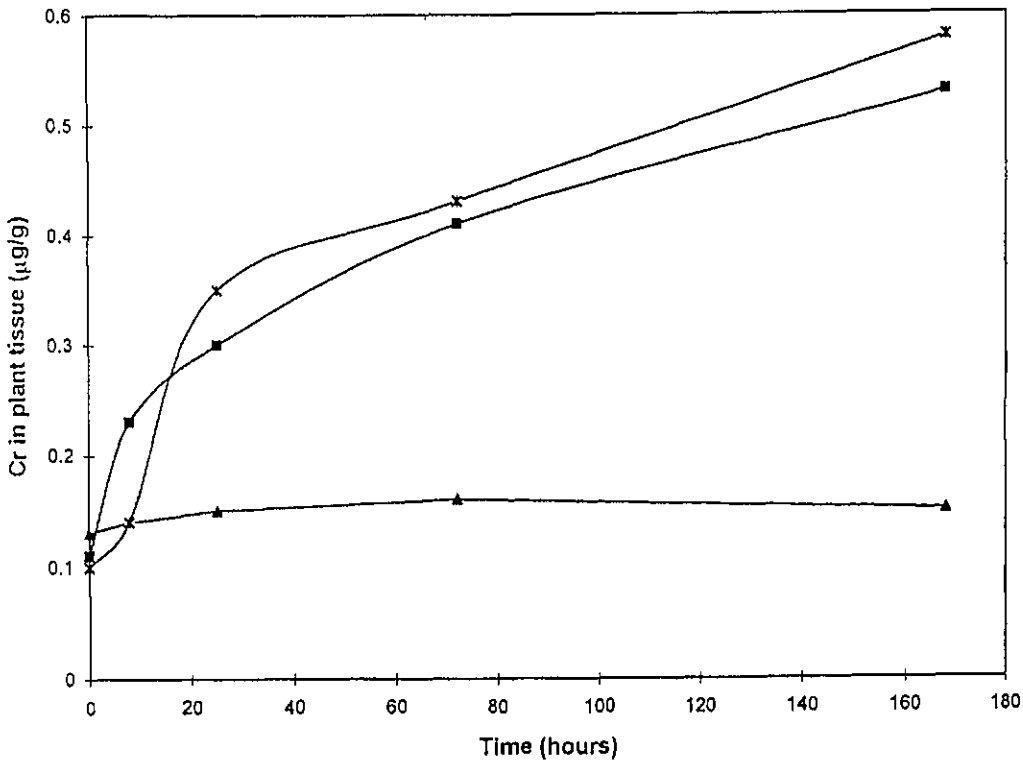

Fig. 6. Kinetics of chromium uptake by cabbage plant (plant leaves): -■-, chromate; -▲-, Cr(III); -*-, Cr(III) oxalate complex.

the same species grown in the same environment may vary dramatically (1–5 times). In such studies radio tracer techniques have been the most popular [28,29]. The following experiment was designed to evaluate the feasibility of using solid sampling ETAAS as an alternative technique in measurements of plant metal uptake. Nine approximately equal cabbage plants were transferred from Arnon-Hoagland nutrient solution in triplicate into three different solutions (9×10^{-3} M Na_2HPO_4, 6×10^{-3} M KH_2PO_4 and 5×10^{-3} M NH_4NO_3) containing 500 ng cm^{-3} of chromium in the form of either chromate, chromium(III) chloride or chromium(III) oxalate. Plants were left in these solutions for one week under continuous aeration, constant temperature and illumination. Biopsy sampling was performed on leaves and roots by the procedure described in Section 3.2.2 at zero time, 8 h, 24 h, 72 h and at the end of the experiment (168 h). Samples collected from all three plants in the same solution and taken at the same time were combined, dried, pulverized and analysed by the slurry sample introduction technique. Temporal variations of chromium content in cabbage roots and cabbage leaves due to different chromium speciation in the nutrient solution are illustrated in Figs. 5 and 6, respectively. It is not the scope of this paper to discuss in detail the results of this experiment, but what should be emphasized is the necessity of metal speciation in the nutrient solution and its effect on the uptake rate and transport index (100 × metal shoot content/total metal content). This has often been neglected, which explains the conflicting results reported for chromium in the literature [30].

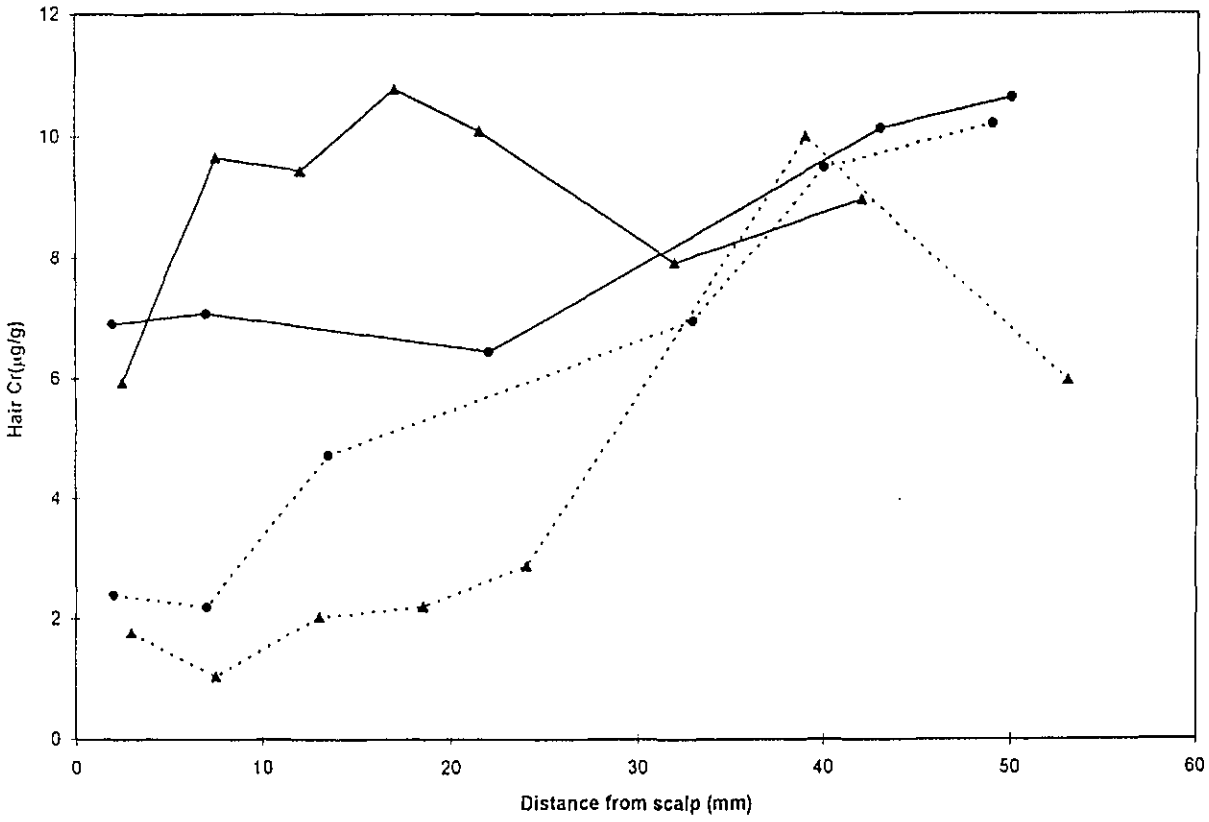

Fig. 7. Effect of hair washing on longitudinal hair Cr profiles typical of tannery workers: ●, subject 1; ▲, subject 2; (——, unwashed hair; – – –, washed hair).

3.3. Measurements of metal concentration gradients along the hair versus bulk metal content

In the majority of biomedical research reports where hair analysis has been used to assess the status of the subject in respect to that particular element, the possibility of trace element variation along the hair length has usually been disregarded. The latter has been emphasized by Renshaw et al. [21,22], Alder et al. [23], and Stauber and Florence [31] who demonstrated the advantages of measuring longitudinal hair profiles for correct interpretation of analytical data for diagnostic purposes. The concentration of the majority of trace elements investigated by these authors was found to increase from root to tip.

Bulk trace element hair contents represent only an average value over a certain period of time. These values may be strongly dependent on hair lengths and take no consideration of the relative proportion of trace elements incorporated in the hair structure after the hair has been formed. Therefore the bulk hair value would generally overestimate the "metabolic hair trace element value". Measurement of longitudinal trace element hair profiles should have several advantages over the bulk trace element hair content. (i) Assuming trace element incorporation takes place predominantly during hair formation (keratinization), it would provide short time variation in the status of the subject with respect to that particular element. (ii) Providing postkeratinization trace element incorporation is significant in comparison to metabolic incorporation, the relative extent of the former may be estimated by evaluation of the distribution pattern of that particular element along the hair length. (iii) Considering some specific details, analysis of longitudinal trace element hair profiles may provide insight into various physiological processes in the organism such as absorption, metabolism and excretion by perspiration.

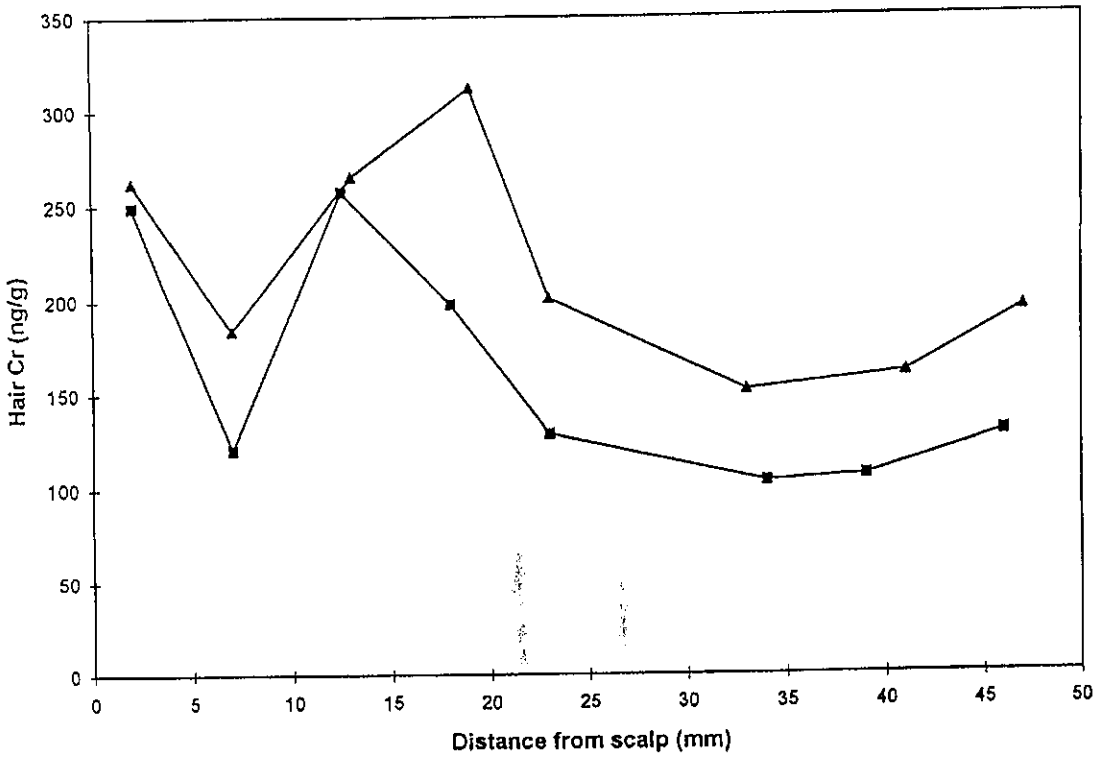

Fig. 8. Variation of hair Cr content with distance from scalp due to the change of habitat (for an occupationally unexposed person). –■–, washed hair; –▲–, unwashed hair.

The technique proposed in this paper has an advantage over those proposed by Renshaw et al. [22] and other workers [23,31] in that it can measure segments of hair 2–4 mm in length without substantial technical problems. Longitudinal hair profiles of chromium, lead and cadmium were measured. The subjects involved in these measurements were mainly children aged 10–14 of both sexes from different residential areas in Slovenia. In addition, the hair of tannery workers was also measured for chromium content.

3.3.1. Chromium in hair

In Fig. 7 longitudinal chromium hair profiles of two tannery workers are illustrated. The solid lines represent unwashed hair and the dotted lines washed hair, both taken from the same hair bundle. Tannery workers are exposed to substantial amounts of chromium at the workplace and elevated levels for blood, urine and hair chromium have been reported in the literature [32,33]. However, the hair levels reported differ significantly. Providing the hair was washed before analysis by the same procedure [24], the bulk hair chromium value will depend strongly (as is evident from Fig. 7) on the length of hair taken for analysis and the chromium distribution along the hair length. Extrapolated "zero length" hair chromium values are generally significantly lower in comparison to the 25 mm length bulk values and should be used as the best estimate of the true metabolic chromium value of tannery workers. An interesting feature relating to the difference between washed and unwashed hair can be observed from Fig. 7. It appears that a substantial amount of chromium can be washed from the hair by relatively non-aggressive solvents (*n*-hexane–ethanol) only from the proximal part of the hair and very little or none from the distal part. Since very few visible chromium-containing particles were identified on

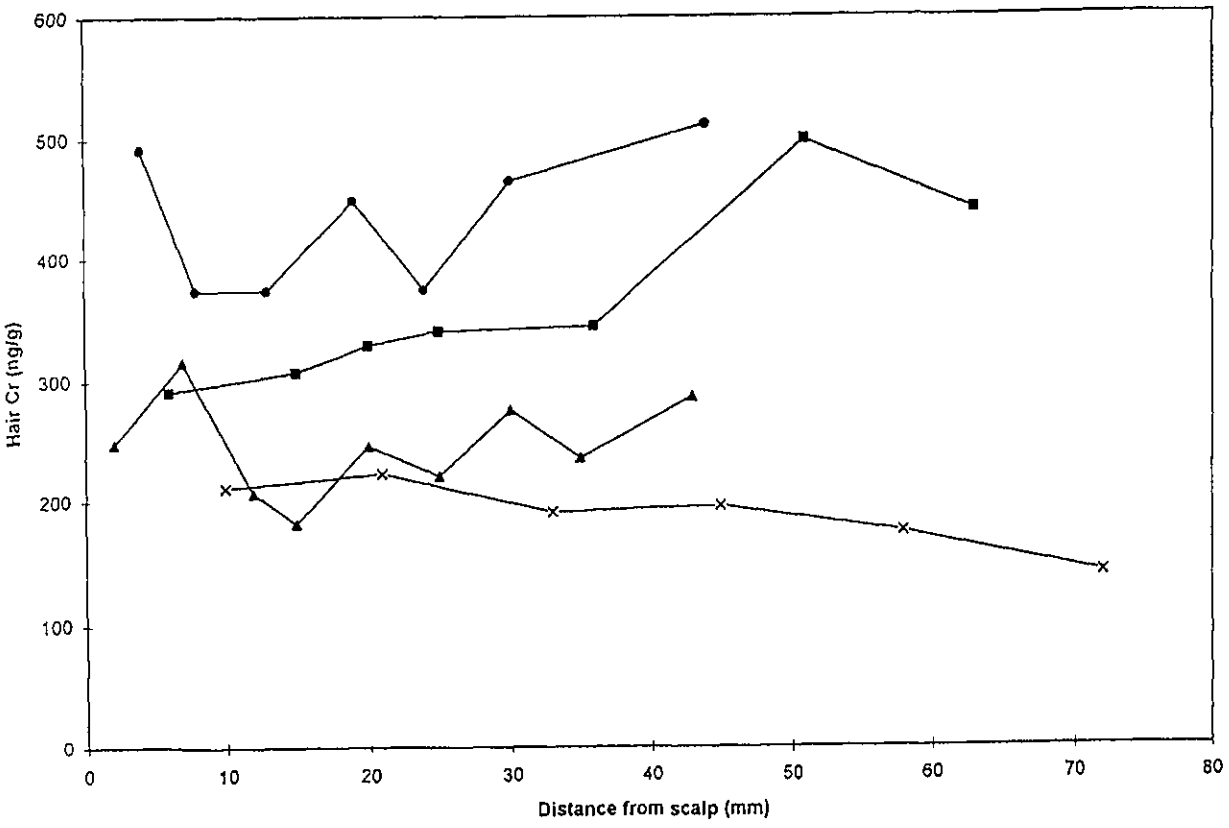

Fig. 9. Longitudinal hair Cr profiles of unwashed children's hair: ■, ×, girls: ▲, ●, boys.

the hair surface before washing, the main contribution to chromium incorporated into the hair structure after hair formation may result from the secretion of the eccrine glands.

Longitudinal hair chromium profiles of occupationally unexposed subjects (boys and girls) illustrated in Figs. 8 and 9 in general display random variation of hair chromium content with distance from the scalp. However, a pronounced increase in chromium concentration with hair length towards the distal part may also be observed (see Fig. 9), but the concentration gradients are significantly smaller than that found for tannery workers. The longitudinal chromium hair profiles of washed and unwashed hair of the same subject (Fig. 8) show a similar pattern, although the washed hair has somewhat lower chromium levels. These findings are in agreement with the results reported by Hambidge et al. [34]. The lower chromium level in the distal part of the hair of the subject in Fig. 8 may be explained by the different nutritional intake (tap water chromium content) due to the change of habitat. Namely, assuming an average hair growth rate (15 mm per month) the regions of high and low chromium hair levels coincide well with the time intervals subject spent at different locations. Further work related to this problem is in progress and will be described elsewhere.

3.3.2. Lead in hair

Lead is one of the major toxins due to its generally widespread use (gasoline, paints, lead batteries, etc.) and its presence in particular occupational environments. The appearance of acute and chronic plumbism has been found to be quite frequent among children and professional lead workers. Several clinical features, blood-Pb, urine-Pb and various laboratory measurements have been

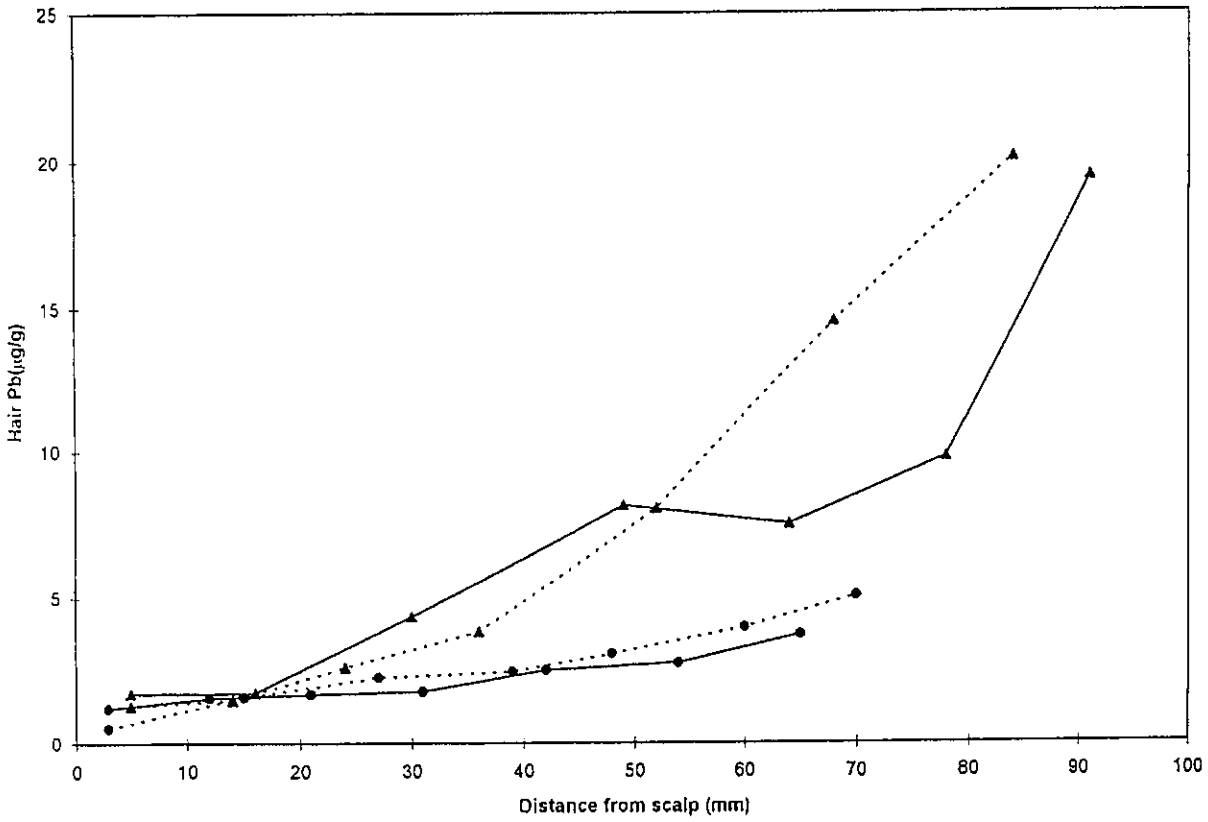

Fig. 10. Influence of hair washing on variation of hair Pb content with distance from scalp: ▲, resident of lead smelter area; ●, city resident; (——, unwashed hair; – – –, washed hair).

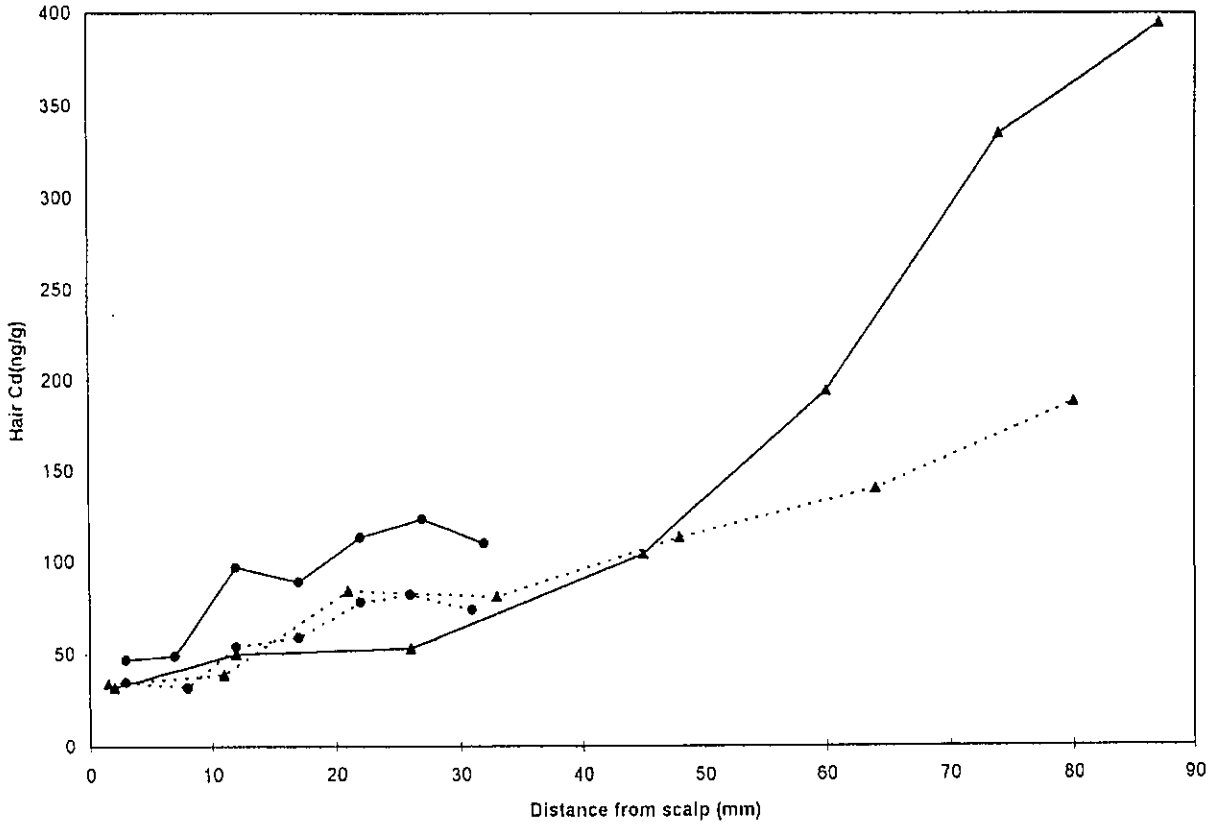

Fig. 11. Influence of hair washing on variation of hair Cd content with distance from scalp: ▲, resident of lead smelter area; ●, city resident; (——, unwashed hair; – – –, washed hair).

employed for diagnostic purposes. Additionally, determination of lead in scalp hair was demonstrated to be a valuable diagnostic tool in mild or chronic lead intoxication [35], and in monitoring human environmental exposure [36]. A reasonable correlation between blood and hair Pb levels was reported for environmentally exposed persons [36].

In these studies, sectional analysis of hair was also performed to identify the history of lead intoxication. The longitudinal hair profiles were plotted by measuring sections of hair 10–25 mm long representing a rather coarse resolution, which thus might produce inaccurate results. The reported hair lead levels (average of a definite length) claimed to be characteristic of a normal subject therefore differ widely and are relatively high.

This paper describes the effect of hair washing (*n*-hexane–ethanol–water) and ambient lead level on longitudinal hair lead profiles. In Fig. 10 variation of hair lead content along the hair length is illustrated for two subjects from urban and lead smelter residential areas. The yearly (1994) average levels of lead in ambient air (particles <2.5 μm) at these locations were 44 $\mu g\ m^{-3}$ and 215 $\mu g\ m^{-3}$, respectively. It may be concluded from Fig. 10 that the ambient air lead level has a significant influence on both longitudinal hair lead profile and proximal hair lead content. However, it is not clear whether hair washing has any influence upon hair lead content. The variations of the difference between the hair lead content of washed and unwashed hair along the hair length may be accounted for by the different lead levels of individual hairs in the bundle. According to Renshaw et al. [22], variations in the lead content between single hairs of the same individual can be as high as ± 100% particularly at the distal part.

3.3.3. Cadmium in hair

Cadmium scalp hair determination has frequently been reported to be a good indicator of environmental or occupational exposure, but this possibility was not universally accepted [36]. However, a poor correlation has been found between hair cadmium and cadmium in different organs (liver, kidney, spleen, lung) and therefore hair cadmium was not considered to be a good index of body burden. It should be pointed out that literature data on hair cadmium (unexposed population) cover an extremely large range (0.17–5.1 $\mu g\ g^{-1}$) and it is reasonable to suspect that not all the data are correct. Data on hair length used in these analyses are incomplete, and no results on longitudinal cadmium hair profiles of normal or exposed subjects are available in the literature.

Longitudinal cadmium hair profiles of children from two geographical locations were measured by the proposed technique. The concentration of cadmium in the ambient air at these locations was practically equal (0.43 $\mu g\ m^{-3}$).

The effect of hair washing on longitudinal cadmium hair profiles is illustrated in Fig. 11. In contrast to lead (see Fig. 10) cadmium hair profiles from both locations show an approximately equal concentration gradient along the hair strand. Moreover, proximal cadmium hair values are almost identical, which is compatible with an equal level of environmental cadmium exposure. Although the washed hair cadmium values seem to be consistently lower in comparison to unwashed hair in one case, the latter cannot be universally accepted. Namely, the difference between cadmium hair contents of washed and unwashed hair of the second subject (longer hair) varies tremendously along the hair length and may well be explained by the large variations in the cadmium content of individual hair [22].

4. Conclusions

Direct atomization of solid samples employing a laboratory assembled electrothermal atomic absorption spectrometer was demonstrated to be a feasible approach in the analysis of plant tissue and hair samples. A minor modification may be necessary to perform these analyses on those commercial instruments which are not specially designed to handle solid samples. Slurry sample introduction was found to be a powerful technique for routine, high accuracy determination of trace elements in botanical and hair samples and homogeneity testing. Both the enhanced solubility of the analyte and reduction of particle size were found to affect the precision of measurement; however, the latter showed the stronger effect. Generally, no further improvement in the precision of measurement can be obtained with slurries of finely ground material ($D_{90} \leqslant 30\ \mu m$) by more complete extraction of the analyte.

Alternatively, the direct sample introduction approach, was indispensable in the analysis of very small samples (<1 mg) such as tissue biopsy samples in the determination of trace element distributions. Direct analysis of solid samples for the trace elements investigated (Cr, Mn, Pb, Cd) and others by ETAAS was generally found to give excellent results for special applications in plant physiology and biomedical research. This has been demonstrated by measurements of the kinetic of chromium uptake by cabbage and its distribution in plant tissue, and by measurements of longitudinal hair trace element profiles.

A considerable difference in uptake and translocation of chromium was noticed between chromium(III), chromium(VI) and negatively charged, low molecular weight chromium(III) complex. Chromium and manganese species were found to be fairly uniformly distributed in plant leaves but not in the roots.

Direct analysis of 2–4 mm hair segments employing the proposed sampling strategy enabled measurement of longitudinal concentration gradients of chromium, lead and cadmium in the hair of tannery workers, and of children (age 10 to 14) from residential areas characterized by different levels of lead in ambient air. These results were demonstrated to be useful in the correct interpretation of analytical data for hair chromium, lead and cadmium in terms of nutritional intake, environmental and occupational exposure. First proximal hair segment values provide more reliable data on true metabolic trace element incorporation than

the conventionally employed average trace element contents of the whole hair length.

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