II Sources of, and Analytical Advances in, Trace Inorganic Constituents in Food

By C. J. Pickford
ENVIRONMENTAL AND MEDICAL SCIENCES DIVISION, AERE, HARWELL,
NR. DIDCOT, OXON.

1 Introduction

This review seeks to highlight some of the significant advances that have occurred in recent years concerning trace inorganic constituents in foodstuffs. Some specific sources of trace-element contamination have also been considered in some detail. Because of the very great current interest in those elements, the toxic metallic elements have been dealt with in greater detail than the non-toxic and non-metallic elements.

In 1977 Crosby[1] reviewed the determination of metals in foods, giving an excellent general picture of the subject up to that time. Biennial reviews are published in *Anal. Chem.* and are a valuable source of applications data.

A. Essential Trace Elements.—Underwood[2] has classified trace elements into three groups: (*a*) those essential for higher animals, (*b*) those possibly essential, and (*c*) the non-essential elements. The definition of 'essentiality' has been dealt with in some detail by Cotzias,[3] and the elements that are generally included in this group are Cr, Co, Cu, F, Fe, I, Mn, Mo, Ni, Se, Si, Sn, V, and Zn. Ti, Pb, As, and Cd[4] are under investigation, with other elements, as being possibly essential.

B. Toxic Trace Elements.—Other elements have no known function in higher organisms but are always present without apparent effect. Some elements, however, are known to be harmful, and their presence may cause toxic, carcinogenic, or other undesirable effects. Many essential elements are toxic at higher concentration levels. The list of these toxic or carcinogenic elements includes[4] As, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Ni, Pb, Sb, Se, Sn, Te, Tl, V, and Zn.

The specificity of many essential- and toxic-element effects is such that it is important to have an accurate knowledge of the distribution of many of these elements at all parts of the food chain, starting from environmental levels, either natural or polluted, through foodstuffs of animal or vegetable origin, to man himself.

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[1] N. T. Crosby, *Analyst*, 1977, **102**, 225.

[2] E. J. Underwood, 'Trace Elements in Human and Animal Nutrition', Academic Press, New York, 1977.

[3] G. C. Cotzias, 'Trace Substances in Environmental Health', ed. D. D. Hemphill, University of Missouri, Columbia, 1967, p. 5.

[4] G. H. Morrison, *CRC Crit. Rev. Anal. Chem.*, 1979, **8**, 287.

C. The Origins of Environmental Levels of Trace Elements.—Ultimately, the distribution of trace elements in our environment, and hence food chain, may be traced back to the average composition of the Earth's crust. Data for some elements of interest are given in Table 1.[5] Concentrations range from high levels of elements such as Si of nearly 30% to levels of 0.002 $\mu g\ g^{-1}$ and lower for Te and rarer elements.

Table 1 *Distribution of some typical essential and toxic trace elements in the Earth's crust*[a]

Element	*Average concentration in earth's crust*/$\mu g\ g^{-1}$
Ba	250
Cr	200
V	150
Zn	132
Ni	80
Cu	70
Sn	40
Pb	16
Be	6
As	5
Sb	1
Tl	0.6
Hg	0.3
Bi	0.2
Cd	0.13
Se	0.09
Te	0.002

[a] Data from ref. 5.

Mobilization of trace elements may occur by a number of routes, such as mining of mineral aggregates, volcanic activity, *etc*., for volatile elements, and leaching processes, to give the raised levels we refer to as 'environmental pollution'.

D. Typical Levels Found in Food.—These may vary from the $< 0.001\ \mu g\ g^{-1}$ level for elements such as V to levels of up to 300 $\mu g\ g^{-1}$ for essential elements, such as Zn, and some other elements present as a result of contamination, such as Sn. There are many sources of data (*e.g*. ref. 6) for trace-element levels in food; those quoted were gathered from member states of the European Economic Community by the Health and Safety Directorate of the CEC. Typical data are given in Table 2.[6]

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[5] B. Mason, 'Principles of Geochemistry', John Wiley and Sons, 1959.

[6] J. Bouquiaux and M. Langevin, EUR 5730C, Health & Safety Directorate, Luxembourg, 1977.

Table 2 *Typical levels*/mg kg^{-1} *of trace inorganic constituents in food*[a]

As	0.001	→	0.1	
Cd	0.003	→	0.3	
Cr	0.01	→	3.0	
Cu	0.1	→	30	
Hg	0.003	→	0.1	
Pb	0.03	→	1.0	
Sn	0.03	→	10	(up to > 300)
Zn	1.0	→	100	

[a] Data from ref. 6.

E. Atypical Levels in Food.—As well as the typical levels quoted, some food products may sometimes contain high levels of certain trace elements. Attention is focused upon this, of course, when the element is toxic, as in the case of Hg in marine fish, up to 3.1 μg g^{-1},[7] Cd in brown crab meat, 10 μg g^{-1},[8] and Pb in acid canned foods such as fruit, 10 μg g^{-1}.[9] It is clear that there are two different problems involved: (*a*) contamination of food by processes such as canning and (*b*) concentration of environmental levels of trace elements by various organisms. In the latter case high environmental levels as a result of man's activities will be concentrated even further. A specific example of this is the concentration of methylmercury in shell fish, from water contaminated by acetaldehyde plant operation[10] in the Minamata Bay in Japan.

2 Sources of Inorganic Constituents

A. Major Sources of Trace Inorganic Constituents.—The major sources of trace inorganic constituents in food can be grouped together to common origins in many cases. Sources for some typical elements are given in Table 3, from data contained in reference 6.

B. Lead from Automotive Exhaust.—The use of lead additives in petrol to raise the octane rating has been a source of much debate in recent years, and a number of studies have pointed to the potential damage caused to the environment. Most of the concern has arisen over lead levels in air and dust, but in view of the raised lead levels found in soils and plants growing near to major roads[11] and the subsequent passage to man *via* the food chain this should also be of concern. Food is normally considered to be the major source of lead to industrially

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[7] A. V. Holden, 'Mercury Contamination in Man and His Environment', IAEA Technical Report No. 137, Vienna, 1972.

[8] B. Otto, 'The Levels of Selected Trace Elements in Food', May 1975, document prepared by the Commission of the European Communities.

[9] J. Bouquiaux, Report of working group, July 1974, C.E.C. document V/F/1966/74e.

[10] L. T. Kurland, S. N. Faro, and H. Sielder, *World Neurol.*, 1960, **1**, 370.

[11] H. L. Cannon and J. M. Bowles, *Science*, 1962, **137**, 765.

Table 3 *General sources of inorganic constituents in food*[a]

Element	*Source*
As	Combustion, pesticides, veterinary products
Cd	Zinc smelters, battery industry, pigments, solders
Cr	Plating, pigments, tanning, dyeing
Cu	Industrial use, pesticides
Hg	Mining, volcanic activity, chloralkali, electrical industry, pesticides and fungicides, dentistry
Ni	Alloys, plating
Pb	Natural, combustion, paints, industry
Sn	Canning

[a] Data from ref. 6.

unexposed individuals, with an average intake of 200—300 μg day^{-1} [12] so that any increase in this amount is viewed with some trepidation .One particular study[13] has sought to establish the pathways of lead from petrol to man by using an isotopically abnormal form of lead in tetraethyl-lead added to petrol in the Turin area. By analysing air, water, particulate, food, and blood samples an attempt is being made to correlate blood lead levels with petrol consumption over a fixed period of time.

C. Trace-element Contamination from Sewage Sludge.—There is some concern that the repeated application, at excessive rates to the soil, of sewage sludge containing trace elements may eventually cause injurious or toxic effects to either crops or animals.

Sewage sludge may contain high levels of Cu (0.8%), Ni (0.33%), Zn (up to 4.9%), Pb (up to 0.3%), and Cd (up to 0.15%), expressed as percentage dry weight, from 42 sewage sludges from England and Wales.[14] In the U.S.A. levels of up to 0.34% have been reported[15] for Cd; such high levels of trace elements are traceable to industrial sources operating within the zone covered by the sewage facilities. Non-industrial areas have much lower levels in general,[16] although substantial levels of Zn are still reported.[17] Much of the concern about the health consequences of this problem has centred on Cd owing to its high toxicity and

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[12] K. R. Mahaffey in 'The Biogeochemistry of Lead in the Environment', Elsevier/North-Holland Biomedical Press, 1978.

[13] S. Facchetti, International Conference on 'Management and Control of Heavy Metals in the Environment', London, Sept. 1979.

[14] M. L. Berrow and J. Webber, *J. Sci. Food Agric.*, 1972, **23**, 93.

[15] D. R. Zenz, B. T. Lynam, C. Len-Hing, R. R. Rimkus, and T. D. Hinesley, Metropolitan Sanitary District of Greater Chicago, Dept. of Research and Development, Report No. 74-20, 1975.

[16] 'Survey of Sewage Sludge Composition', Department of the Environment, 1978, unpublished.

[17] D. Purves, paper presented at 'Management and Control of Heavy Metals in the Environment', London, Sept. 1979.

ability to replace Zn in the body, with dangerous consequences. Page *et al.*[18] have reported data for pea, wheat, and corn uptake from sludge-amended soils as a function of Cd content of the sludge, pH, and plant species. These data show that more Cd is mobilized at a lower pH and that the highest Cd concentration was found in the leaves, rather than the seeds or grain, of the plant. However, the concentration of Cd in peas grown at a lower pH (4.4—5.2) was practically linearly related to Cd added from the sewage sludge.

Davis and Coker[19] have reviewed the agricultural effects of Cd in sewage sludge used as fertilizer. They consider the difficulty of assessing the extractable part of the Cd present to assess the portion which is taken up by plants.

The guidelines used at present to control Cd levels in sewage sludge used as fertilizer are cautious to allow for the limited knowledge currently available about uptake rates, *etc.* European legislation allows from 10 to 167 g ha^{-1} yr^{-1} to be added to agricultural land, whereas the U.K. suggests a soil increase of up to 2.3 p.p.m. to be achieved over 30 years or more.

3 Analytical Methods

A. Sample Preparation.—Before a sample can be analysed, some form of pretreatment is almost invariably required. Samples are generally dried to constant weight and then either analysed directly or wet or dry ashed and the ash or a solution of it is analysed by a solution technique. A full appraisal of preparation methods for food can be found elsewhere.[1]

B. Atomic Absorption Spectroscopy.—The breakdown made by Crosby[1] of published analytical applications showed that in the years up to 1977 a.a.s. had been by far the most popular technique used for trace inorganic constituents in food. An examination of recent literature confirms this trend. The techniques of hydride generation and electrothermal a.a.s. are becoming particularly popular as a result of improved instrumentation.

The hydride generation a.a.s. technique has been used for the analysis of As, Se, Sb, Te, and certain other elements. Evans, Jackson, and Dellar[20] have recently described a comprehensive procedure for determining Sb, As, and Sn in foodstuffs using this technique combined with a specific wet oxidation step. Fiorino *et al.*[21] have used a similar hydride generation technique coupled with an argon-entrained hydrogen flame for As, Se, Sb, and Te in food. Their results for As and Se are compared with data obtained by i.n.a.a. (instrumental neutron activation analysis), a.a.s., fluorimetry, and spectrophotometry and show excellent agreement. A similar study for As and Se has been made by Ihnat and Miller.[22]

[18] A. L. Page, A. C. Chang, and F. T. Bingham, as in ref. 17.

[19] R. D. Davis and E. G. Coker, as in ref. 17.

[20] W. H. Evans, F. J. Jackson, and D. Dellar, *Analyst*, 1979, **104**, 16.

[21] J. A. Fiorino, J. W. Jones, and S. G. Capar, *Anal. Chem.*, 1976, **48**, 120.

[22] M. Ihnat and H. J. Miller, *J. Assoc. Off. Anal. Chem.*, 1977, **60**, 1414.

There is no doubt that, despite interferences that may arise during the hydride-forming step from transition metals and other species, the hydride-generation a.a.s. technique is becoming very widely used and is one of the most convenient techniques for these elements.

Electrothermal a.a.s. is now very extensively used for the determination of certain trace metals in acid digests of food material. The very high sensitivity attained by this technique permits direct determination of trace elements such as Pb in food digests at normal environmental levels,[23,24] although many workers prefer to use an extraction process to separate heavy metals before electrothermal a.a.s. determination (*e.g.* ref. 25). In addition to improving sensitivity even further, a solvent-extraction step has the added advantage of reducing the matrix and background effects which are often associated with this technique.

Very much work is still done using flame a.a.s. combined with an extraction technique to improve detection limits where necessary. Allenby *et al.*[26] have published a routine screening procedure for Cd, Cu, Pb, and Zn in food for levels down to 0.02 $\mu g\ g^{-1}$, using such a procedure with an optional DDC/heptan-3-one extraction step. Using similar procedures, many workers claim that it is unnecessary to use electrothermal rather than flame a.a.s. since the extraction step increases the sensitivity by a sufficiently large factor.

C. Inductively Coupled Plasma Optical Emission Spectrometry.—This technique (i.c.p.o.e.s.), originally pioneered by Greenfield[27] in the U.K. and by Fassel and co-workers in the U.S.A.,[28] is rapidly becoming one of the most widely used multi-element techniques for trace metals. Although the capital cost of the equipment is high, a large number of elements may be determined simultaneously in a sample, so that the unit cost per element determination may be much lower than with a.a.s. The technique has been extensively reviewed by Barnes.[29]

Detection limits from i.c.p.o.e.s. are similar to those from flame a.a.s. for many elements, although much better for refractory elements such as B, Zr, *etc.* There has been only a limited investigation of interference effects so far (ref. 29 and refs. therein), but it would appear that chemical effects such as the phosphate/calcium effect seen in a.a.s. are reduced or absent, but that spectral interference, especially when direct-reading spectrometers are used with wide slit widths, occurs as in other forms of emission spectrometry.

Irons *et al.*[30] have compared the technique with *X*-ray fluorescence spectrometry (x.r.f.) amongst several laboratories, using digests of NBS standard reference materials such as Bovine Liver and Orchard Leaves for the comparison. They conclude that i.c.p.o.e.s. is the more sensitive technique for most elements

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[23] D. Harbach and H. Diehl, *Z. Anal. Chem.*, 1978, **290**, 145.

[24] Z. Grobenski, M. Melcher, and B. Weltz, *Z. Anal. Chem.*, 1978, **290**, 144.

[25] R. W. Dabeka, 25th Canadian Spectroscopy Symposium, Sept. 1978, Quebec, Canada.

[26] P. Allenby, J. W. Robertson, and F. C. Shenton, *J. Assoc. Public Anal.*, 1977, **15**, 61.

[27] S. Greenfield, I. L. W. Jones, and C. T. Berry, *Analyst*, 1964, **89**, 713.

[28] R. H. Wendt and V. A. Fassel, *Anal. Chem.*, 1965, **37**, 920.

[29] R. M. Barnes, *CRC Crit. Rev. Anal. Chem.*, 1978, **7**, 203.

[30] R. D. Irons, E. A. Schenk, and R. D. Giauque, *Clin. Chem.*, 1976, **22**, 20218.

except Ni and Pb. I.c.p.o.e.s. requires a liquid sample, x.r.f. a solid pellet; this may be advantageous or not, depending on the sample type. Accuracy and precision were found to be similar for the two techniques, although the much simpler data evaluation required for i.c.p.o.e.s. meant that even a very small minicomputer was sufficient.

A.a.s. and i.c.p.o.e.s. have been compared by Munter *et al.*[31] for a number of food materials. They presented a large amount of data obtained with an i.c.p.o.e.s. system for standard reference materials and other food products, previously analysed by 15—23 other laboratories by a.a.s. They conclude that the inductively coupled plasma is a near-ideal multi-element source, although they point out that lower detection limits are needed for the elements Cr, Cd, Ni, and Pb in order to reach normal levels of these elements.

D. X-Ray Fluorescence Spectrometry.—This technique is much used for environmental monitoring (*e.g.* air filters) but has not found a wide application for the analysis of food, perhaps owing to the high cost of the equipment and the fact that samples and standards must be closely matched to obtain meaningful data. Giaque[32] describes the use of the technique for a number of elements in biological materials and compares the technique in another publication[30] to i.c.p.o.e.s. Proton-induced x.r.f. (p.i.x.e.) has been used by Campbell[33] to determine trace elements in wines and complex biological matrices.

Pelletization of freeze-dried solids has been applied to milk samples[34] and other types of foods[35] and appears to provide a convenient means of sampling in many cases.

E. Neutron Activation Analysis.—This multi-element technique is more sensitive than x.r.f. or i.c.p.o.e.s. for many elements and is essentially blank free, although it does require access to irradiation facilities. Generally, sensitivities are lower if no chemical separation is made (i.n.a.a.), and the presence of large amounts of Na, *etc.*, may require chemical separation to be carried out, at a much greater cost.

Schelenz[36] determined 25 elements in human diet by this route, and with Diehl[37] he described the application of the technique to the determination of up to 28 elements in food and similar materials, with detection limits in the range 10^{-6}—10^{-11} g for a 0.15 g sample. They report matrix effects for chromium when it was measured in potato flour.

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[31] R. C. Munter, R. A. Grande, and P. C. Ahn, *ICP Information Newsletter*, 1979, **5**, 368.

[32] R. D. Giaque, F. S. Goulding, J. M. Jaklevic, and R. H. Pehl, *Anal. Chem.*, 1973, **45**, 671.

[33] J. L. Campbell, B. H. Orr, A. W. Herman, L. A. McNelles, J. A. Thompson, and W. D. Cook, *Anal. Chem.*, 1975, **47**, 1542.

[34] A. L. Langhorst, N. A. Bonner, F. Bazau, and D. R. McIntyre, U.S. Energy Res. Dev. Adm. Rep., 1976.

[35] R. T. King, *J. Sci. Food Agric.*, 1977, **28**, 631.

[36] R. Schelenz, *J. Radioanal. Chem.*, 1977, **37**, 539.

[37] J. F. Diehl and R. Schelenz, *Lebensm.-Wiss. Technol.*, 1975, **8**, 154.

Other determinations using this technique involve extensive chemical separation and are better described in that context.

F. Other Analytical Techniques.—The summary by Crosby[1] of the citation of analytical methods used for food shows that, in addition to the techniques mentioned, flame photometry, spectrophotometry, titration, and electrochemical methods are the only others to be used to any extent. The use of these techniques is restricted to mainly routine applications using standard published methodologies.

G. Separation and Preconcentration Techniques.—There are many circumstances where the sensitivity or selectivity of a method is such that some form of separative or preconcentration procedure must be used. This is particularly true of the toxic trace elements such as Pb, Cd, and As, and many publications (*e.g.* refs. 25 and 26) deal with the a.a.s. determination of these elements in food after chelation and solvent extraction. Such a separation does, of course, raise the sensitivity of flameless a.a.s. even further, permitting interference-free determination at low environmental levels.

With the increasing availability and use of multi-element instrumental methods, there is a particular need for preconcentration methods that separate elements *en bloc* from interfering matrix elements. Thus Menke[38] used ion exchange to preconcentrate trace elements from food, prior to x.r.f. determination. Van der Sloot[39] has studied the use of activated charcoal as a collecting medium for trace metals, either in simple acid solution or chelated with ammonium pyrrolidine dithiocarbamate, the final determination being made by i.n.a.a.

Chelating resins have also been used for preconcentration of trace elements from various matrices before determination by x.r.f.[40] or i.c.p.o.e.s.[41] Similar materials have been used[42] for the trace determination of metals in foods and other materials by a.a.s.

The concentration of trace impurities plays an important role in neutron activation analysis, either before the activation step[43] or afterwards, although in this case the recovery can be incomplete since inactive carriers are generally added to improve, and assess, the yield obtained.

Although chemical separation or preconcentration techniques may lengthen an analysis somewhat, this may be at least partially offset if a group of elements is simultaneously separated and determined.

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[38] H. Menke, *Fresenius' Z. Anal. Chem.*, 1977, **286**, 31.

[39] H. A. van der Sloot, ECN-1, Petten, Netherlands, 1976.

[40] D. E. Leyden, G. H. Luttrell, W. K. Nouidez, and D. B. Werho, *Anal. Chem.*, 1976, **48**, 67.

[41] R. M. Barnes and J. S. Genna, *Anal. Chem.*, 1979, **51**, 1065.

[42] D. S. Hackett and S. Siggia, 'Environmental Analysis', Academic Press, New York, 1977, p. 253.

[43] M. H. Yang, P. Y. Chen, C. L. Tseng, S. J. Yeh, and P. S. Weng, *J. Radioanal. Chem.*, 1977, **37**, 801.

4 Future Trends

A. Speciation of Trace Elements.—The importance of the exact form of a trace element with regard to its toxicity, and hence the maximum allowable concentration that may be present in food, has been recognized for some time. The methyl form of mercury is known to be the more toxic form,[44] compared to inorganic mercury, and specific analytical techniques are capable of distinguishing between the two forms.[45] In the case of mercury, conversion of inorganic mercury to methylmercury has been reported in *Nature*[46] and explains the build-up of organic mercury in fish products such as tuna, which was recently the source of much publicity.

Similarly, the toxicity of chromium depends on the valence state of the element. This is reflected in the different air levels allowed for Cr^{III} and Cr^{VI}.

Considered to be of sufficient importance, the topic of metal speciation merited a session at the recent conference 'Management and Control of Heavy Metals in the Environment' in London, September 1979. Florence and Batley[47] have reviewed the possible approaches for chemical speciation based on chemical modelling procedures or analytical measurements following separation. A number of other publications at the above conference dealt with metal speciation in aquatic systems, and there seems no doubt that future work on foodstuffs will be directed towards determination of the form as well as the concentrations of metal species.

There are, of course, considerable difficulties in extension of this topic to food. Most meat, vegetable, *etc.* products, unlike water, require considerable chemical pretreatment before analysis, with the attendant risks of changing the form of the metal present.

B. Synergistic Effects.—Synergistic effects already play a role in trace-element toxicology, *e.g.* the role of Se in Hg toxicity has been studied,[44] the uptake of Pb is enhanced from Ca- or Fe-deficient diets,[48] and in agriculture the inter-relationship of Mo and Cu is well known.[49] It seems reasonable to suppose that this topic will also assume greater importance in the trace-element chemistry of food. It is easy to envisage the legislative nightmare that could result.

In the study of synergistic effects, multi-element analytical techniques such as i.c.p.o.e.s., x.r.f., or i.n.a.a. are clearly advantageous, and the present widespread use of computers for complex data handling renders the task of establishing inter-element correlations rather easier.

C. New Elements.—As more understanding is obtained about the effects of trace

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[44] E. J. Underwood, 'Trace Elements in Human and Animal Nutrition', Academic Press, New York, 1971, and refs. therein.

[45] L. Magos, *Analyst*, 1971, **96**, 847.

[46] S. Jensen and A. Jernelow, *Nature*, 1969, **223**, 753.

[47] T. M. Florence and G. E. Batley, *CRC Crit. Rev. Anal. Chem.*, 1980, **9**, 219.

[48] K. R. Mahaffey, T. A. Banks, C. L. Stone, S. Capar, J. Compton, and M. Glubik in 'Proc. Int. Conf. Heavy Metals Environ.', Toronto, Canada, 1977.

[49] A. T. Dick, *Aust. J. Agric. Res.*, 1954, **5**, 511.

elements on man, toxic effects of 'new' trace elements are discovered. Thallium, long known as a toxic element in factory situations but only recently being considered in any detail, is now becoming of interest to environmentalists (see, for example, ref. 50) and, no doubt, other elements will create similar interest in the future.

Naturally the interest in new elements reflects to a certain extent the 'state of the art' of analytical techniques. Thallium is, as it happens, a rather difficult element to determine at trace levels by most of the techniques available for instrumental analysis, so that until recently determination of this very toxic metal at environmental levels was a difficult analytical task.

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[50] S. Tooze, *Ecologist*, 1980, **5**, 163.