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The Distribution of Heavy Metals in Soil and Metal Uptake into Vegetation, at Beaumont Leys Sewage Farm, Leicester

Part I: Analytical Methodology and Metal Distribution in Soil

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Beaumont Leys sewage farm was one of the first large sites to be subjected to a comprehensive chemical investigation. Approximately 2500 samples of soil were examined for up to 15 total and available metals. In the early 1970s little analytical expertise was available in this specialised field and the AAS instrumentation available was unsophisticated.

The original analytical methods have been critically evaluated and detailed improvements have been incorporated into methods for reassessing the soil metal concentrations over a small area of Beaumont Leys due for imminent redevelopment.

The importance of an adequate knowledge of the distribution and intensity of contamination on a site in advance of any decisions on its future use is now increasingly recognised. Such information is particularly needed when redevelopment for sensitive uses such as housing is proposed.

Earlier work suggested that the concentration of some metals showed little decline with increasing soil depth. This paper details further investigations which strongly suggest that the metallic content of Beaumont Leys soils is not mobile and remains in the topsoil.

Beaumont Leys is an area three miles north-west of Leicester which was occupied from the 1890s to 1966 by the City of Leicester sewage works. Sludge was spread on adjacent farmland and the effluent irrigated over much of the remainder of the 810 hectare site, with the consequence that the heavy metal concentration in the soil is now significantly greater than the surrounding area.

In 1970–71 this laboratory began the analysis of soil samples for heavy metals in order to assess any potential hazard which would affect its future development. The sampling points were the intersections of a 200 ft grid[1,2].

Since 1972, private and local authority housing, together with industrial buildings, have been progressively constructed on the site. The allocation of land uses was based on the soil analyses carried out in 1970–71. Between 1972 and 1976 further soil samples were analysed to provide additional information.

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As part of its research programme on the problems of contaminated land* the Central Directorate on Environmental Protection of the Department of the Environment (D.O.E.) commissioned several studies based on the Beaumont Leys site. One of these was to monitor the concentrations of metals in soils in specific areas of the development before and after stripping/mixing/respreading, and another involved the uptake of metals into vegetables from Beaumont Leys soil. The Leicestershire County Analyst's Laboratory contracted to carry out this work in 1978.

From the start of the contract it was agreed that any further work involving soil sampling on the Beaumont Leys site should be designed around the 200 ft grid design of 1971. Although it would have been possible to base further sampling on the National Grid, correlation with the earlier studies would then have been almost impossible.

The initial work is reported in three sections: (1) analytical methodology; (2) the survey to check earlier results, and (3) an investigation into the distribution of metal concentration down the soil profile.

Analytical Methodology

The original survey was carried out in 1971 in considerable haste. The atomic absorption spectroscopy (AAS) equipment then available had no form of background correction, microprocessors still being a thing of the future. By 1978, methods for the determination of toxic metals in soils had developed enormously and the significance of the results in terms of hazards for future land uses was beginning to be realised. There was by that time some guidance on significant levels of metals in soil[3] and the analytical methods chosen had to be capable of producing meaningful results at these concentrations.

An effort was therefore made in this work not only to reduce possible variabilities in the preparation and analysis but also to check the previous earlier data.

"TOTAL" METALLIC CONTENT OF SOIL

This was defined as that fraction of the metallic content of soil which was readily extractable with strong mineral acid.

The method used by Pike *et al.*[1,2] involved ashing the sample at 450°C to destroy the organic matter before extracting the metals from the ashed residue with boiling 5 M hydrochloric acid.

This method suffered from two major drawbacks. Firstly, the ashing stage involved considerable manipulation and furnace space was limited and not always available. There seemed to be merit therefore in eliminating this ignition stage completely, if possible. Secondly, when 5 M hydrochloric acid was boiled, aggressive fumes of the acid were evolved. By reducing the concentration of the acid to 2 M, this problem could be much reduced.

It was then necessary to check whether 2 M hydrochloric acid was as effective as 5 M in extracting metals from soil. The results obtained for three soils from

* "Contaminated land" has no official or statutory definition but is generally understood to mean land where the presence of chemical contaminants in the soil may give rise to hazards to people, plants, animals, buildings or underground services e.g. water.

Beaumont Leys are shown in Table I. They show that ashing soils before acid treatment is unnecessary as there is no significant increase in the extraction efficiency of the system for most metals. Furthermore, it was shown that the amount of copper extractable with acid was considerably reduced by ashing, possibly due to the formation of insoluble cuprosilicate compounds on heating[4].

The 2 M hydrochloric acid extraction method was then evaluated against other extractants using samples of six soils prepared by Agricultural Development and Advisory Service (ADAS), Shardlow, Derbyshire:

1. Nitric acid digestion. This Severn-Trent Water Authority method was similar to that devised using hydrochloric acid above. It was considered appropriate to carry out parallel extractions using 20 per cent. nitric acid solution. The results are summarised in Table II.

2. Nitric and sulphuric acid digestion. This was included to check whether the more volatile elements, such as arsenic, were lost when using the other methods (Table II).

3. Wet oxidation using perchloric acid and/or nitric acid. These acids are often regarded as the most effective extractants available. At that time these methods were not in use at the County Analyst's Laboratory. However ADAS, Shardlow, who had routinely used the method[5] did take part in a small collaborative exercise in which the six soils were analysed in quadruplicate by both laboratories. Table III summarises the results.

EQUIPMENT

Glen Creston Cross Beater Hammer Mill; Gallenkamp wrist shaker; Atomic Absorption Spectrophotometer—Instrumentation Laboratory, Models 151 or 357 fitted with deuterium arc background correction; Hydride generation kit—Instrumentation Laboratory; Glassware used was Pyrex or Quickfit from Corning Ltd; Fan Oven—Baird & Tatlock set at 105°C; pH Meter—EIL model 5046.

REAGENTS

All chemicals used were of analytical grade.

1. *Acetic acid:* 0·5 M solution.
2. *A.A.S. Elemental Standards:* 1000 mg of each element per litre.
3. *Ethylenediaminetetra-acetic acid (di-ammonium salt) (EDTA):* 0·5 M solution.
4. *Hydrochloric acid:* 2 M solution.
5. *Hydrochloric acid:* 0·07 M solution.
6. *Sulphuric acid:* S.G. 1.84.
7. *Nitric acid:* S.G. 1.42.
8. *Nitric acid:* 20 per cent. solution by volume.
9. *Ammonium oxalate:* Saturated solution.

TABLE I

EFFECT OF ASHING ON EXTRACTION OF METALS FROM SOIL

	Ashed at 450°C then extracted with 2 M HCl	2 M HCl extraction	Per cent. metal extracted without ashing
Soil A			
Cadmium	2·90	2·95	102
Copper	65	92	142
Nickel	61	63	103
Lead	196	207	106
Zinc	332	340	102
Soil B			
Cadmium	1·00	0·90	90
Copper	31	41	132
Nickel	35	32	91
Lead	78	77	99
Zinc	150	145	97
Soil C			
Cadmium	1·75	1·95	111
Copper	45	59	131
Nickel	49	45	92
Lead	90	88	98
Zinc	229	214	94

Results calculated as milligrams element/kg dry soil.
Each result is the mean of two replicates.
Mean recovery from the three soils (per cent.): Cadmium, 101; Copper, 135; Nickel, 95; Lead, 101; Zinc, 98.

TABLE II

EXTRACTION OF METALS FROM SOIL: PRECIS OF VARIOUS METHODS. MEAN RESULTS ON SIX STANDARD SAMPLES

	2 M Hydrochloric acid mean	20% nitric acid mean	HNO_3/H_2SO_4 mean
Arsenic	43	42	46
Barium	858	842	<0·1
Beryllium	1·2	1·3	1·7
Cadmium	11·7	12·2	13·0
Chromium	545	553	557
Cobalt	19	18	14
Copper	449	456	458
Lead	870	882	118
Manganese	349	355	—
Molybdenum	10·4	10·4	—
Nickel	111	110	123
Silver	20	11	5·8
Tin	132	29	—
Vanadium	70	62	88
Zinc	1178	1188	1233

The above results are expressed as mg element/kg dry soil. Detailed results are given in Tables XI–XIII.

TABLE III

DETERMINATION OF METALS IN SOIL: COLLABORATIVE WORK BETWEEN LEICESTERSHIRE COUNTY COUNCIL AND ADAS

Element	L.C.C.	ADAS
Arsenic	43	47
Cadmium	11·7	12·2
Chromium	545	547
Copper	449	432
Lead	870	737
Nickel	111	124
Zinc	1178	1223

Six soils prepared by ADAS, Shardlow, each analysed in quadruplicate, mean values of the 24 results are tabulated.

Leicestershire County Council calculated as mg element/kg dry soil. ADAS calculated as mg element/litre of air dried soil.

TABLE IV

TOTAL METAL CONTENT OF SOIL SAMPLES. (200 FT GRID POINTS) COMPARISON OF PRESENT AND EARLIER SURVEY

		Arsenic			Cadmium		Chromium	
No.	pH 1978	1971	1978	Barium 1978	1971	1978	1971	1978
Dh35	7·1	14	17	77	4·0	2·3	197	195
Dh36	6·9	3	21	108	2·0	3·9	108	350
Di 31	7·3	5	7	72	1·6	0·6	31	29
Di 32	7·2	4	15	92	4·2	2·0	412	100
Di 33	6·8	15	16	103	6·0	3·5	232	159
Di 34	6·8	17	19	103	4·0	3·5	236	206
Di 35	5·6	19	15	124	6·0	4·4	431	498
Di 36	5·7	16	14	124	4·0	5·6	202	568
E 31	6·7	7	11	51	3·0	1·8	132	88
E 32	6·8	17	17	98	4·0	3·5	132	230
E 33	6·5	13	16	108	5·0	3·9	179	227
E 34	6·6	12	16	92	4·0	2·0	126	233
E 35	6·6	16	21	112	6·0	3·4	830	465
E 36	5·9	14	23	124	9·0	6·2	1410	1010
Ea 31	7·2	8	17	77	2·2	1·4	102	114
Ea 32	7·1	20	17	71	6·0	1·7	226	173
Ea 33	6·6	10	21	98	3·0	4·1	46	305
Eb 31	6·2	11	16	66	3·5	2·5	193	195
Eb 32	6·6	15	17	67	3·0	1·6	98	133
Eb 33	6·0	16	16	82	4·0	2·2	470	337
Mean		12·6	16·1	92	4·2	3·0	290	281
Range:								
Min.		3	7	51	1·6	0·6	31	29
Max.		20	23	124	9·0	6·2	1410	1010

TABLE IV (CONTINUED)

	Copper		Lead		Mercury		Nickel		Zinc	
No.	1971	1978	1971	1978	1971	1978	1971	1978	1971	1978
Dh35	80	61	130	128	0·4	0·9	75	41	355	317
Dh36	44	86	90	198	0·1	1·7	80	65	162	339
Di 31	20	31	40	31	0·1	0·03	55	25	83	89
Di 32	100	51	412	100	0·3	0·3	105	49	355	205
Di 33	134	70	145	120	0·8	0·4	105	64	355	277
Di 34	92	82	155	149	0·8	0·6	90	65	260	298
Di 35	120	101	245	265	2·1	1·0	120	85	420	423
Di 36	68	134	180	322	1·1	1·4	90	90	222	404
E 31	63	43	120	84	0·2	0·3	80	44	168	143
E 32	48	70	105	129	0·5	0·5	95	85	168	298
E 33	91	86	110	126	0·4	0·4	95	102	270	338
E 34	63	62	105	143	0·2	0·5	75	71	187	226
E 35	130	89	315	191	2·0	0·8	110	93	500	307
E 36	198	156	202	398	—	1·9	110	121	600	569
Ea 31	48	45	95	94	0·2	0·4	80	51	118	164
Ea 32	116	58	165	317	0·4	0·4	105	72	295	205
Ea 33	37	110	70	164	0·1	0·6	55	112	156	360
Eb 31	73	64	110	110	0·3	0·4	90	71	216	245
Eb 32	48	54	105	118	0·5	0·4	95	62	147	185
Eb 33	52	66	215	170	0·9	0·7	120	63	250	257
Mean	81	76	156	168	0·6	0·7	92	72	264	285
Range:										
Min.	20	31	40	31	0·1	0·03	55	25	83	89
Max.	198	156	412	398	2·1	1·9	120	121	600	569

Procedure

MOISTURE

Since the soil metal results were to be reported as the concentration of the element in the dry soil, the residual moisture was determined. About 2 g of the air-dried soil was accurately weighed and dried at 105°C to constant weight.

EXTRACTION WITH 2 M HYDROCHLORIC ACID

The air-dried test soil (5 g ± 0·01 g) was weighed into a 400-ml beaker, mixed with 70 ml of 2 M hydrochloric acid, and boiled on a hot plate for one minute. The beaker was removed and allowed to stand for a further two minutes. The supernatant liquor was filtered through a 12·5-cm, No. 111 paper into a 250-ml calibrated flask. This extraction process was repeated twice more, passing the extract through the same filter. The residue and filter were thoroughly washed with water until all traces of acid were removed and the washings added to the flask. After cooling, the mixture was made up to 250 ml. For high zinc concentrations, this solution was further diluted, while for low cadmium levels, the solution had to be concentrated five times before the estimation of the metallic content.

EXTRACTION WITH 20 PER CENT. NITRIC ACID

This was identical to the hydrochloric extraction above, with the substitution of 20 per cent. nitric acid solution for 2 M hydrochloric acid.

TABLE V

HEAVY METALS IN SOIL SAMPLES (BEAUMONT LEYS—200 FT GRID POINTS). METALS SOLUBLE IN 0·5 M ACETIC ACID

No.	Cadmium	Copper	Nickel	Zinc
Dh35	1·3	5·1	8	106
Dh36	2·0	7·4	16	128
Di 31	0·4	3·2	2	16
Di 32	1·1	4·1	10	68
Di 33	1·4	4·6	13	91
Di 34	1·8	5·7	15	88
Di 35	2·0	5·8	27	134
Di 36	2·8	10·0	30	154
E 31	0·7	4·1	8	36
E 32	1·4	4·0	16	100
E 33	1·5	6·0	27	110
E 34	0·8	4·3	18	67
E 35	1·6	7·4	29	96
E 36	2·7	10·0	37	178
Ea 31	0·6	3·2	8	34
Ea 32	0·6	4·1	13	46
Ea 33	1·7	6·0	28	100
Eb 31	1·2	5·5	20	78
Eb 32	0·6	4·1	12	45
Eb 33	1·0	5·2	16	62
Mean	1·4	5·5	17	87
Range:				
Min.	0·4	3·2	2	16
Max.	2·8	10·0	37	178

DIGESTION WITH NITRIC/SULPHURIC ACID

The air-dried soil (5 g ± 0·01 g) was weighed in a 250-ml conical flask to which was added 5 ml of nitric acid (S.G. 1.42) and 10 ml of sulphuric acid. The mixture was heated to boiling point on a hot plate, more nitric acid being added dropwise as long as the solution tended to darken. Boiling was then continued until white fumes were evolved, the solution was cooled, and 10 ml of saturated ammonium oxalate solution were added. The mixture was re-heated until white fumes were again copiously evolved. It was then cooled, diluted carefully with water, transferred quantitatively to a 100-ml flask and made to the mark with water.

ATOMIC ABSORPTION SPECTROSCOPY (AAS)

The metallic content of the soil extract solution prepared above was estimated by flame AAS, using conditions and instrument settings set out in the manufacturer's literature.[6] Arsenic and antimony were determined using the hydride generation technique and mercury by the cold vapour method[6].

The instruments were calibrated using appropriate metal standard solutions diluted from concentrated solutions containing 1000 mg of each respective element per litre.

TABLE VI

HEAVY METALS IN SOIL SAMPLES (BEAUMONT LEYS—200 FT GRID POINTS). METALS EXTRACTABLE USING 0·07 M HCL

No.	Antimony	Arsenic	Barium	Cadmium	Chromium	Lead	Mercury
Dh35	0·19	1·1	66	2·3	6·9	22	0·01
Dh36	0·14	1·5	84	3·4	10·6	37	0·01
Di 31	<0·05	0·3	70	0·6	1·1	7	0·01
Di 32	<0·05	1·5	63	2·1	4·7	16	0·02
Di 33	<0·05	1·3	82	3·0	6·2	16	0·01
Di 34	<0·05	0·7	68	3·3	7·8	23	0·02
Di 35	0·14	1·1	86	4·0	8·8	39	0·02
Di 36	<0·05	1·3	88	5·5	10·7	50	0·01
E 31	0·09	1·0	41	1·4	3·1	12	0·02
E 32	0·09	1·5	62	3·1	8·3	14	0·01
E 33	0·26	1·4	75	4·0	6·4	13	0·01
E 34	0·05	0·8	65	1·9	5·9	15	0·02
E 35	<0·05	1·1	65	3·4	9·8	11	0·01
E 36	0·37	1·5	75	5·7	11·9	44	0·01
Ea 31	<0·05	1·4	52	1·5	4·6	15	0·02
Ea 32	<0·05	0·8	59	1·7	5·9	17	0·01
Ea 33	<0·05	1·6	64	4·1	8·0	25	0·02
Eb 31	<0·05	1·9	47	2·6	6·8	17	0·02
Eb 32	0·27	1·6	47	1·5	5·0	16	0·02
Eb 33	<0·05	1·3	64	2·2	7·1	25	0·02
Mean	0·11	1·2	66	2·9	7·0	22	0·015
Range:							
Min.	<0·05	0·3	41	0·6	1·1	7	0·01
Max.	0·37	1·9	88	5·7	11·9	50	0·02

QUALITY ASSURANCE

The Association of Public Analysts' Quality Assurance Protocol[7] had not been published at the time of this work, and no true standard soil with quoted metal concentrations could be located.

The production of six soils for collaborative work with other laboratories and for use in quality control went some way to satisfy the need for quality assurance. One standard soil was included with each batch of soils to be analysed; the details of this scheme are to be published in a later paper.

Discussion

For cadmium, chromium, copper, nickel, lead and zinc, the difference between the 2 M HCl and 20 per cent. HNO_3 extractions was found to be insignificant. 2 M HCl was superior for the extraction of silver and tin.

The 2 M HCl system described above used relatively innocuous chemicals and the extractions could be carried out in the open laboratory (although the boiling stage is recommended to be carried out under a fume hood) without causing discomfort to laboratory staff.

The nitric acid–sulphuric acid digest had little advantage over the other two systems to compensate for its extra manipulative effort. Only beryllium and vanadium showed any increase but there was a significant decrease in silver, barium and more importantly lead.

TABLE VII

HEAVY METALS IN SOIL SAMPLES (BEAUMONT LEYS—200 FT GRID POINTS). PERCENTAGE METAL EXTRACTED WITH VARIOUS EXTRACTANTS

		Antimony	Arsenic	Barium	Cadmium	Chromium	Copper	Lead	Mercury	Nickel	Zinc
Mean total metal content mg element/kg ds.		—	16·1	92	3·0	281	76	168	0·67	72	350
Metal available in 0.5 M acetic acid	Mean concn.	—	—	—	1·36	—	5·5	—	—	17·7	87
	per cent. of total	—	—	—	45·3	—	7·2	—	—	24·6	24·9
Metals extracted by Toys (Safety) Regs. Method	Mean concn.	0·11	1·22	66	2·86	6·98	—	21·7	0·015	—	—
	per cent. of total	—	7·6	71·7	95·3	2·5	—	12·9	2·2	—	—

TABLE VIII

AVAILABLE COPPER IN SOIL (BEAUMONT LEYS). COMPARISON OF EXTRACTION METHODS

	Mean values on 10 soils	
	Copper (*mg/kg* dry soil)	Per cent. extracted
Total	70·9	100
0·5 M acetic acid extractable	4·8	6·8
Diammonium EDTA extractable (L.C.C.)	34·6	48·8
Diammonium EDTA extractable (ADAS)	44·3	62·5

The collaborative study with ADAS suggested that for the six soils analysed the 2 M hydrochloric acid method was at least as good as the widely used nitric acid–perchloric acid wet digestion, although it was acknowledged that the absolute efficiency of system could not be measured due to the unavailability of "standard" soil samples at the time.

Conclusion

It is improbable that any system would quantitatively extract all metals from soil but the 2 M hydrochloric acid extraction method is a simple, rapid and efficient system which compares favourably with many of the better known methods which use more hazardous chemicals and frequently are more time-consuming.

1971–78 Surveys

Topsoil samples were taken at 20 selected intersections of the 200 ft grid as established in 1971 in order to assess the comparability of the data from the original survey to that of 1978.

PREPARATION OF SOIL SAMPLES

The soil samples were collected from the site by members of the Leicester City Engineers Department. The surface vegetation was removed, and cores from the top six inches of soil were taken and submitted to the laboratory in polythene bags. Generally the samples weighed about 1 kg; on arrival at the laboratory stones greater than 0·5 cm in size and any extraneous vegetable matter was rejected. The soils were air dried at room temperature (not exceeding 30°C), milled to pass a 4 mm screen, and stored in polythene bags to await analysis.

pH DETERMINATION

Soil pH values were determined by an empirical method. Ten grams of the air-dried soil were mixed with 50 ml of distilled water, and after allowing to stand for 1 h, the pH was determined on the solution using the appropriate pH meter.

TABLE IX

DISTRIBUTION OF METALS IN SLUDGED SOIL. MEAN RESULTS OF SIX SOILS

(a) Total Metals (mg/kg of dry soil)

Depth	Cadmium	Chromium	Copper	Lead	Nickel	Zinc
Topsoil	27	1005	642	1484	236	2376
Top/Sub	8·6	548	313	783	122	1438
1 m	0·4	32	27	20	38	102
2 m	0·5	26	25	19	41	73
3 m	0·4	24	23	19	40	77

(b) Available Metals (mg/kg of dry soil)

Depth	Cadmium	Copper	Nickel	Zinc
Topsoil	7·4	48	111	1150
Top/Sub	2·8	28	49	660
1 m	0·2	2·9	2·1	24
2 m	0·1	4·8	3·2	16
3 m	0·1	3·2	2·0	14

TOTAL METALS

The determination of the total metals using the 2 M HCl method was carried out together with a nitric acid/sulphuric acid digest for the determination of arsenic. For practical details see above. Mercury was determined using the method of Iskandar *et al.*[8]

TABLE X

DISTRIBUTION OF METALS IN SOIL FROM THE EFFLUENT IRRIGATION AREA. MEAN RESULTS FROM SIX SOILS

(a) Total Mean (mg/kg of dry soil)

Depth	Cadmium	Chromium	Copper	Lead	Nickel	Zinc
Topsoil	3·5	310	77	163	76	227
Top/Sub	1·7	166	43	90	51	195
1 m	0·3	25	20	13	26	57
2 m	0·3	23	21	13	32	60
3 m	0·3	25	21	10	32	73

(b) Available Metals (mg/kg of dry soil)

Depth	Cadmium	Copper	Nickel	Zinc
Topsoil	1·1	3·4	20	92
Top/Sub	0·6	2·9	12	60
1 m	0·1	3·2	1·1	18
2 m	0·1	3·0	1·3	14
3 m	0·1	3·5	1·4	13

AVAILABLE METALS

The available concentration of a metal in the soil is that proportion of the total concentration present which can be readily taken up from the soil into plant tissues.

All methods used to determine available metals are of essence empirical, but the following two have been widely accepted.

Acetic acid (0·5 M) solution has been used to assess the degree of possible metal deficiency in soil for agricultural uses[5] and via the zinc equivalent concept in which zinc, copper, nickel are estimated, to assess soil phytotoxicity. (For work on Beaumont Leys, cadmium was added to the list of elements to be determined.)

During the period of the D.O.E. contract it became known that ADAS had changed their method of determining available copper to one involving 0·05 M diammonium EDTA[5] and consequently some analyses using this method were also carried out.

(More recently, MAFF have announced their intention to phase out the "zinc equivalent" concept and to discontinue the use of the term "available" generally in favour of "total" metals extracted with nitric acid–perchloric acid[9].)

Practical details. Zinc, copper, nickel and cadmium are extracted using a 0·5 M acetic acid solution. Air-dried soil (5 g ± 0·01 g) was weighed into a flask to which was added 200 ml of 0·5 M acetic acid. The mixture was shaken, using a wrist-shaker, for 30 min, filtered, and the filtrate analysed by AAS.

For copper, air-dried soil (10 g ± 0·01 g) was shaken for 1 h with 50 ml of 0·05 M EDTA (diammonium salt) solution, and filtered. Copper was determined in the filtrate by AAS.

EXTRACTABLE METALS

Concern was expressed over the hazard to children with the condition known as pica living at Beaumont Leys. In an attempt to assess the effect of any consumption of soil, use was made of the method in the Toys (Safety) Regulations 1974[10] which, though not ideal, was selected as being the most appropriate method of analysis immediately available.

Practical details. For the determination of lead, cadmium, chromium, barium, arsenic, antimony and mercury, soil was extracted with fifty times its weight of 0·25 per cent. HCl (0·07 M) as in the method given in the Toys (Safety) Regulations 1974[10]. The results are listed in Tables VI and VII.

Discussion

The mean results of the total metal determinations for the 20 soil analyses in 1978 were comparable to those found in 1971. In no case, taking the 1978 figure rather than that from the earlier survey, would the overall recommendations as

to the reclamation of the site have been changed. In most cases the precision of the means is remarkable, although the problems of accurate re-sampling are demonstrated by the variation of the results from individual sample points.

The most available metal would appear to be cadmium, with almost half being extracted by 0·5 M acetic acid. Approximately 25 per cent. of the nickel and zinc are in the available form but less than 10 per cent. of the copper was found to be so.

If 0·05 M $(NH_4)_2$ EDTA is used then about half the total copper becomes available: i.e. approximately five times the value found using 0·5 M acetic acid. ADAS now use the 0·05 M diammonium EDTA system for both zinc and nickel as well as copper, and the EDTA method has been specified by Interdepartmental Committee on the Redevelopment of Contaminated Land[11] for the determination of phytotoxic metals.

The analytical method found in the Toys (Safety) Regulations 1974 is designed to predict the extractability of toxic material from paint on toys and therefore results obtained from other systems must be examined with a good deal of circumspection. A significant proportion of barium was found to be extracted with 0·07 M HCl from the soil; cadmium was almost quantitatively soluble in the acid solution. The results obtained were well below the permitted maximum levels of soluble metals found in paint; however, there could be a considerable difference in the weight of paint on a toy compared to the weight of soil a child might ingest.

Conclusion

This study confirms that different extraction systems release very different concentrations of metal and that the interpretation of such data should be made with considerable caution within the realms of contaminated land studies.

Concentration of Soil Metal with Increasing Soil Depth

Data from the 1971 survey,[1,2] suggested that for some metals including cadmium, the concentration in the soil remained more or less constant to a depth of 2 m.

It was agreed that further investigation was required in order to confirm the results.

PRESENT WORK

Twelve sample points on the 200 ft, grid were selected, six on the sludge spreading area, and six from the effluent distribution area. Samples were taken from the topsoil and then, by means of a mechanical digger, at the topsoil/subsoil interface, and at depths of 1, 2 and 3 m respectively. The samples were prepared and analysed using the methods above.

ANALYTICAL METHODOLOGY

Total cadmium, chromium, copper, nickel, lead and zinc were determined using the 2 M HCl extraction method and available cadmium, copper, nickel and zinc were determined using acetic acid as detailed above.

RESULTS

These are presented in precise form in Tables IX and X.

Discussion

It is clear from the results obtained on both sludged land and from the effluent irrigation area, that the metals associated with the sewage remain in the topsoil, even though Beaumont Leys was run for many years as a working farm, and consequently much of the area was regularly ploughed.

The 1971 survey results have not been confirmed and it is possible that the depth samples, which were obtained using a hand auger, were contaminated with topsoil and therefore produced misleading results.

Sampling the topsoil at Beaumont Leys will give the highest concentration of metals; it would appear unlikely that contamination will be further concentrated in the surface soil and cause future problems.

The pattern of results that has been obtained from the analysis of Beaumont Leys soil may not be characteristic of all sites, but may well be true for sewage-sludged land.

APPENDIX

TABLE XI

TOTAL METALS IN STANDARD SOILS: 2 M HYDROCHLORIC ACID EXTRACTION
(mg/kg of dry soil)

Soil No.	1	2	3	4	5	6
Arsenic	14	26	10	20	25	166
Barium	95	1100	200	1500	1700	550
Beryllium	0·9	1·2	0·9	1·1	1·2	2·0
Cadmium	1·3	8·8	—	31	28	0·8
Chromium	131	720	42	1120	1225	33
Cobalt	18	17	17	17	20	29
Copper	64	400	63	975	900	295
Lead	104	970	160	1275	1355	1370
Manganese	390	325	450	250	320	360
Molybdenum	5	5	5	10	18	20
Nickel	34	99	20	232	234	47
Silver	2·8	21	1·3	50	44	2·0
Tin	20	115	15	270	290	85
Vanadium	43	53	40	58	70	160
Zinc	174	1465	149	2530	2360	391

Tabulated results are means of duplicate analyses.
—, no analysis carried out.

TABLE XII

TOTAL METALS IN STANDARD SOILS: 20 PER CENT. NITRIC ACID EXTRACTION
(mg/kg of dry soil)

Soil No.	1	2	3	4	5	6
Arsenic	13	27	8	21	25	157
Barium	53	1050	200	1500	1650	550
Beryllium	1·2	1·3	0·9	1·0	1·2	2·4
Cadmium	1·5	9·9	—	32	29	0·9
Chromium	149	720	44	1125	1255	24
Cobalt	17	15	19	15	17	22
Copper	61	415	53	1000	945	260
Lead	96	970	152	1275	1405	1395
Managnese	420	315	490	255	315	325
Molybdenum	5	5	5	10	23	15
Nickel	35	102	20	235	236	33
Silver	2·5	13	1·3	25	24	2·3
Tin	5	17	<5	58	70	20
Vanadium	38	43	40	50	55	145
Zinc	177	1465	160	2515	2425	386

Tabulated results are means of duplicate analyses.
—, no analysis carried out.

TABLE XIII

TOTAL METALS IN STANDARD SOILS: SULPHURIC ACID–NITRIC ACID WET OXIDATION
(mg/kg of dry soil)

Soil	1	2	3	4	5	6
Arsenic	19	34	10	32	34	148
Barium	<0·1	<0·1	<0·1	<0·1	<0·1	<0·1
Beryllium	1·5	1·8	1·4	1·6	1·8	2·2
Cadmium	1·2	8·1	0·2	36	32	0·4
Chromium	168	660	62	1140	1270	33
Cobalt	13	13	13	14	16	18
Copper	56	390	64	984	930	326
Lead	34	140	68	164	166	136
Nickel	40	114	27	244	264	47
Silver	4·1	8·5	2·4	7·0	8·6	4·3
Vanadium	93	92	87	67	69	118
Zinc	172	1480	160	2640	2480	468

Tabulated results are means of duplicate analyses.
< = less than.

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A Review of Approaches to the Rapid Analysis of Aflatoxins in Foods

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An overview of methods of analysis for aflatoxins is presented, with an emphasis on practical performance. Currently used TLC and HPLC procedures are outlined and contrasted and recent developments in immunologically-based methods are reviewed in detail. Enzyme-linked immunosorbent assay, "card" tests and affinity column methods are described and compared.

The relative importance in terms of a hazard to food safety of aflatoxin B_1 as a contaminant of nuts and nut products, and of its metabolite aflatoxin M_1 as a contaminant of milk and dairy products, is reflected by the world-wide existence of tolerance levels for aflatoxins in these commodities[1]. In the U.K., control of aflatoxins in edible nuts has to date been through a voluntary code of practice[2] applied by the shippers and manufacturers to the unprocessed nuts using the Tropical Development and Research Institute (TDRI)† sampling plan[3], but a proposal for legislation is pending for this code to be replaced by a limit of 10 μg of total aflatoxin per kg, enforceable at the point of retail sale. Aflatoxin M_1 concentrations in U.K. milk have been reduced to a level not detectable by current analytical methods (less than about 0·01 μg/kg) by regulations controlling the amount of aflatoxin B_1 permitted in animal feedingstuffs[2].

Statutory methods for determination of aflatoxins need to be well established, need to have wide acceptance by the scientific community and need to have been fully validated by collaborative testing before being ultimately adopted in regulations. Such methods are available for determining aflatoxins in foods and have been fully endorsed and tested by organisations including the Association of Official Analytical Chemists (AOAC)[4], the American Oil Chemists' Society (AOCS), the International Union of Pure and Applied Chemistry (IUPAC) and the European Community (EC)[5]. A review of officially approved methods has been compiled by Park and Pohland[6], and Horwitz and Albert[7] have discussed the overall reliability of aflatoxin assays. However, the adoption and testing of procedures for analytical methods are very slow, and thus it takes a considerable time for advances in analytical techniques eventually to become embodied in regulatory protocols.

In this review we consider established methods of aflatoxin analysis and then examine some of the new techniques that have been recently introduced in this

† Now called "The Overseas Development Natural Products Institute".

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very rapidly developing area, and which offer the prospect of simpler, more rapid means of determining aflatoxins in foods. These new approaches, we believe, will in time have a considerable impact on enforcement. They will enable aflatoxin determinations to be carried out more easily without requiring the skills based on extensive experience in the area that have been hitherto necessary. The fact that the new approaches are far more rapid should also enable monitoring to be undertaken on a larger scale than has been previously possible, thus to some extent overcoming the sampling problems associated with uneven distribution of toxins in contaminated commodities. Alerting the public analyst at an early stage to these changes that are taking place will not affect the speed of change with respect to Statutory Methods but should at least offer the option of adopting better methods for initial screening for aflatoxins in foods. Thus the usually low percentage of positive samples can be identified rapidly and then confirmed using stipulated regulatory methods.

Chemical Methods

Early methods for aflatoxins were laborious: samples were ground and defatted in a soxhlet, the toxins extracted by solvents, cleaned up on silica gel columns and determined by paper chromatography. Subsequent developments included a switch to TLC and extraction of toxins directly into non-lipophilic solvents but otherwise techniques remained essentially unchanged for about 20 years. Modern chemical methods for aflatoxins rely on the same basic principles although the cleanup column is likely to be purchased ready packed (Sep-Pak, Bond-Elut or similar).

The choice of chromatographic technique for quantification is not completely straightforward. For historical reasons, TLC has been the standard approach for aflatoxin analysis and the gradual introduction of HPLC procedures for the final determination had to compete with existing thoroughly validated and widely-used TLC-based methods[3]. The trend has been towards increased use of HPLC; 30 per cent. of participants in the 1986 International Agency for Research on Cancer (IARC) aflatoxin M_1 check-sample exercise[8], compared to 12 per cent. in 1980[9]. For the 1986 aflatoxin B_1 in peanut meal check-sample exercise[10], 26 per cent. used HPLC (including 5 per cent. who employed the AOAC CB cleanup[4]); other preferences were: AOAC (CB method) 29 per cent.; AOAC (BF method[4]) 10 per cent.; EEC Official method 7 per cent.; others 28 per cent. Both normal and reversed phase HPLC methods have been devised but the central problem common to both is the strong dependence of the fluorescence of aflatoxins B_1 and G_1 on their physical environment.

Direct reversed phase HPLC is limited because B_1 and G_1 fluorescence is quenched in aqueous solvents. This may be circumvented by chemical derivatisation of the terminal double bond to give analogues of the "2" toxins. Precolumn addition of water using trifluoroacetic acid catalysis is suitable if only a few samples are to be analysed, and provided that the sample clean-up is efficient; for larger numbers postcolumn reaction with iodine-saturated water becomes more convenient[11]. With either approach, preliminary confirmation of toxin identity may be achieved by chromatographing samples both with and without derivatisation. In normal phase HPLC, a solvent system incorporating

toluene and formic acid has been shown not to quench toxin fluorescence while an alternative approach is to pack the fluorimeter flow cell with silica gel. The fluorescence of B_1 or G_1 dynamically adsorbed to the silica surface is not affected by the solvent conditions.

However, although quantification of mycotoxin standards is significantly improved using HPLC, it is also true that inter-laboratory coefficients of variation for full analyses, which are typically 30–60 per cent. (obtained from collaborative studies and check sample exercises), do not differ greatly whether HPLC or TLC is employed for the determination step[9]. This may be an artifact of the data caused by the difficulties of statistical analysis on relatively small numbers of results, when a few near-outliers can markedly influence the calculated standard deviation.

An alternative explanation lies in the fact that analysts employing HPLC have tended to use a wide range of clean-up methods compared to those utilising TLC—the latter having predominantly opted for the AOAC CB procedure or the similar EC method. Thus extra variability at the clean-up stage may have offset the anticipated improvement in quantitation. This view is supported by the results of the relatively few (11) laboratories using the CB cleanup with HPLC for peanut meal in the 1986 IARC check-sample exercise, where the average coefficient of variation was only two thirds that of other groups. McKinney[12] concluded that the inadequate validation of aflatoxin standard solutions was the principal factor responsible for that part of the coefficient of variation in excess of the component intrinsically due to the analytical technique. The American Society for Testing and Materials (ASTM) has evaluated the quantitative precision of HPLC analysis[13] and found that standard deviations of 3–8 per cent. may be expected, depending upon the complexity of the mixture to be separated. The corresponding figures for TLC with densitometric and visual quantification are approximately 10 and 25 per cent. respectively.

The most significant advantage of TLC is that it can be a very inexpensive technique, although in its more sophisticated forms it requires a substantial capital investment in items such as spotters and densitometers. In addition, if one-dimensional TLC gives adequate resolution, a considerable number of samples may be analysed in parallel on one plate. Should two-dimensional TLC be found necessary, several plates can be developed simultaneously. Thus results may be obtained rapidly by TLC, even when taking into account the time required for spotting the plates. Furthermore, confirmation of the identity of a B_1 spot is readily achieved by derivatisation on the plate followed by a second development.

The principal disadvantages of TLC analysis are its lack of potential for automation and the subjective nature of the quantification step. Autosamplers permit unattended running of HPLC equipment and allow the sample throughput of this serial method of analysis to be as great as with TLC. Furthermore, the recent development of short, very high efficiency columns has demonstrated the capability of HPLC to provide extremely rapid results. Use of a densitometer overcomes the quantification problem but at a cost equivalent to that of a simple HPLC system. However, densitometers also offer a marked

improvement in sensitivity over visual quantification. On a standard TLC plate, the detection limit is about 200 pg of B_1 but with a densitometer, 40 pg are readily detected. This is similar in both sensitivity and cost to HPLC with a high quality fluorimeter. Using HPTLC, the detection of standards is improved by an additional factor of 3–4 due to the low dispersion of the chromatographic zones, but because of the small spot size necessary to achieve this performance problems arise when spotting sample extracts containing any appreciable amount of co-extractives such as lipid. HPTLC for this reason has not yet found any widespread application in aflatoxin analysis, although a method for M_1 in cheese has been described[14].

Because of the general growth in the use of HPLC, many laboratories possess the necessary instrumentation and could therefore perform aflatoxin analysis in this way should it appear to offer definite advantages over the more conventional TLC methods. Comparing HPLC and TLC techniques, a similar high degree of competence is necessary with either when establishing procedures and validating methods, but it is sometimes not appreciated that although TLC may be carried out using very simple equipment, it then demands greater operator skills and attention to detail in use than does HPLC.

Separation by HPLC may be preferred for other reasons. One factor to consider is safety, where liquid chromatography offers greater protection because toxins are maintained in solution and contaminated silica dust does not arise. One potentially important advantage of HPLC lies in its suitability for on-line clean-up of crude extracts using either column switching or some form of robotics. It is possible that this will ultimately be seen as one of the more compelling reasons for employing HPLC rather than TLC as the analytical technique. Whatever method is used for the final measurement, the purity of the residue obtained from the sample will have a major influence upon both the detection limit achievable and also the degree of confidence which may be placed on the result. The application of HPLC to the analysis of aflatoxins (and other mycotoxins) has been reviewed recently[15]. This paper also explores the chromatographic consequences of varying parameters such as the column packing and mobile phase.

Methods of Analysis for Aflatoxins (A.O.A.C. and Others)

There are a number of formally adopted methods for determining aflatoxins in foods, and although it is not the purpose of this paper to review these procedures in detail, an overview of the various steps involved in sample preparation and clean-up is necessary to appreciate fully the advantages offered by the newer techniques.

CB METHOD FOR AFLATOXINS IN NUTS AND NUT PRODUCTS

In Table I the AOAC, CB method[4] is shown in schematic form. This involves an initial blending of the nuts or nut product with chloroform for extraction, filtration and/or centrifugation and then a silica gel column chromatographic clean-up prior to TLC determination of the aflatoxins. Blending and extraction will be unavoidable with any analytical procedure for handling solid foodstuffs

TABLE I

THE CLEAN-UP PROCEDURE FOR AFLATOXINS USING THE AOAC CB METHOD

1. Grind sample.
2. Shake 50 g for 30 min with chloroform (250 ml) and water (25 ml).
3. Filter, collect 50 ml.
4. Load onto a deactivated silica gel column.
5. Wash with hexane (150 ml), then anhydrous diethyl ether (150 ml).
6. Elute toxins with 150 ml methanol/chloroform (3 + 97).
7. Evaporate, add 200 µl acetonitrile/benzene for TLC.

and it is unlikely that any changes can be implemented to improve this stage of the assay. Water slurrying methods are not new but offer advantages in preparing representative samples.

However, the column chromatographic clean-up stage, as well as being time-consuming, is one of the most critical points in the assay, influencing both the recovery of aflatoxins and the freedom of the final extract from co-extracted interferences. This stage requires care in activation of column materials, and skill in elution of the final extract without loss of the toxin. Thus improvement of this stage could be of significant advantage to the assay as a whole.

The CB and EC methods are very similar but there are indications that the EC method[5] gives slightly higher recoveries than does the CB. The differences are:

1. The EC method stipulates prior defatting of samples containing more than 5 per cent. of fat, whereas the CB does not.
2. For the column cleanup:
   a. The CB method employs silica gel dried for 1 h at 105°C and then deactivated with 1 per cent. of water, while the EC uses silica gel as supplied.
   b. The chloroform sample extract is loaded directly onto the CB column which is then washed with hexane. For the EC procedure, the extract and hexane are premixed.

The BF method[4], which incorporates a methanol/water extraction simultaneously with petroleum ether defatting, followed by partition of aflatoxins in the methanolic phase into chloroform (without any column cleanup), is simpler and more rapid as it does not involve a column cleanup stage but recoveries are typically 10–30 per cent. lower than for the EC or CB procedures.

STUBBLEFIELD METHOD FOR AFLATOXIN M_1 IN MILK

This procedure[16] for determining aflatoxin M_1 in milk is in principle similar to that of the CB method for aflatoxins in nuts and nut products, except that preliminary sample handling of a liquid is somewhat different and aflatoxin M_1 requires different elution conditions from the silica column. Again it is clear that the stages of the assay requiring improvement are at the point of extraction and column clean-up. A problem often encountered with solvent extraction of milk is the formation of unbreakable emulsions and elimination of this step is highly desirable. A schematic description of the Stubblefield procedure is shown in Table II.

TABLE II

THE CLEAN-UP PROCEDURE FOR AFLATOXIN M_1 IN MILK USING THE AOAC STUBBLEFIELD METHOD

1. Shake CAREFULLY milk (50 ml) + chloroform (120 ml) + saturated sodium chloride (5 ml) for 75 sec.
2. Separate, evaporate.
3. Load onto a deactivated silica gel column (2 g).
4. Wash with toluene/acetic acid, 9:1 (25 ml), hexane (15 ml), acetonitrile/ether/hexane (2:3:5) (25 ml).
5. Elute toxins with acetone/chloroform (2:3) (50 ml).
6. Evaporate, add 100 µl of chloroform for TLC.

Pre-packed Cartridges for Clean-up

Although not formally adopted within any of the "Official" methods there has been widespread use of commercial disposable cartridges to replace the laboratory-packed silica gel columns[17,18]. A wide variety of pre-packed cartridges are available, such as "Sep-Pak" (Waters Assoc., Milford, Mass., U.S.A.), Bond-Elut (Analytichem Internat., Harbor City, CA, U.S.A.) and "Baker-10 SPE" disposable columns (J. T. Baker Chemical Co., Phillipsburg, NJ, U.S.A.). These all differ in the design of the polypropylene plastic cartridge, but all are available containing either silica or modified silica packings (essentially similar from different manufacturers) of which the C_{18}-bonded phase is the most widely used for aflatoxin analysis.

For aflatoxin B_1 analysis of peanut butter a typical procedure[17] involves methanol/water extraction as in the BF method, filtration, defatting into hexane and then liquid/liquid extraction into chloroform. The chloroform extract is evaporated to dryness, re-dissolved in dichloromethane and loaded onto a pre-washed unmodified silica gel cartridge. The cartridge is washed and aflatoxins eluted with chloroform/acetone (9:1) prior to analysis. The use of the cartridge has the advantage that time is saved in column preparation (activation is generally not required) and solvent volumes are much smaller, making a saving in material costs and reducing washing and elution times.

Pre-packed cartridges are also amenable to batch-wise operations, for example using a vacuum manifold to handle a number of cartridges simultaneously (not all cartridges are interchangeable because of the different end fittings employed), or in a rather more sophisticated form with fully automated systems such as the "AASP" system (Varian Assoc., U.S.A.). The recoveries by conventional column chromatography and Sep-Pak are essentially similar and little difference is generally discernible in the extent of co-extractives in the chromatograms. The main difference is in the speed of this stage which takes of the order of 60 min using a laboratory column (not including the time required for packing the column), but only 5–10 min using the Sep-Pak.

For milk analysis, in a similar manner to the above, it is possible to improve the Stubblefield procedure clean-up simply by replacing the silica gel column with a silica Sep-Pak or equivalent. However, as the sample in this case is a liquid, methods have been proposed which avoid the initial liquid/liquid partition stage. In these procedures the milk is diluted with water, and then applied directly to a C_{18} cartridge[19,20]. After washing the cartridge with various

solvents the aflatoxin M_1 can be eluted either in a sufficiently clean form for direct analysis[19], or can undergo further chromatographic clean-up prior to final analysis[20]. The Ferguson-Foos and Warren procedure[20] using a Sep-Pak has recently undergone an AOAC collaborative trial and is to be recommended for adoption as a first action method[21].

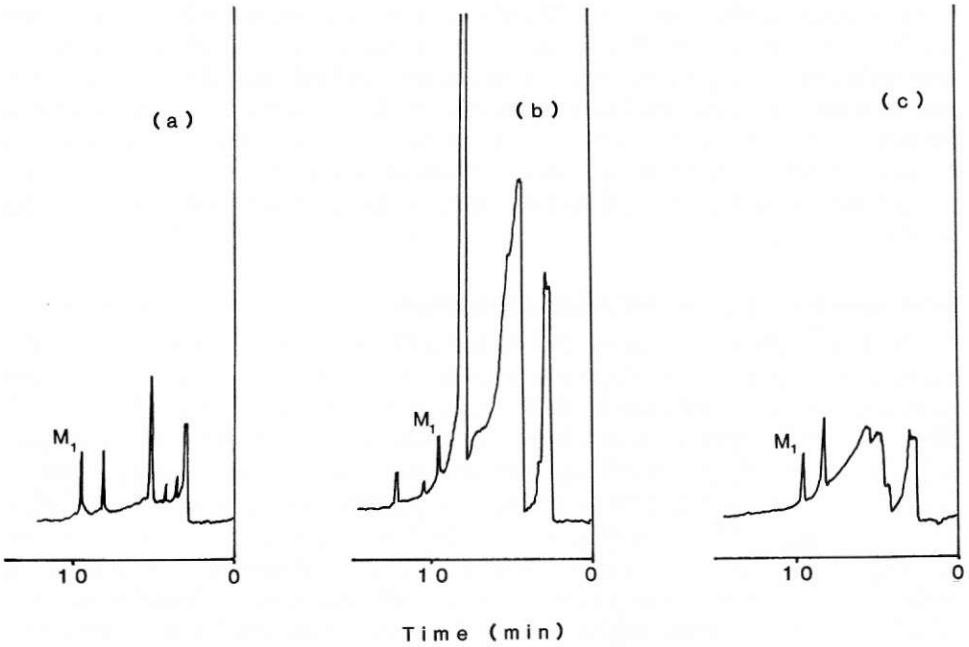

Fig. 1. HPLC chromatograms for aflatoxin M_1 in milk comparing different clean-up approaches. (a) Stubblefield Method, (b) Extrelut extraction (method of Gauch *et al.*[22]) (c) Sep-Pak extraction and clean-up (method of Takeda[19]). Whole milk, samples containing about 0·02 μg of aflatoxin M_1 per kg, analysed after clean-up on a 5 μm Spherosorb ODS column, eluting with water-acetonitrile-methanol (6 : 3 : 1) at a flowrate of 0·75 ml/min. Fluorescence monitoring with excitation at 355 nm and emission at 433 nm.

An alternative approach to liquid/liquid extraction is the use of pre-packed columns containing a bed of inert material which absorbs the sample extract and holds it stationary while aflatoxins or other lipophilic solutes are partitioned into immiscible organic solvents flowing through the column. Such columns as Extrelut (Merck 11737) or ChemTubes (Analytichem) contain diatomaceous earth (or kieselguhr) with a large pore volume but a granular structure. The packing is contained in a polypropylene syringe-style column, the elution rate of solvent being controlled in the Extrelut version by a cannula attached to the outlet. Different sizes of column are available for handling up to 300 ml of aqueous extract. This type of column has been used for aflatoxin M_1 analysis in milk[22], applying the sample to the column after an initial protein precipitation with concentrated hydrochloric acid. Typical chromatograms for aflatoxin M_1 in milk are shown in Figure 1 to illustrate both the Sep-Pak and the Extrelut approach to extraction compared with the more conventional methods. Although all three methods would appear to give acceptable chromatograms the

one with the least co-extractives was obtained by the Takeda method[19] (Sep-Pak) and this was also found on balance to offer the greatest advantages in terms of relative costs and time-savings[23].

One cautionary note with respect to pre-packed cartridges concerns the variation in activity of packing materials which may occur between batches of cartridges. Furthermore, manufacturers reserve the right to alter the properties of their packing materials without notice. It is therefore essential to test each batch before use, by checking the recoveries obtained with standards. There are also differences in performance between pre-packed cartridges of the same nominal packing material but obtained from different manufacturers—in the authors' experience distinct differences are encountered in the purity of extracts obtained from different brands of C_{18}-modified silica cartridges when used to extract the same spiked milk sample, but analysed under otherwise identical conditions.

Immunological Methods for Aflatoxin Analysis

The first radioimmunoassay (RIA) for aflatoxin B_1 was described in 1976, whereas commercial development (based on enzyme reactions rather than radioactivity) occurred only in 1984. Since then, development has been rapid. Three distinct types of immunological assay have emerged: (a) batchwise quantitative methods based on immunoassay, in this case enzyme-linked immunosorbent assay (ELISA); (b) semiquantitative but rapid methods suited to the analysis of single samples, such as the "card" test; and (c) affinity column cleanup. All methods are based upon the specific and very strong association between an antigen and its antibody. Although aflatoxins are small molecules and therefore not immunogenic themselves they may readily be coupled to carrier proteins and antibodies raised against them. Antibodies from different sources differ widely in their properties and cannot be treated as equivalent. Cross-reactivity values (a measure of the specificity of an antibody) and rate constants for the binding "reaction" are not always available but give important information about the antibody. Some antibodies interact essentially only with B_1, others may uniformly recognise a wide range of aflatoxins and their adducts with nucleic acids and proteins. Most will interact with a number of aflatoxins, some more strongly than others. As a result no immunological assay is optimal for all purposes.

IMMUNOASSAY

The quantification of trace levels of compounds in biological matrices has been performed routinely in clinical chemistry for many years by immunoassay. Chu[24] has reviewed the application of immunoassay to mycotoxin analysis. Aflatoxin analyses by radioimmune assay have been reported in the literature for some time but these have not been developed commercially, probably because the food industry is reluctant to accept radiochemically-based methods. Recently, single or double antibody enzyme-linked immunosorbent assays (ELISA) for aflatoxins have been launched by a number of companies. Mortimer *et al.*[25] have analysed 129 commercial peanut butter samples for

aflatoxin B_1 using a prototype of one of these assays, and found a good correlation with values determined by CB/HPLC.

Equipment of varying levels of sophistication is available for ELISA because of its widespread use in clinical chemistry. At its simplest all that is needed is a basic spectrophotometric plate-reader. Relatively inexpensive commercial ELISA plate readers are obtainable, but additional filters may be needed to achieve maximum sensitivity. It is also possible (at considerable expense) to purchase fully automated ELISA workstations.

ELISA methods typically employ an initial aqueous methanol extraction followed by a simple dilution of the extract in buffer, with aliquots of this solution being pipetted into the wells of a microtitre plate. This is a standard format, transparent, plastic unit (about 12 × 8 × 1·5 cm) with a 8 × 12 array of wells (7 mm diameter × 10 mm deep) moulded into it. In the commercial aflatoxin ELISA kits the plate is supplied with a dried layer of aflatoxin-protein conjugate adsorbed onto the surface of the wells.

In a double-antibody ELISA, sample or standard is added to each well of the plate, followed by a limited amount of anti-aflatoxin antibody (first antibody). The plate is then incubated, usually at ambient temperature, to allow antibody-antigen binding to occur. Toxin in solution, which originated from the sample or standard, competes for the first antibody against aflatoxin anchored to the plate via the protein layer in the well. Thus the more aflatoxin there was in the sample, the smaller the amount of first antibody that will remain bound to the plate. Aflatoxin-antibody complexes in solution are discarded, and the plate is thoroughly washed. A second antibody is then added, which recognises and binds to the anti-aflatoxin antibody now attached to the plate wall. This second antibody additionally has an enzyme, for example alkaline phosphatase or horseradish peroxidase, covalently attached to it. Finally, an enzyme substrate is added to the well. After incubation, the optical density of the coloured product of the enzymic reaction is measured using the plate-reader. A high optical density reading indicates a high level of enzyme present during the incubation and thus a high level of second antibody bound to the wells. In turn this indicates that a high proportion of the first antibody added became bound to the wells and, conversely, that there was only a low concentration of aflatoxin in the sample.

In a single antibody ELISA, the colour-producing enzyme is conjugated directly to the first (anti-aflatoxin) antibody. This has the advantage of eliminating one step from the ELISA, making the assay quicker, but the major disadvantage is that more first antibody is used, and as this is the most expensive component of the ELISA, the overall cost of the ELISA is increased. The enzyme-antibody conjugates used in double antibody ELISAs are cheap and readily available commercially.

Aflatoxin concentrations in samples are usually calculated by reference to a set of standards run on the same plate. Typically, 16–24 wells are used for the standard curve, although one kit replaces this with a single reference material occupying just three wells and supplies a ready-drawn standard curve. For fast screening, each sample extract could be run just once, although application in duplicate or triplicate would be more normal and desirable. Thus one plate has

the capacity for up to 93 samples, although 25–30 would be more usual. Not many laboratories need to analyse this number of samples simultaneously and later versions of ELISA kits use strips of 2 × 8 wells, together with a rack to hold them in the standard format, so that it is practical to assay a smaller number of samples. This inevitably increases the unit cost of an assay as the standard curve must be run every time. Since quantification of aflatoxin concentrations is obtained within a defined range of the on-plate calibration curve, samples falling off the curve must be re-analysed. Sensitivity of existing ELISAs is limited to about 2 μg/kg and separate assays are needed if both B_1 and total aflatoxins are required. In addition the careful validation of the assay required for each foodstuff analysed could be expensive. The ELISA stage may be fully automated (current capital cost about £20,000) but would be difficult to integrate with sample extraction. Manual performance of ELISA is relatively tedious and yet demanding, and thus makes special demands on staff.

Thus ELISA is best suited to large batches of samples, when throughput with the standard 96 well plate format may be up to 10-fold higher than with conventional chemical methods. Extraction is the limiting step. Material costs are about £3–4 per sample. Currently, the coefficient of variation (20–50 per cent.) is similar to that obtained with chemical methods, although it is probable that this will be reduced in the future, as analysts gain more experience with ELISA, and the commercial kits, which are still very new, are further developed.

AFFINITY COLUMNS FOR AFLATOXIN CLEAN-UP

Aflatoxin affinity columns, although immunologically-based, may if required be used simply as a substitute for current chemical methods of clean-up. Apart from the column itself, no specialised apparatus is necessary. The columns consist of an anti-aflatoxin antibody bound to a gel material (about 50–100 mg of gel on a dry weight basis) contained in a small plastic cartridge. The principle of the extraction is that the crude extract is forced through the column and the aflatoxins are left bound to the recognition site of the immunoglobulin. Extraneous material can be washed off the column with water or aqueous buffer, and the aflatoxins are finally obtained in a purified form by denaturing the protein gel with an elution solvent such as methanol. Columns are commercially available, marketed in forms such as the "Easi-extract" column (Microtest Research Ltd, York, U.K.) and the "Aflatest" column (May and Baker Diagnostics Ltd, Glasgow, U.K.). These columns exhibit differences in their performance for recovery of each of the different aflatoxins, reflecting the specificity of the antibody employed.

For the analysis of a peanut butter sample using a typical affinity column the procedure would be as follows: Peanut butter (10 g) is blended with methanol/water, 60 : 40 v/v (20 ml) for 2 min at high speed. After centrifugation, an aliquot of the supernatant is diluted 12-fold with water. The affinity column is pre-washed with 10 ml of water and then the diluted sample is slowly applied using a plastic syringe. The column is washed with a further 10 ml of water, the washings being discarded, and the aflatoxins are eluted from the column with 2 ml of methanol or acetonitrile. With a sufficiently sensitive detector, or with a

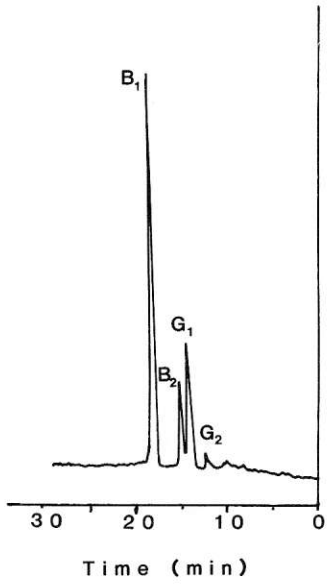

Fig. 2. HPLC chromatogram illustrating an immunoaffinity column clean-up for aflatoxins in a compound animal feedingstuff. Feedingstuff containing manioc and citrus pulp, and contaminated at ca. 15 μg/kg with aflatoxin B_1. HPLC analysis using a 5 μm Spherisorb ODS column eluting with water-acetonitrile-methanol (6:3:1) at a flowrate at 0·7 ml/min. Post column derivatization with iodine-saturated water at 70°C in a 0·3 × 5000 mm stainless steel loop. Fluorescence monitoring with excitation at 355 nm and emission at 433 nm.

highly contaminated sample, water can be added to the eluent and the sample analysed by HPLC directly. Otherwise, the eluent is evaporated to dryness and the residue re-dissolved in a suitable solvent for HPLC or TLC analysis. Few extraneous peaks are observed. This was particularly evident when affinity columns were used for the analysis of aflatoxins in a compound animal feed. Although this material contained manioc and citrus pulp which are frequent sources of interference in aflatoxin analysis using conventional clean-up, the chromatogram interference-free shown in Figure 2 was obtained after affinity column clean-up.

Quantification of all four major aflatoxins simultaneously is achievable and the cartridge format lends itself to partial automation on equipment designed for solid phase extraction. It is also possible to collect the eluted toxin and use a standard UV spectrophotometer to determine its concentration. Alternatively the eluent is passed directly from the column through a Florisil "mini-column" (which may be obtained prepacked from the affinity column manufacturers); the toxin adsorbs in a tight band and is estimated visually by comparison with standards under long-wave UV to give a value for total aflatoxins. The immunoaffinity columns cost about £5 each; no information is available about re-usability.

Affinity column clean-up has been shown in this laboratory to be particularly effective in the analysis of milks, as the liquid sample may be passed directly through the column and essentially pure aflatoxin M_1 finally eluted. Recoveries from naturally contaminated samples were monitored by use of the standard

reference milk powders[26] available from the Community Bureau of Reference (BCR) of the EC. Expressed as a percentage of the certified value, together with the associated coefficient of variation and number of measurements involved, the results were 93·2 ± 5·0 per cent. ($n = 9$) and 76·8 ± 4·2 per cent. ($n = 6$) for reconstituted milks containing 0·076 and 0·031 μg of aflatoxin M_1 per kg respectively. The detection limit depends primarily upon the volume of milk that can be passed through a column; with skimmed milks this can be up to 1000 ml and with this size sample a limit of 50 pg/l is attainable[27]. In contrast, current chemical methods typically achieve 10 ng/l.

OTHER RAPID TESTS

Another test to fall into the category of immunoaffinity methods is the EZ-Screen "Card test", a joint venture by two American companies (Environmental Diagnostics and International Diagnostics). The test gives a rapid visual indication of whether the sample exceeds a specified concentration of aflatoxin. Levels of 5 or 10 μg/kg are specified, depending upon the test conditions. The antibody currently employed measures the sum of B_1, B_2 and G_1. It has only a low cross reactivity for G_2, which however is not often found in samples. The principle of operation is essentially the same as described above for a double antibody ELISA. The card consists of a sandwich of blotting paper between two sheets of plastic, with the overall size and shape of a credit card. There are two holes (ports) in one of the plastic faces, allowing drops of test liquids to soak in, passing through a mesh onto which is bound the anti-aflatoxin antibody. A defined volume of sample extract is placed on one of these ports, and a control solution on the other. Aflatoxin in the original sample is indicated by the absence of a blue colouration formed in the ports after the addition of aflatoxin-conjugated enzyme. The control is used to confirm that the system is working. All samples containing more than the specified concentration of aflatoxins give the same result (the sample extract may be diluted and retested if necessary to give a better idea of actual aflatoxin levels), while samples containing less aflatoxin give a graduated colour response and can be estimated visually to within about 25 per cent.

The system is very convenient to use and a single sample may be assayed in 20 minutes. Material costs are £4–8 per sample depending upon throughput. The test is not readily amenable to automation, but may be performed by untrained personnel. The only equipment required is the blender for extracting the sample and with access to power the test may be carried out in the field. When 520 raw peanut samples were analysed at the buying point by non-technical personnel, only 12 results were in wide disagreement with subsequent HPLC analysis. Only 6 of those could be directly attributed to errors associated with the card. In a further laboratory comparison of the card test on 618 samples of peanuts, 95·8 per cent. were in close agreement with the values determined by HPLC (R. J. Cole, personal communication). In this laboratory the test cards have been found to give good recoveries with a wide range of dried fruit. The cards have a shelf life of at least six months when refrigerated in the unopened pack; a more rapid reduction in sensitivity is experienced as the cards age following opening.

Conclusions

Successful chemical analysis of aflatoxins depends upon constant awareness of the critical nature of many of the techniques involved. Laboratories which cannot maintain these skills because of limited sample throughput will benefit from the availability of immunoassay. Each assay type has its advantages and drawbacks. ELISA demands more care from the analyst than the other methods described here. ELISA and the "card" test are both suited to large scale screening; the choice between them for this application depends on factors including the site of the analysis, the personnel and equipment available and the type of result required. Until either has been adequately validated it will be desirable to confirm positives by other methods.

The "card" test is likely to gain favour because of its simplicity, with affinity column clean-up perhaps used to confirm positives. Experience with a wide range of samples is lacking but there are indications that the affinity column will give acceptable results with both foods and feeds and this is likely to be true for the "card" test too. The affinity column is a welcome addition to the chemical analytical laboratory, considerably speeding up clean-up. It, too, requires validation. Immunoassay will make an increasing impact on aflatoxin analysis. None of the methods has yet been validated adequately but because of the level of interest this is likely to come about in the near future.

All chemical aflatoxin assays depend critically upon the preparation of fully standardised reference solutions and analysts are strongly urged to follow the AOAC procedures. It is unwise to depend upon bought-in samples claimed to contain a specific concentration of aflatoxin. Some of the immunological methods do not require standardisation but determination of recoveries and detection limits will still depend on the availability of accurate standards. It is essential also to take advantage of certified naturally-contaminated standard reference materials (SRMs) where these are available. The BCR currently has available three levels of M_1 in milk powder[26] (0·76, 0·31 and <0·05 μg/kg) and plans to add B_1 SRMs of peanut butter, peanut meal and animal feed incorporating manioc and citrus pulp. Use of these materials adds considerably to overall confidence in the performance of any aflatoxin assay.

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Book Reviews

THIN-LAYER CHROMATOGRAPHIC R_f VALUES OF TOXICOLOGICALLY RELEVANT SUBSTANCES ON STANDARDIZED SYSTEMS. By Deutsche Forschungsgemeinschaft (DFG) and the International Association of Forensic Toxicologists (TIAFT). VCH, 1987. ISBN 0-89573-685-9. 223pp. Price approx.: £24.

The book tabulates hR_f (i.e. $R_f \times 100$) values of 1100 toxicologically-relevant substances from ten Standardised TLC Systems using silica or modified silica as the stationary phase. The substances include drugs, illicit products, pesticides and endogenous compounds. There is an outline of the methods used to choose and standardise the TLC systems, five mathematical parameters being employed to compare the separating power. These bring together the features of distribution of R_f values, their reproducibility and the correlation of chromatographic properties when more than one system is used. Sensitivity, time for analysis and cost do not vary greatly between systems but the measurement of discriminating power and identification power was used for comparison purposes. Separate chapters describe the running of the systems and the measurement of the hR_f values. There is a chapter section listing all substances in alphabetical order with their hR_f values for all ten systems together with their CAS registry numbers, otherwise the sections are divided into the data from individual systems.

The presentation of the book is clear and provides a valuable reference source for the forensic chemist.

G. J. DICKES

THE LEISHMANIASES IN BIOLOGY AND MEDICINE, Vol. I, BIOLOGY AND EPIDEMIOLOGY, pp. 551, £69; Vol. II, CHEMICAL ASPECTS AND CONTROL, pp. 392, £55. Edited by W. PETERS and R. KILLICK-KENDRICK. Academic Press, London, 1987.

Sir William Leishman identified the protozoa responsible for many of these infections, which include blackhead in turkeys, kala-azar, sleeping sickness etc. As well as identifying the causative organisms, he developed his useful bacteriological stain, and it is after him that this wide group of ailments is named.

Leishmaniasis is one of the six major communicable diseases in the UNDP/World Bank/WHO Programme for Research and Training in Tropical Diseases. The Wellcome Trust organised an informal conference on the subject, and these books have resulted from participants putting their work together.

Volume I deals with morphology, physiology and epidemiology of the diseases, and whilst of enormous interest to an expert in Tropical Medicine, has little to recommend it to the practising analyst.

Volume II is of interest to Analysts largely for two chapters dealing with chemotherapy, i.e. Chapters 17 and 18, which describe the use of antimonial, 8-aminoquinoline, allopurinol and various other compounds. However, details of metabolism of these drugs are regrettably lacking, and this does reduce the value of the book.

The two volumes are of value to a library, but as already stated, although they may well be of enormous interest to specialists in Tropical Medicine, could not be advised as anything but very interesting reading to the average chemist unless his laboratory was attached to a Public Health Laboratory. In certain areas overseas where these diseases are endemic, however, they could become required reading.

The books are well written and well printed, with no errors which were readily discernible.

G. V. JAMES

QSAR IN ENVIRONMENTAL TOXICOLOGY II. Edited by KLAUS E. KAISER. D. Reidel Publishing Co., Dordrecht, 1987. 465 pp. Price: £67.95.

QSAR, or quantitative structure-activity relationships, in chemistry, biology, toxicology and environmental sciences has grown strongly with the appearance of new books and new journals in each field. As a result, workshops have been held and the second, held at McMaster University, Ontario in 1986, has resulted in this hardback book, edited by Dr Klaus Kaiser of the Canadian National Water Research Institute.

A total of 31 papers are reproduced, the whole being amply supplied with tables, figures, etc. Each paper opens with an abstract and is completed with pertinent references.

Test organisms dealt with include bacteria, various fish, yeast, rats and birds, whilst factors covered include surface area, weight, and fat content, in relation to a broad spectrum of chemical compounds from metals to pesticides.

The papers are well written and the book is well and clearly printed, being packed with information relevant to studies of the environment, as well as to investigations into atmospheric and aquatic pollution problems, and, of course, to the work of biologists in Water Authority Laboratories.

The book is completed with 4 Indexes, i.e. the usual Author and Subject ones, and also a chemical formula Index and a Chemical Abstracts Service Registry Number Index which could be invaluable.

This is the second book in the series and others are promised in the future. They can be recommended.

G. V. JAMES

ANALYTICAL PROFILES OF DRUG SUBSTANCES, Vol. 16. Edited by K. FLOREY. Academic Press, 1987, Orlando, Fl., U.S.A. 771 pp. Price: US$ 65.

The contributors are drawn from the ranks of pharmacy, hospitals, industry and universities from a wide geographical range, including India, Canada, U.S.A., Holland, Italy, and Saudi Arabia.

The Editor, from the Squibb Institute for Medicine Research, saw a need to supplement official standards and also to collect information scattered through literature, etc., about metabolism and methods of synthesis or breakdown, either physical or chemical.

This volume covers 17 different substances, and each chapter describes the therapeutic effect, physical properties, sources, identification, synthesis, and methods of analysis, with of course, references to original papers. The articles are well illustrated with spectra (IR, UV, Mass, NM, etc.), and methods of assay and impurity limits are described. The articles have illustrations, where appropriate, as well as tables of e.g. R_f values.

The book is completed by a cumulative substance index of the contents of this and the previous 15 volumes. It is very well produced and worth the approximately £40 to any analyst who deals widely with drugs, either for the National Health Service or for forensic purposes.

G. V. JAMES