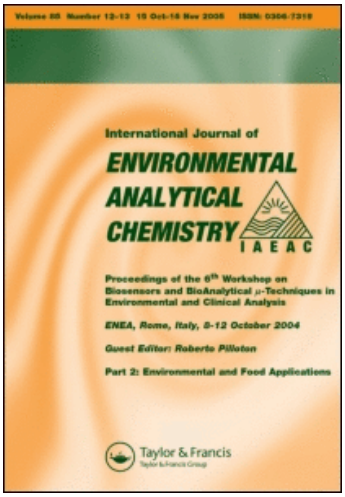

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Determination of Lead, Cadmium, Copper and Zinc in Biological Materials by Anodic Stripping Polarography

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The high sensitivity of anodic stripping polarography and the simple equipment used make this technique very suitable for determining certain toxic elements, in particular lead and cadmium, as well as essential elements such as zinc or copper. These are found in biological and environmental systems in the concentration range of between nanograms per gram and a few hundred micrograms per gram of sample. The amount of sample required for one analysis is of the order of 100 mg, therefore the blank values introduced by the oxidizing mixture do not represent a serious limitation. After the decomposition of the sample by wet ignition no further separations are required. Copper and lead are determined from a solution made 1.0 M with respect to HCl. For zinc and cadmium the solution is buffered to pH 4.9–5.1.

Values are presented for a set of samples among which are standard kale, orchard leaves, and bovine liver. The uptake of lead, zinc, and cadmium has been measured in carrots grown in the environment of a lead-mining area near Mežica, Slovenia. The results are compared with those from a non-exposed site.

Anodic stripping polarography (ASP) has only recently been introduced as a technique for determining the two very toxic environmental contaminants lead and cadmium; some other elements, including copper and zinc, which belong to the essential element group, can also be determined using appropriate sample aliquots. The technique covers the concentration range of

interest (10^{-8} M solutions) with adequate accuracy and precision and does not require expensive equipment.

Only a few papers dealing with the determination of trace metals in biological samples by ASP have been published so far. Both direct ashing[1-6] and wet ignition[7,8] have been used for the decomposition of the sample.

In the two last-mentioned methods, however, the elements are separated by extraction prior to their determination by ASP. In the present investigation it was shown that the technique can be applied directly to the solution obtained following wet ignition of the sample. By properly adjusting the medium, lead and copper can be determined from one aliquot of the solution and zinc and cadmium from another.

EXPERIMENTAL

Apparatus and Reagents

Current-potential curves were recorded with a polarograph (Type PO4, Radiometer, Copenhagen) at a rate of change of applied e.m.f. of 0.2 V/min. A sitting mercury drop (~6 mg) was used as indicator electrode. The volume of the electrolysis cell was 14 ml but the actual volume of the electrolysis solution was always 5.0 ml. The external reference electrode was a saturated calomel electrode (SCE) connected to the electrolysis solution by a salt bridge filled with agar-agar and 2 M potassium chloride solution. During electrolysis the solution was stirred with a glass stirrer at 400 rev/min. Oxygen was removed by bubbling pure argon ($O_2 < 0.01\%$) through the solution for 7 to 10 min.[9]

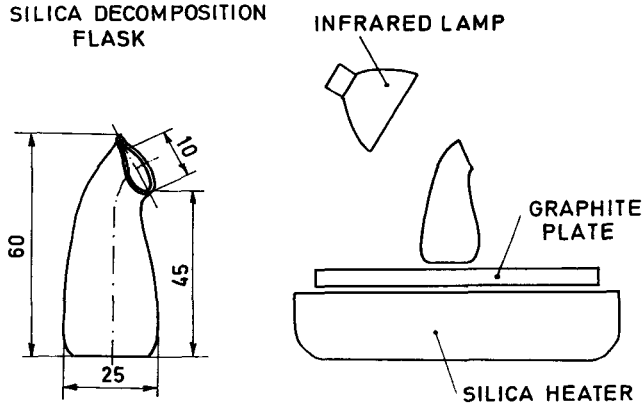

FIGURE 1 Silica decomposition flask and heating arrangement.

The amount of sample taken for one analysis was typically 100 mg. Decomposition was carried out in a silica container of about 10 ml as shown in Figure 1. Its upper part is narrow and bent in order to minimize losses by sputtering during the oxidation. The weighed sample was transferred into the container, the acid mixture was added, and then placed on a graphite plate fixed over a silica heater; the evaporation of the acid was assisted in the final stage by an additional infrared lamp (above), as shown in Figure 1.

All acids and sodium acetate used were of suprapure quality (Merck); H_2O_2 and NaOH were p.a.; water was first dionized and then doubly distilled from a silica still.

PROCEDURE

(a) Determination of Copper and Lead

The biological sample ($\sim$100 mg) is transferred into the silica decomposition flask, 0.5 ml nitric acid (d = 1.40 g/cm^3) and 0.2 ml sulphuric acid (d = 1.84 g/cm^3) are added, and the flask is heated on the graphite plate until all nitric acid has evaporated. The temperature is carefully increased until fumes of SO_3 start to form. Hydrogen peroxide (30%) is added down the wall of the flask until the dark solution clears. The total amount of peroxide required to complete the decomposition is between 0.5 and 0.8 ml.

The solution is finally evaporated to dryness. The residue is dissolved in 5 ml of warm 1 M HCl, taking care that all solids are removed from the walls by using a silica spatula. The cooled solution is transferred to the electrolysis cell and oxygen flushed out with argon.

The electrolysis is carried out at -0.8 V versus SCE for 3 to 10 min, depending on the concentrations of copper and lead in the solution. The current-potential curve is then recorded between -0.8 to 0.0 V versus SCE at a constant rate. The oxidation current peak for copper is at approx. -0.2 V and for lead at -0.4 V versus SCE, respectively. In sample with a high lead-to-copper ratio, resolution is improved if copper is electrolysed at -0.45 V (Figure 2).

The concentrations of copper and lead have been determined by the method of standard addition (the standard was added before the decomposition of the sample) as well as from calibration curves. A typical calibration curve is reproduced in Figure 3. The technique as described is applicable to concentrations of copper and lead as low as 10^{-7} M (0.006 mcg/ml) and 2.10^{-8} M (0.004 mcg/ml), respectively. At lower concentrations the accuracy and precision deteriorate due to reagent blanks. The amount of sample has to be increased if the concentrations of lead and copper in the material are lower than 0.2 ppm and 0.3 ppm, respectively.

Cadmium can also be determined from the same solution by carrying out the electrolysis at −0.85 V versus SCE; the cadmium maximum in this medium is approx. −0.6 V versus SCE. However, the accuracy and precision of results for cadmium are poorer due to the high reduction current of

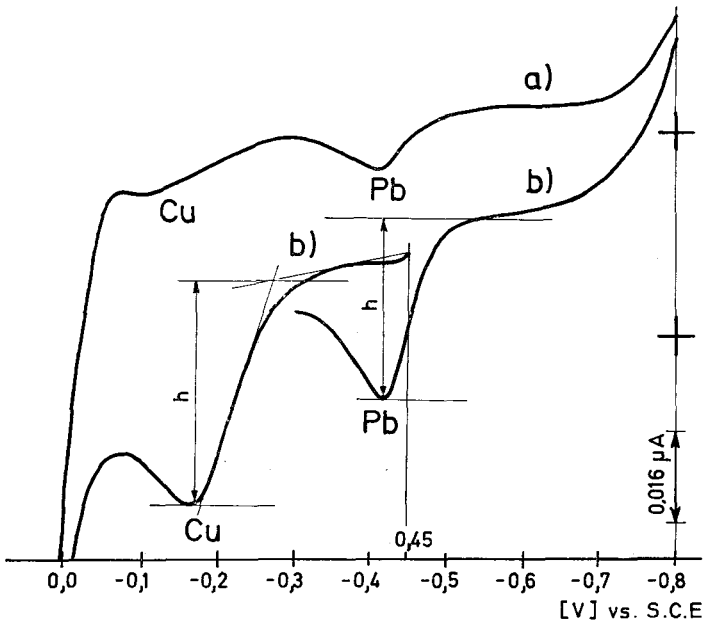

FIGURE 2 Current-potential curves for copper and lead ions in the acid solution. (a) blank; (b) solution containing 2.10^{-7} M Pb^{2+} and 2.10^{-6} M Cu^{2+}. Electrolysis time 3 min. Curve (a) electrolysis at −0.80 V versus SCE for lead and copper; curve (b) copper only electrolysis at −0.45 V versus SCE.

hydrogen ions (Figure 2). Weakly acid solutions are preferable. Therefore, the conditions for cadmium were modified to determine it together with zinc as described below.

(b) Determination of Cadmium and Zinc

A separate 100-mg aliquot of the sample is taken for the determination of zinc and cadmium. The same procedure as applied to copper and lead is followed, as far as the dissolution of the sulphuric acid residue in 5 ml of 1 M HCl is concerned. The solution is then transferred quantitatively into a 10-ml volumetric flask, 1–2 drops of methylorange are added, and the solution is

carefully neutralized against this indicator, first with 2.5 N and later with 0.1 N NaOH and/or 0.1 N HCl. Finally the pH is adjusted to 5.3 by adding 0.5 ml of a 0.5 M sodium acetate-acetic acid buffer followed by addition of water to the mark. After mixing, 5 ml of the solution is pipetted into the electrolysis cell, oxygen is removed by bubbling argon through the solution for 7 to 10 min, and the solution is electrolysed for 3 to 10 min at −1.30 V

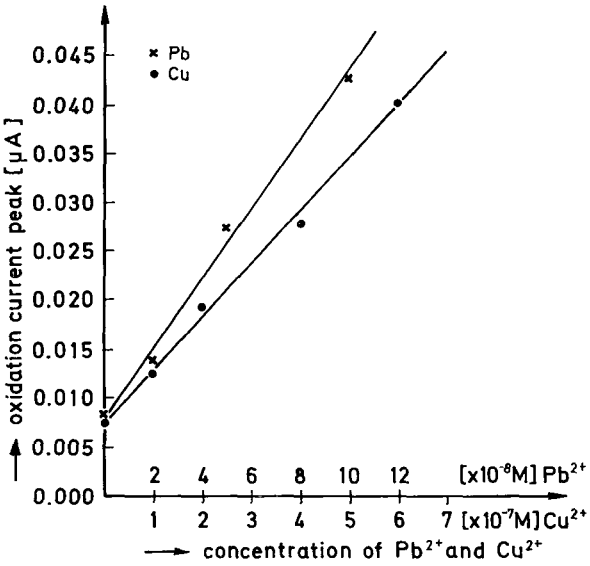

FIGURE 3 Calibration curves for copper and lead. Electrolysis time 10 min at −0.8 V versus SCE.

versus SCE for zinc and at −0.9 V versus SCE for cadmium, respectively. In most biological samples there is frequently a large excess of zinc with respect to cadmium. Therefore the current-potential curves are recorded separately for zinc and cadmium (Figure 4). The standard addition method as well as calibration curves have been used (Figure 5). Practical sensitivity limits for these two elements are 5×10^{-7} M (0.03 mcg/ml) for Zn and 10^{-8} M (0.001 mcg/ml) for Cd, equal to 3 ppm Zn and 0.1 ppm Cd when using 100 mg of sample for one determination. This limit can be further lowered for cadmium by prolonging the electrolysis time. In the case of zinc this is not feasible because of high blanks for this element. The amount of sample has to be increased adequately if the zinc concentration is lower.

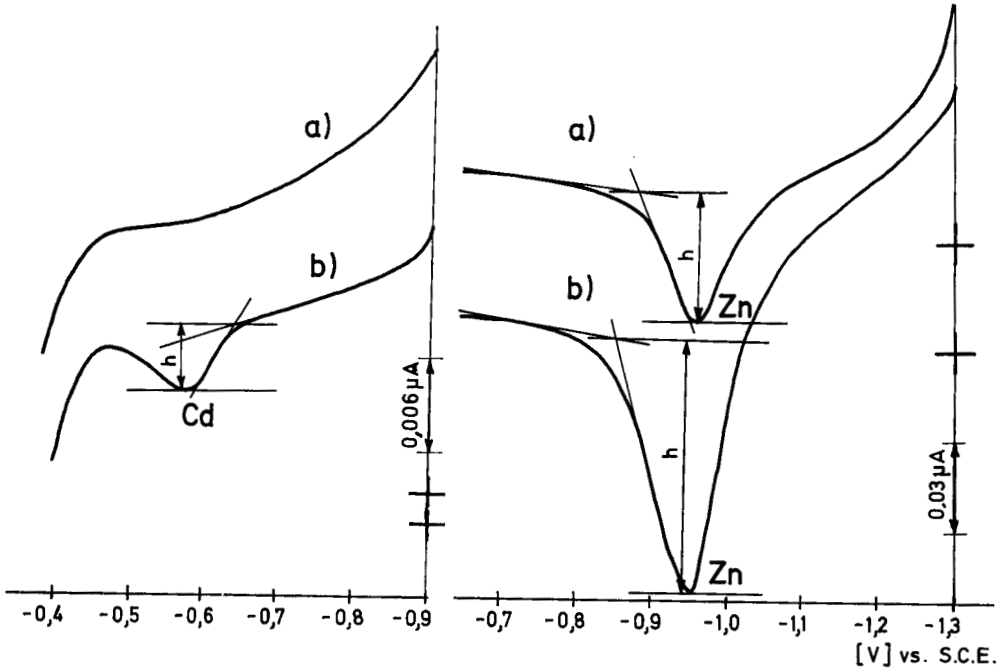

FIGURE 4 Current-potential curves for cadmium and zinc. (a) blank; (b) solution containing 5.10^{-8} M Cd^{2+} and 5.10^{-7} M Zn^{2+}. Electrolysis time for cadmium 6 min at -0.90 V versus SCE, for zinc 3min at -1.3 V versus SCE.

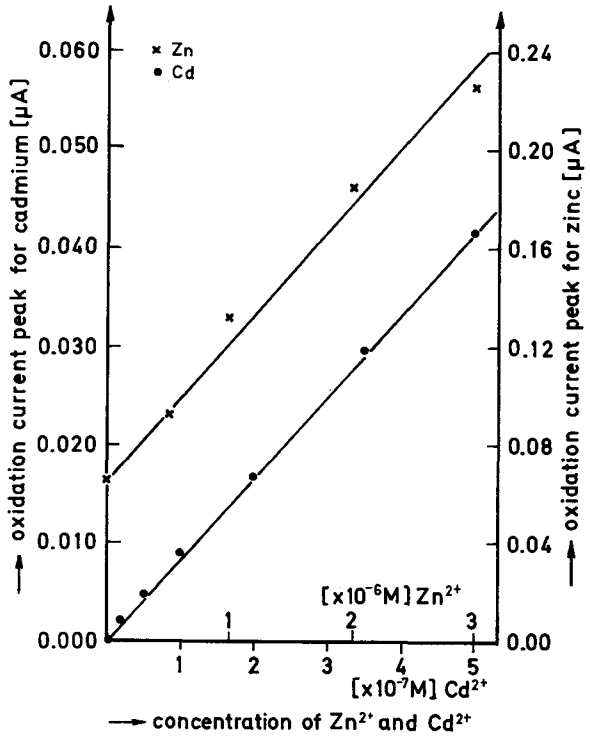

FIGURE 5 Calibration curves for cadmium and zinc. Electrolysis time for cadmium 6 min at −0.9 V versus SCE, for zinc 3 min at −1.3 V versus SCE.

RESULTS AND DISCUSSION

For accurate determinations of metals in biological materials quantitative mineralization of the sample is very important. Data in the literature indicate that results are low for all four elements if ashing is used for mineralization.[10] According to Doshi *et al.*[11] losses for zinc and cobalt occur already at the ignition temperature of 400°C. Therefore, wet ashing is preferable provided that the reagents used are extremely pure and that blanks are determined or known.

High cathodic currents were observed if the decomposition was not complete. Best results were obtained when using a mixture of sulphuric and nitric acids and assisting the oxidation by dropwise addition of H_2O_2 into hot solution. Final solutions were either clear or there was a small amount of solids (calcium sulphate or silica) left when following this procedure. Per-

chloric acid is not suitable for plant materials because of a considerable precipitation of the sparingly soluble potassium perchlorate formed.

It is also necessary to evaporate the solution to dryness after the mineralization has been completed in order to avoid cathodic currents at somewhat lower potentials, which interfere with the determination of zinc. These are very likely due to traces of either organic substances or of hydrogen peroxide.

The choice of the supporting electrolyte is another critical parameter. Newberg and Christian[2] claim that copper, lead, and cadmium can be determined in biological materials by simply ashing the sample and dissolving the ash in distilled water. We found low results for all four components if either ashing or wet ignition, followed by evaporation, was used and then the residue taken up in distilled water or in an acetate buffer solution pH 5–6.5. The latter medium was investigated more thoroughly because it would allow simultaneous determination of all four components.[12] In a series of experiments carried out with pure solution to which sulphate ions were added, we found low results for both lead and zinc in a medium buffered with acetate. Replacing acetate by potassium chloride leads to loss of accuracy for lead and copper. Therefore it was decided to determine the latter two elements in 1 M hydrochloric acid which dissolves practically all the residue. Biological samples always contain considerable amounts of calcium, magnesium, and iron; neutralization of solutions containing hydrochloric acid (prior to the determination of cadmium and zinc) leads to the formation of hydroxyl salts or hydroxides. Experiments with synthetic solutions to which amounts of calcium, magnesium, iron, potassium, and phosphate had been added comparable to those normally found in this type of sample (corresponding to 3% Ca, 1% Mg and 0.1% Fe, 2% K and 1.6% P in the original sample) have shown that a precipitate does not form if the pH does not exceed 5.5. The lower pH limit, on the other hand, is given by the high reduction current of hydrogen ion which appears in solutions of pH less than 4.7; this interferes with the measurement of the oxidation current peak for zinc (Figure 4). This interference begins to appear at pH 5.3 if the concentration of zinc is less than 5×10^{-7} M, but this was not the case with any of the samples analysed so far.

Using the above procedure, solutions could be analysed 10^{-8} M with respect to lead, copper, and zinc provided that the blank values for these elements were negligible. In the case of cadmium there is a linear dependence of maximum anodic current with concentration in the broad range from 10^{-8} M to 10^{-5} M. Of other ions mentioned (Ca, Mg, Fe, K, HPO_4^-) only the last-mentioned affects the anodic maxima of cadmium (Figure 6) as well as of zinc in solution at pH 5, but does not influence lead and copper peaks in 1 M hydrochloric acid. In order to minimize the influence of the varying concentration of these interferences present in different biological samples on the

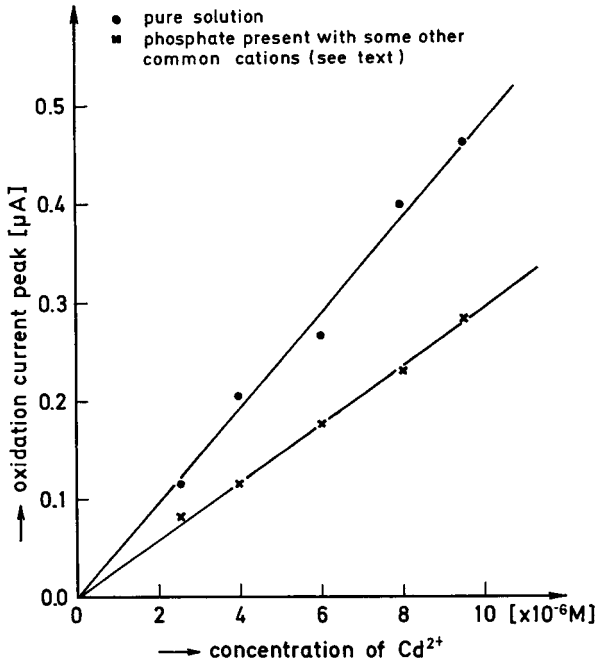

FIGURE 6 Influence of phosphate on the calibration curves for cadmium ion in the concentration range $2.5.10^{-6}-9.5.10^{-6}$ M. Electrolysis time 3 min at −0.9 V versus SCE.

accuracy of the results, they have all been analysed by the use of calibration curves as well as by the method of standard addition. The data presented in Tables I and II for eight different biological samples indicate good agreement between the two approaches. The results all appear to be within the limits of precision achievable by using ASP.

For some of the materials in the tables, results obtained by other techniques are included for comparison and show good agreement. The first five samples were in the form of homogeneous powder. The last three were obtained from samples cut to cubes of approx. 5 mm edge, and freeze-dried. The lower precision reflects the inhomogeneity of these samples due to differences in concentration within different sections of the plant. (Compare the rel. S.D. in Tables I and II for the freeze-dried carrot sample with the other three carrot samples.)

The high concentration of lead and arsenic in orchard leaves probably indicates that they might have been sprayed at some stage with preparations containing these two elements. It is known that arsenic(III) interferes in lead

TABLE I

Results for copper and lead in biological samples by anodic stripping polarography (ASP). For copper, values obtained by neutron activation analysis (NAA) and atomic absorption spectrophotometry (AA) are included for comparison

Sample	Copper					Lead		
	ASP			NAA	AA	ASP		
	Calibration curve		Stand. add.			Calibration curve		Stand. add.
	(ppm)	(%)[a]	(ppm)	(ppm)	(ppm)	(ppm)	(%)[a]	(ppm)
Orchard leaves[b]	(7)[c] 11.2	9.2	(3) 12.7	(5) 10.8	(7) 12.9	(10) 44.0	5.2	(3) 43.4
Tomato leaves[b]	(6) 7.7	6.6	(4) 7.7	(5) 9.4		(6) 4.5	2.8	(3) 3.2
Bovine liver,[b] freeze-dried	(6) 197	6.4	(6) 195	(9) 191	(8) 205	(4) 0.36	6.9	(4) 0.39
Kale[d]	(6) 5.2	11.8	(4) 5.0		(4) 4.3	(7) 2.3	10.3	(3) 2.9
Carrot, freeze-dried	(8) 8.4	4.9	(3) 8.6			(6) 1.2	10.5	(4) 1.1
White carrot	(5) 2.4	12.7	(2) 2.3			(4) 0.57	23.3	(3) 0.71
Carrot 3	(7) 4.0	15.3	(6) 4.1			(6) 15.3	19.3	(5) 15.4
Carrot 6	(7) 3.0	11.6	(3) 3.3			(6) 3.4	23.5	(3) 3.6

[a] Rel. S.D.

[b] These samples were kindly supplied by the U.S. National Bureau of Standards.

[c] Figures in parentheses give the number of determinations.

[d] This sample is an international reference sample kindly supplied by Professor H. J. M. Bowen.

TABLE II

Compositive results for zinc and cadmium in biological samples by ASP. For zinc, values obtained by AA are included

Sample	Zinc				Cadmium		
	ASP			AA	ASP		
	Calibration curve		Stand. add.		Calibration curve		Stand. add.
	(ppm)	(%)[a]	(ppm)	(ppm)	(ppm)	(%)[a]	(ppm)
Orchard leaves[b]	(7)[c] 28.3	9.3	(3) 29.8	(5) 29.5	(6) <0.1		(3) <0.1
Tomato leaves[b]	(6) 62.9	2.7	(6) 62.9	(5) 61.0	(6) 2.4	9.1	(5) 2.1
Bovine liver,[b] freeze-dried	(7) 118	3.7	(4) 119	(5) 124	(5) <0.1		(5) <0.1
Kale[d]	(6) 29.4	5.2	(3) 28.5		(6) 0.75	10.3	(6) 0.72
Carrot, freeze-dried	(7) 21.3	4.9	(3) 20.8		(5) 0.68	10.5	(2) 0.74
White carrot	(4) 12.4	26.0	(2) 12.1		(4) 0.31	13.3	(4) 0.28
Carrot 3	(6) 41.6	4.5	(3) 39.9		(6) 1.7	17.6	(5) 1.5
Carrot 6	(6) 31.5	4.5	(2) 30.2		(6) 1.3	24.5	(4) 1.6

[a] Rel. S.D.

[b] These samples were kindly supplied by the U.S. National Bureau of Standards.

[c] Figures in parentheses give the number of determinations.

[d] This sample is an international reference sample kindly supplied by Prof. N. J. M. Bowen.

determinations due to the same half peak potential during its oxidation. However, following wet ignition of the sample arsenic was expected to be in the pentavalent state. To prove this the effects of arsenic(III) added to the oxidizing mixture were investigated.

Measurements of the oxidation current peak of 8×10^{-6} M lead solution alone and of the same solution containing as much as 10^{-4} M added As(III) before treatment with the oxidants, and then following the whole procedure, were 0.344 μA and 0.355 μA, respectively, thus showing evidence of negligible interference. At the potential used during electrolysis there is evidently no reduction of As(V). Compared to lead, pentavalent arsenic has its anodic maximum at a much more positive potential, but since it is not reduced under the conditions used it does not affect the accuracy of lead determination.

Using this method, we could clearly demonstrate not only considerable differences in heavy metal concentration in plant tissues but also a strong dependence upon the area where they were grown. Although concentrations of copper in carrot samples do not vary essentially, lead, zinc and cadmium differ in a much wider range and are particularly high in samples 3 and 6 which were grown close to a lead zinc mine. The precision and accuracy make the technique suitable for environmental studies involving uptake studies and distribution measurements.

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