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A PRELIMINARY STUDY OF ALUMINIUM AND THE TEA BUSH

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All bio-geochemical *) studies should include a detailed account of the uptake and physiological effects of the element concerned on at least one plant species. The aluminium relationships of the garden hydrangea, *H. macrophylla,* were explored in this respect to a limited extent by the writer [3]) twenty years ago but as a corollary to a comprehensive survey of aluminium in the plant world [4]) [6]) a more detailed investigation seemed desirable. Since the tea bush, *Camellia sinensis,* is the aluminium-plant with the greatest economic value it was chosen as the species to study. It was believed that its connexions with aluminium might eventually have some bearing on its cultivation. The work described below was largely undertaken at Rothamsted Experimental Station in 1948–50 as part of a Colonial Development and Welfare Research Scheme and was later followed up in Uganda.

HISTORICAL

Bertrand and Levy [2]) in 1931 appear to have been the first to record a high aluminium content for tea. They examined 76 species of food and garden plants and found that prepared Ceylon tea contained 465 ppm †) aluminium as against a mean of 11 ppm for the rest. The next year Yoshii and Jimbo [38])

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*) Bio-geochemistry may be simply defined as the natural history of individual chemical elements [17]).

†) All analytical data cited in this paper have been recalculated to parts per million (ppm) or milligrams per kilo of oven dried material.

published the results of their survey of the occurrence of aluminium in the plant kingdom. This included old leaves of the tea bush which contained 2370 ppm aluminium. It must be stated here that the tea family (*Theaceae*) had been shown to be aluminium accumulating much earlier in 1922 by Hallier [15]) and in 1927 by von Faber [7]) but they did not test the tea bush. In 1934 the present writer determined aluminium in old leaves from the tea bush growing in one of the economic greenhouses at the Royal Botanic Gardens, Kew. Only 392 ppm aluminium was found. High figures are cited in American literature by Robinson [28]), as much as 9300 ppm aluminium being found by Hou. McMurtrey and Robinson [22]) in a review of neglected soil constituents state that concentrations nearly as high as 20,000 ppm are possible although they give no specific examples. Russian workers have only recently been aware of the ability of the tea bush to take up large amounts of aluminium. The earliest mention of this is by Polynov [26]) in 1944 and although he refers to it later [27]) he does not quote any figures. Quantitative data for aluminium in Russian tea, as far as can be ascertained, have only very recently been published. Parfenova and Troitskii [24]) in 1951 recorded 3220 ppm aluminium for healthy mature tea leaves and 1310 ppm for unhealthy leaves.

CONSTANCY OF ALUMINIUM IN THE TEA BUSH

In order to verify the foregoing and to obtain some idea of the constancy of high aluminium uptake by the tea bush a series of samples were taken from Kew Herbarium specimens which had originally grown in countries not included above. The results *) are tabulated below.

It is evident from Table I and the results of previous workers that considerable variation occurs in the amount of aluminium accumulated by the tea bush but it is a constant feature. This variation may be due to differences in age of leaf, age of tree, genetic constitution, rainfall, altitude or soil. The effect of these factors will now be assessed.

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*) Details of the method of analysis are given in the appendix.

TABLE I

Location	Collector	Aluminium ppm
Kew Herbarium specimens		
India		
Kodaikanal, Pulney Hills, South India	Souliere	8,360
Pashok, Darjeeling, North India	—	*ca* 10,000
China		
Yunnansen	Maire 1094	7,860
Mid-west Yunnan	Forrest 26029	6,820
North Yunnan	Forrest 27384	8,850
No locality	Fortune 165	2,650
Tibet		
South East Tibet	Forrest 18886	11,500

ALUMINIUM CONTENT AND AGE OF LEAF AND TREE

The low figure of the commodity sample analysed by Bertrand and Levy indicates that young leaves do not contain anything like the amount of aluminium present in old leaves. In order to confirm this and at the same time study the other factors concerned with aluminium uptake three extensive series of samples were obtained from Ceylon, Tanganyika and Kenya respectively which included as many different jats and clones as possible. A selection of the results obtained is presented for each series in Tables II, III and IV.

It is abundantly clear from the above data that the tea bush takes up aluminium throughout its life, even when left to grow into a tree and stores it in the leaves. The greater part of the storage takes place during the seven months following maturity (Table II). Young leaves contain only about 100 ppm and this increases with genetic variation to between 5,000 and 16,000 ppm in leaves about to fall. In the flush (*i.e.* the commercial part of the plant) the aluminium content increases progressively from bud to mature leaf — from 50 ppm to 1500 ppm. A normal well plucked flush (bud and two leaves) would contain between 150 and 250 ppm which is perfectly harmless to consumers of the beverage. Aluminium content might well be used as an index of "Goodness of Pluck" for the rise from the second leaf to the third and fourth is steep:

TABLE II

Ceylon: St Coombs, Talawakelle											
Clone	Mean yield lbs/ acre	Blister blight	Age After pruning months	Aluminium, parts per million							
				Flush		Banji				Mature	
						1st leaf		2nd leaf			
9	1033	0–1	7	170	116	44	84	132	229	10500	9750
			25	39		83		213		8650	
			37	138		125		343		10100	
25	1753	3–4	7	lost	125	300	221	840	711	8500	11300
			25	130		133		582		9300	
			37	120		260		spoilt		16000	
26	523	2–3	7	140	118	lost	180	335	403	10600	young leaves ?
			25	107		180		380		1205	
			37	100		180		495		1285	
777	991	1–2	7	74	100	197	182	334	368	5150	9460
			25	93		201		352		14300	
			37	133		149		420		8940	
934	1029	1–2	7	102	209	118	212	272	534	7400	7830
			25	282		285		625		9650	
			37	243		232		705		6430	
1114	1170	1–2	7	292	207	139	166	386	306	5560	7620
			25	121		194		225		7900	
			37	208		187				9400	
1294	676	4	7	43	66	105	101			2780	3480
			25	57		59				1580	
			37	99		140				6100	
1526	—	—	7	46	82	492	526			7550	7370
			25	35		565				7200	
			37	165		89				7350	

Observations:

		Mature	Banji	Bud
Clonal Features:	High Al	Clones 25, 9, 777, 26	25, 934, 1526	934
	Medium Al	,, 931, 1114, 1526		
	Low Al	,, 1294		

Notes: Yields are for the 1st pruning cycle.

Blister blight 0 = Disease absent.
1 = White blisters on leaves.
2 = Many white blisters on leaves, no stem infection.
3 = Many young stems damaged, but not killed throughout length.
4 = Young stems seriously damaged, many completely destroyed.

Rainfall: 95 inches.
Soil: Yellowish brown on Red Loam.
Altitude: 4,500 ft.

TABLE III

Tanganyika						
Variety or jat	Location	Age years	Aluminium ppm		Phosphorus ppm	
			Youngest	Oldest	Youngest	Oldest
Assamica	Amani Village	47	4640	7550 (9)	1120	1200
Unknown	Amani Herbarium	46	7500	8400 (9)	1050	800
Unknown	Amani, Tea Corner	36	2520	4500 (9)	1430	1100
Manipuri ex Nyasaland	Kwamkoro	20	5200	5480 (9)	1370	1420
Bazaloni ex Pangledjar Java	,,	20	4380	6200 (7)	1440	1800
Jatinga–Manipuri (thick)	,,	20	6750	8600 (7)	2430	1280
Manipuri ex Ceylon	,,	20	5160	15400 (6)	1020	1020
Dahootea Assam	,,	20	8130	10800 (7)	945	1290
Dahootea Assam	Amani	17	2570	8350 (7)	985	930
Jatinga Assam	,,	17	9420	17100 (7)	880	960
Jingamira Assam	,,	16	7950	12600 (7)	1500	1570
Manipuri ex Tingamira Est.	,,	16	10800	11700 (7)	2000	1750
Assamica ex Keroyalla Est.	,,	16	2880	10400 (6)	1260	1670
Black Manipuri	Monga, Karimi Est.	4	61	591 (4)	2500	1310
Rajghur	,, ,, ,,	4–5	577	2880 (4)	2680	875
Pure Assamica	,, ,, ,,	4–5	711	1950 (4)	2980	1500
China jat	,, ,, ,,	1	214	8540 (4)	4620	1560
Assamica	Amani Embryo	47	13			
,,	,, Testa	47	69			
,,	,, Carpel	47	293			

Notes: Samples were taken from long mature shoots; figures in brackets are the number of leaves per shoot, all of which were analysed, the gradation in Al and P were usually quite regular from youngest to oldest leaf.

Rainfall: 76 inches. Soil: Red Loam. Altitude: 2989 feet.

TABLE IV

Kenya									
Variety or jat	Site	Alt., feet	Rain, inches	Age, years	Soil		Al ppm	P ppm	Mn ppm
Large mature leaves									
China Hybrid	Kaisugu Est.	7300	68	12	Black on murram		3100	1670	5600
Assam–Manipuri	,, ,,	7300	68	13	,, ,, ,,		2950	1670	4000
Bokel India	,, ,,	7300	68	22	,, ,, ,,		5120	1810	1800
Assam Indigenous	Kapkorech Est.	6800	65	12	Red loam		1900	1100	5500
Bokel India	,, ,,	6800	65	20	,, ,,		10200	2300	8800
Ex Uganda	Kapkimolwa Est.	6200	57	4			350	1390	170
Manipuri	Kericho Est.	6950	71	50	Red loam		3310	1710	5900
Manipuri, Tingamiri	Buret T. Co.	6200	65	25	Red on murram		2060	1800	3000
Small half-mature leaves									
China Hybrid	Ngambuya	5500	40	7	Red loam		3200	1460	n.d.
,, ,,	Kaganjo	5500	40	14	,, ,,		3320	1480	1270
,, ,,	Kanyenyeni	6600	60	2	Volcanic tuff		2050	1950	n.d.
,, ,,	Karuri	7100	65	18	,, ,,	young leaf	2240	2140	n.d.
,, ,,	Igonta, Fort Hall	6200	50	14	Red loam	young leaf	1930	2580	n.d.
,, ,,	Maguru School	5700	45	9	,, ,,		1100	2000	n.d.
,, ,,	Gatara	6300	52	10	,, ,,		625	2690	n.d.
Young leaves									
China Hybrid	Kagunduini	6500	48	20	Red loam	bud 1st leaf 2nd leaf	455	2760	1340
,, ,,	,,	6500	48	20	,, ,,	3rd leaf	1040	2440	2300
,, ,,	Karuri Plot	7100	65	18	Volcanic tuff	thin banji leaf	312	2670	1360
,, ,,	Manunga	7200	65	2	,, ,,	tip	712	3540	620
,, ,,	,,	7200	65	2	,, ,,	3rd leaf	1250	3470	n.d.
,, ,,	Gathagara	6300	50	25	,, ,,	bud 1st leaf 2nd leaf	712	4170	715
,, ,,	,,	6300	50	25	,, ,,	3rd leaf	1250	3050	770
,, ,,	,,	6300	50	20	,, ,,	bud 1st leaf 2nd leaf	680	3160	1180
,, ,,	,,	6300	50	20	,, ,,	3rd leaf	895	1640	1850
Assam Indigenous	Limuru	7340	52	19	Chocolate loam	bud 1st leaf	115	2220	0
,, ,,	,,	7340	52	19	,, ,,	2nd leaf	440	2150	0
,, ,,	,,	7340	52	19	,, ,,	bud 1st leaf	295	3070	0
,, ,,	,,	7340	52	19	,, ,,	2nd leaf	580	2160	0
						bud 1st leaf	250	4050	0
						2nd leaf	520	—	

n.d. = not determined.

250 ppm to 900–1500 ppm. Contamination with dust is inevitable but it can easily be removed by vigorous and repeated shaking with 50% alcohol or acetone acidified to about 1% with nitric acid; rubbing whole leaves with cotton wool soaked in this solution followed by vigorous shaking completely removes both dust and soil splash.

ALUMINIUM AND GENETIC CONSTITUTION

The results for Ceylon and Tanganyika show that certain clones and strains or jats are more vigorous accumulators of aluminium than others. For example clones 25 and 777 from Ceylon and the Jatinga Assam jat from Tanganyika approach the maximum figure of 20,000 ppm quoted by McMurtrey and Robinson. The Indian Tea Research Institute at Tocklai, Assam recognises three distinct sub-species of tea: — *Camellia sinensis* var. *assamica,* the large leafed Indian tea; var. *sinensis,* the small leafed China tea and var. *cambodiensis,* the thick leafed, drooping tree not used in commerce. It was considered that samples from these extreme varieties grown on the same kind of soil might show extreme differences in aluminium content. Material was obtained from

TABLE V

Assam, India
Tocklai Experimental Station, Cinnamara

Variety	Stem	Young leaves	Banji leaves	Mature leaves	Dropping leaves
			Aluminium ppm		
cambodiensis	130	309	396	4500	19500
sinensis	188	155	166	4000	10000
assamica	112	331	512	2820	4450
			Phosphorus ppm		
cambodiensis	445	3340	2700	1240	1610
sinensis	425	3180	3190	1090	565
assamica	438	3930	3010	1800	1730
			Manganese ppm		
cambodiensis	190	760	800	2000	1650
sinensis	410	480	770	3800	2100
assamica	140	560	700	1500	1200

Altitude: 284 feet.
Rainfall: 81 inches.
Soil: Yellowish brown alluvial loamy sand.

Tocklai and analysed for aluminium. The results are submitted in Table V. The genecontrolled nature of aluminium uptake is again strikingly confirmed.

ALUMINIUM AND BLISTER BLIGHT

Another character of the tea bush which is controlled by genes is its susceptibility to the disease "blister blight". It was suggested by Eden [10]) and Lamb [19]) that aluminium might be connected with this disease, but in the first set of Ceylon samples (Table II) there was no correlation. A later set of samples was secured from the Tea Research Institute of Ceylon to examine aluminium content and extremes of resistance and susceptibility to blister blight. The results obtained showed clearly that the differences in aluminium content are not related to blister blight in any direct manner. The results are tabulated below: —

TABLE VI

Ceylon, St. Coombs, Talawakelle	
Blister blight	Aluminium ppm *)
Highly resistant	2100
Highly susceptible	5500
Susceptible but non-fermenter	1230

*) Mean of three determinations each.

ALUMINIUM AND TEA YELLOWS

It is appropriate here to consider aluminium accumulation of the tea bush and the sulphur deficiency symptoms known as "tea yellows" [34]). Since excess of aluminium in many other genera produces a permanent yellowish green colour in the dry leaves [5]) it was thought that some, at least, of the yellowness in tea yellows might be linked with aluminium. A set of samples was accordingly obtained from the Mlange Tea Experimental Station, Nyasaland and analysed in the usual manner. The most noteworthy feature of the results is a very definite increase of aluminium in the affected flushes — a mean of 640 ppm as against 380 ppm in the healthy flushes. The manganese contents of the old leaves of the healthy plant are the highest ever recorded for any healthy species. The high phosphorus figures for the slightly and moderately affected leaves are probably due to fertilizer treatments.

TABLE VII

Nyasaland
Mlange Tea Experimental Station

Yellows status	Age of leaf	Al ppm	Mn ppm	P ppm
Healthy	Flush, first leaf	260	1100	2810
	Flush, second leaf	500	630	2870
	Mature	7900	9150	1500
	At point of dropping . . .	10600	9700	1450
Slightly affected	Flush, both leaves	390	1400	2900
	Mature	4900	3000	4100
	Older	12500	3700	6400
Moderately affected . .	Flush, first leaf	360	1100	2550
	Flush, second leaf	2800	1700	2740
	Mature	7300	3570	7300
	Older	5400	3000	3300
Severely affected	Flush, both leaves	1300	400	3800
	Flush, both leaves	1550	1100	2070
	Mature	1670	1600	1900
	At point of dropping . . .	7300	3500	1100

Altitude: 2125 feet.
Rainfall: 62 inches.
Soil: Red Loam. pH 5.2–6.0.

DISTRIBUTION OF ALUMINIUM IN THE TEA BUSH

Before discussing the relationships between aluminium uptake and climate or soil it is relevant first to study some of the more simple physiological phenomena of distribution within the bush and interactions with other elements. The data in Table V indicate that aluminium is likely to be stored largely in the leaves just as it is in the hydrangea and other plants [2]) [3]). In order to confirm this, tea seedlings were grown to about 8 inches tall at Kew and then fed with strong solutions of aluminium and manganous sulphates, (2,000 ppm Al and Mn, 8 doses; 10,000 ppm Al and Mn, 2 doses). After four months the plants were pulled up and sectioned for analysis. Manganese and phosphate were determined in each portion of the plant at the same time as the aluminium. Unfortunately only one replication of four treatments was possible owing to shortage of seedlings. Whatever differences that did occur were so striking that it was considered worthwhile to analyse completely and separately one plant from each treatment. Apart from the distribution data some interesting points were revealed concerning aluminium in relation to manganese and phosphate. Detailed results are given in Table VIII and a summary of the whole experiment in Table IX.

A glance at Table VIII at once reveals that the leaves are the organs where aluminium is stored, very little occurs in the stems or woody roots. The secondary roots contain more aluminium but some of this is soil contamination which could not be washed off. The same distribution pattern occurred in all four plants and also in those examined by Parfenova and Troitskii[24]). Tea fruits from Tanganyika contained only traces of aluminium (reported in Table III).

ALUMINIUM AND ITS RELATIONSHIPS WITH PHOSPHORUS AND MANGANESE

The phosphorus determinations made on the Tanganyika, Kenya and Kew pot samples all indicate that the distribution and mobility of phosphorus in the tea bush is quite normal despite the presence of a large excess of aluminium and manganese, although both elements might conceivably immobilise phosphorus as insoluble phosphates. Growing points such as shoot and root tips contain the largest amounts of phosphorus and this diminishes with age of leaf by translocation through the stem. A certain amount of storage takes place in the stem. The fact that both aluminium and manganese contents of the youngest leaves, stems and woody roots are extremely low probably accounts for the non-interference of these elements with phosphate movement.

With regard to manganese it is interesting to note that the plant treated with manganous sulphate was the tallest and had the largest and thinnest leaves which were slightly chlorotic at the end of the experiment. Unlike the others this plant never entered the dormant "banji" state during the short duration of the experiment but grew continuously.

Certain trends are suggested by the data summarized in Table IX. The aluminium treated plant which was the healthiest and greenest of the set, had the highest weight of tops and underground parts, and the manganous sulphate treated plant although it showed signs of chlorosis was better in both respects than the controls. The

TABLE

Kew pot								
	Control				Aluminium treated			
	Al ppm	Mn ppm	P ppm	Wt g	Al ppm	Mn ppm	P ppm	Wt g
Leaf 1	345	575	1970	.196	920	840	1400	.293
,, 1 + 2								
,, 2 + 3	478	850	1770	.625	1500	1300	1140	.750
,, 3								
,, 3 + 4								
,, 4								
,, 4 + 5	564	810	2260	.493	2960	2610	1760	.710
,, 5								
,, 5 + 6								
,, 6 + 7	596	1170	2290	.475	1630	1650	895	.846
,, 8								
,, 8 + 9	940	1840	2130	.488	1780	1860	1120	.843
,, 9								
,, 10	1620	2010	2030	.026	2100	2160	880	.335
,, 10 + 11								
,, 11					2440	1300	1030	.077
,, 11 + 12	1590	3000	1300	.257				
,, 12 + 13					2650	3000	750	.531
,, 13	2320	2570	1080	.12				
,, 14 + 15	2910	2100	1360	.017	3890	4650	510	.211
,, 16					4660	2280	545	.100
,, 16 + 17								
,, 17 + 18					7500	2750	712	.010
,, 18 + 19								
,, 20								
Flower buds					230	180	2290	.044
Green stems	61	100	2830	.025	296	18	2760	.041
Brown stems	26	85	2120	.867	198	15	2180	1.325
Woody stems	72	108	1600	.814	285	110	855	.884
Stem bases	115	83	1270	.248	208	107	1010	.342
Stems below	76	<16	1470	.143	306	68	1610	.181
Root stocks	174	28	1550	.659	96	41	1550	2.081
Primary roots	280	55	2050	.326	477	18	2430	.248
Secondary roots	615	110	3850	.604	2650	47	4180	.998
1st side shoot leaves	675	700	2110	.448	1660	1400	910	.194
1st side stems	86	120	1820	.903				
2nd side shoot leaves	835	2350	2570	.439				
2nd side stems	81	110	1310	.090				

Woody stem = thick stem to 2 cm above soil surface. Stem base = thick stem from 2 cm above

*) Leaves 1 + 2 of second stem.

†) Leaves of second stem.

VIII

experiment

Manganese treated				Aluminium + Manganese treated				
Al ppm	Mn ppm	P ppm	Wt g	Al ppm	Mn ppm	P ppm	Wt g	
190	6150	4050	.033	2360	25000	5740	.250 *)	Leaf 1
				2200	25000	5860	.198	,, 1 + 2
98	13900	3210	.031					,, 2 + 3
				3330	31000	6600	.134 †)	,, 3
				3340	32000	5370	.249	,, 3 + 4
				2520	14500	5290	.036 †)	,, 4
510	17300	2690	.252					,, 4 + 5
				2740	15700	4920	.058	,, 5
				2420	11700	2100	.502 †)	,, 5 + 6
490	23600	2180	.297	2250	11000	2100	.617	,, 6 + 7
630	21800	1400	.058					,, 8
				3500	10500	2100	.312	,, 8 + 9
790	23400	1070	.248					,, 9
				4270	10200	1920	.155	,, 10
740	20700	1630	.684					,, 10 + 11
				8000	7900	1960	.028	,, 11
								,, 11 + 12
920	19300	1060	.741					,, 12 + 13
								,, 13
780	13800	815	.455					,, 14 + 15
								,, 16
1250	14200	800	.496					,, 16 + 17
								,, 17 + 18
1830	12500	762	.301					,, 18 + 19
2310	8400	630	.081					,, 20
								Flower buds
54	3100	960	.270	230	5100	5910	.228	Green stems
37	965	1650	1.180	566	3600	2250	.347	Brown stems
74	1930	825	.802	267	2100	2200	.403	Woody stems
55	1400	662	.178	408	2180	2540	.097	Stem bases
52	1550	650	.110	615	1800	3550	.076	Stems below
90	710	435	.840	340	2620	4700	.195	Root stocks
102	810	582	.467	1180	910	6010	.042	Primary roots
436	1820	2980	1.170	1310	1640	>6300	.462	Secondary roots
1150	15600	882	.067	3160	23000	1850	.088	1st side shoot leaves 1st side stems
								2nd side shoot leaves
								2nd side stems

to soil surface. Stem below = thick stem from soil surface to 1 cm below.

TABLE IX

Summary of results of Kew pot experiment

Treatments: C = Control, A = Aluminium sulphate, M = Manganous sulphate, A+M = Aluminium and Manganous sulphates

		Aluminium ppm				Manganese ppm				Phosphorus ppm			
		C	A	M	A+M	C	A	M	A+M	C	A	M	A+M
Maxima	Leaves.	2910	7500	2310	8000	3000	4650	23400	32000	2290	1400	4050	5800
Minima	Leaves.	345	920	190	2200	575	840	6150	7900	1080	510	630	1920
Weighted means	Leaves.	896	2100	956	2920	1440	2030	18200	17500	2100	1080	1280	3460
	Stems	64	235	50	385	100	60	1490	3360	1780	1650	1160	3060
	Roots	360	891	385	1030	64	41	3360	1800	3130	3241	3060	5840
Totals	Leaves.	3116	10321	4219	7405	5018	9937	80084	44491	7306	5311	5644	8789
	Stems	146	653	133	443	227	166	3937	3609	4077	4559	3060	3525
	Roots	573	2968	625	721	102	137	2808	1260	4980	8010	4124	4078
Totals per Plant:													
C	Control	3835				5347				16363			
A:	Aluminium sulphate	13942				10240				17880			
M:	Manganous sulphate	4977				86829				12828			
A+M:	Manganous and Aluminium sulphates	8569				49360				16392			

Soils

Treatment	C	A	M	A+M
pH	5.2	4.5	4.6	4.3
Avail. Al ppm . .	n.d.	120	30	84
Avail. Mn ppm . .	n.d.	tr.	970	868
Avail. P ppm . .	17	28	41	34

Additions to soil

C:	nil
A:	284,000 ppm Aluminium
M:	674,000 ppm Manganese
A+M:	224,000 ppm Aluminium + 424,000 ppm Manganese

Dry weight of plant material

	C	A	M	A+M
Tops	5.76	7.67	7.05	3.69
Underground.	1.73	3.41	2.59	.78
Totals	7.49	11.08	9.64	4.47
Wt of soil, g . .	242	225	162	232

combined treatment obviously retarded growth and its high phosphate content was the result of uptake stimulated by the manganese. That toxic manganese is associated with the high phosphate uptake is shown in both stems of the A + M plant. The incipient chlorosis of the manganese treated plant was almost certainly due to an upset of the balance with iron. Aluminium did not prevent toxic symptoms and retarded growth in the A + M plant although the leaf lesions might not have formed if the plants had been kept shaded and the manganese applied in smaller doses over a longer period. Unlike manganese, aluminium is not translocated rapidly to the growing points but is accumulated gradually as the leaves age and lose their phosphate. Since the amount of available manganese in the soil of A + M plant was less than for the M plant and yet the manganese uptake was higher on a percentage basis in the former it is possible that the aluminium may have stimulated absorption of manganese.

The very strongly developed root stock of the aluminium treated plant may prove to be the most significant effect of this element as a good thick tap root or root-stock is most important as a reservoir of starch, without which the tea bush will not flourish and produce a crop.

EFFECT OF RAINFALL AND ELEVATION

Very wide ranges of rainfall figures and altitude are covered by the samples listed in Tables I–VII. It is apparent that aluminium uptake is not affected by either factor. Wherever the tea bush flourishes it will absorb aluminium from the soil and accumulate it in old leaves.

ALUMINIUM AND TEA SOILS

Most planters know that the tea bush will not thrive in limey soils and that the soil reaction has to be below about pH 6.0. All soils contain some calcium in an exchangeable form and soil of pH 6.0 would contain as much as ten milli-equivalents per cent if it were a clay and still tea would grow quite well. Calcium contents of the mature tea leaf are quite considerable (up to 7700 ppm, see also [17]) [18])). It would appear that it is not the excess calcium which is the direct cause of tea failing in carbonate-free soils above pH

6.0 but the lack of something else. Aluminium does not occur in the soil solution in measurable amounts between pH 5.0 and pH 6.5 [25]) but owing to the tenacity with which tea roots hold soil particles it is conceivable that the root mucilage and soil colloids form one system in which cation exchange can take place. Exchangeable aluminium might well be the form which is available to the tea bush especially when the water-soluble form is absent or present in extremely small amounts. The first extractant to be tried for exchangeable aluminium was normal sodium chloride solution. This at once revealed abundant aluminium in all soils on which tea grew successfully and very little or none above pH 6.0. Since a considerable increase in soil acidity takes place with a neutral salt like sodium chloride to pH values well below that of the natural soil, the procedure of Heslep [16]) was tried, using normal calcium acetate buffered to approximately the same pH as the soil. The first set of results confirmed the sodium chloride data but owing to the difficulty in preparing a calcium acetate solution free from aluminium it was decided to use ammonium acetate buffered in the same way *). The original set of soils was extracted with these solutions and the results, although they are somewhat lower, all indicate that above pH 6.0 available aluminium is practically non-existent. These results are represented graphically in Figure 1.

Hut sites, old cattle kraals, termite mounds and ash accumulations are notoriously bad [9]) for tea cultivation on account of their high soil pH values (pH 6.4–8.2). No exchangeable aluminium is found in these soils.

In order to ascertain whether lack of aluminium was responsible for poor growth and yield on patches of soil that had long previously been hut sites, a series of carefully taken leaf samples were supplied by the Tea Research Institute of East Africa, Kericho. Ten flushes (2 leaves and a bud) were taken from each of five good and five adjacent unthrifty bushes. Composite surface soil samples were also examined. The analytical data for these samples are submitted in Table X. Despite the fact that the bushes were of the same height, growing on soil with a reaction well below the limit of pH 6.0, and differed only in lack of flushing ability of the "Hut"

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*) Details of the method are given in the appendix.

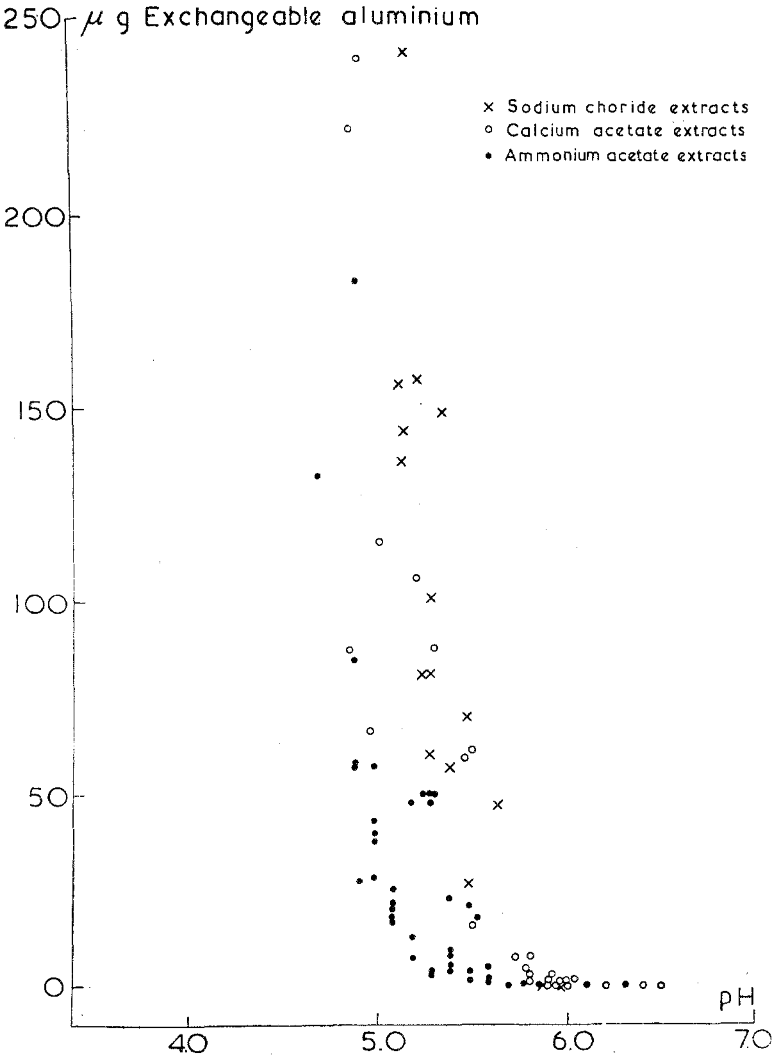

Fig. 1. The relationship of tea soil pH to exchangeable aluminium.

site bushes, there is a significant difference in the aluminium content of both the flushes and the soil, but not of the old leaves. The old leaves were all the fifth from the growing point but there may have been differences in age between them, which caused such wide variation within each set. Total aluminium in the leaves of the two sets of bushes is probably not very different as the flushes would weigh very little. The low content of available soil aluminium may still be the cause of the poor tea on the hut site. If aluminium acts as a root stimulant as is suggested by the Kew pot experiment then a 14-fold difference in available aluminium might be the factor responsible. A large-scale pot experiment is now in progress at Kawanda Research Station in order to test this hypothesis.

TABLE X

Aluminium content of good and poor tea bushes				
	Flushes *)		Old leaves	
	Good	Poor	Good	Poor
	400	217	3440	3040
	484	251	4000	3160
	419	285	3260	3880
	436	450	3890	3050
	350	256	5330	2350
Mean	418	292	3984	3096
Difference between means	126		888	
S.E. of differences	47		437	
L.S.D. for significance at				
$P = 0.05$, ...	107		1008	
$P = 0.01$, ...	156			

*) Ten flushes were taken off each bush but only one was used for analysis (selected at random). This was justified as no significant difference could be found between a single analysis and the mean of ten from the same bush.

Soil

	Good site	Hut site
Exchangeable aluminium (ppm) by NaCl	59	3.0
,, ,, ,, by $(CH_3COO)_2Ca$ . . .	27	0.0
,, ,, ,, by CH_3COONH_4 . . .	21	1.5

The old leaf data of Table X are not in accord with those of Parfenova and Troitskii 24) who ascribe the differences of two patches of Georgian tea to the beneficial effects of aluminium in the better patch. From their soil profile descriptions it would appear that the prime reason for this difference would more feasibly be the waterlogging of the bad patch. Even on acid soil with a reaction well below pH 6.0, as in this instance, waterlogging is disasterous for tea bushes. The plants growing in this bad patch lacked the vigour and root development required for an uplift of sufficient water to convey, in the transpiration stream, much aluminium to the leaves.

ALUMINIUM AN ESSENTIAL ELEMENT FOR TEA

The invariable presence of large amounts of aluminium in the tea bush and of exchangeable aluminium in tea soils poses the question of essentiality. Aluminium has been demonstrated as being beneficial to water-plants by Stoklasa[33]), peas and millet by Sommer[32]), maize by Lipman[21]), solfatara plants by von Faber[14]), volcanic lava plants by Yoshii[37]) and ferns by Tauböck[35]). Only one aluminium-plant has been investigated in this connexion, viz. *Symplocos japonica* by Neger[23]). Aluminium was definitely proved to be essential but he did not prove that the accumulation of large amounts was essential or even beneficial. An interesting function of aluminium, for citrus at least, has been found by Liebig, Vanselow and Chapman[20]) in its preventing copper toxicity symptoms by a process of antagonism in the fine roots. Copper is not excluded nor prevented from migrating to the leaves but its toxicity is entirely nullified. The same effect may occur in tea but the present writer[7]) considered that manganese toxicity effects in the field might be precluded in a similar manner despite the results of the Kew pot experiment – Stocklasa had apparently the same opinion but did not try to demonstrate it. Manganese in excess of about 1,000 ppm of leaf dry weight induces toxic symptoms in most plants but the tea bush, in the field, can take it up to about 10,000 ppm and still remain healthy. The Kew pot plants appeared quite healthy until a large dose of manganous sulphate on a very hot day induced scorch or spotting in the youngest leaves of the plant treated with both manganese and aluminium; these leaves had accumulated 32,000 ppm manganese. Tubbs[36]) in a water culture experiment induced uptake to 15,400 ppm but his plants were defoliating within a week.

It is quite possible that aluminium confers no real benefit on the tea bush for Storey and Leach[34]) grew tea very successfully in water culture solutions under conditions in which very little, if any, aluminium uptake would occur.

The above suggests that a formal statistically controlled experiment is most desirable but time and facilities have not yet been available to the writer in Uganda. However, one simple preliminary experiment has been conducted and results were sufficiently instructive to warrant recording here. This was an alkaline soil trial in the greenhouse at Kawanda Research Station.

ALKALINE SOIL EXPERIMENT

Nine large pots were filled with soil from a tea nursery in which the surviving plants were very stunted (only 6–12″ tall) at 18 months. This soil had a pH of 8.0 and this is probably the limit of soil alkalinity which the tea plant will tolerate. Four plants from this nursery were put into each pot and after they had been established about two months the leaves of two plants in each of three pots were brushed respectively with 1% solution of aluminium sulphate, ferrous sulphate and a 1 : 1 mixture of both. The treatment took place every day for two weeks, then sporadically over the next four weeks. The effect of the aluminium treatment was apparent in the change to patchy dark green of completely chlorotic young leaves. Injections with 1% aluminium and ferrous sulphates of chlorotic leaves on similar plants in the same soil produced green patches at the point of injection by aluminium sulphate within about four days. In only one instance among about 50 injections did the iron solution produce a green spot whereas the aluminium injections were about 70% effective.

After two years, only 2 aluminium, 1 iron and 4 aluminium and iron treated plants were still living, together with 7 controls, 2 of which were on the point of dying.

This experiment showed that aluminium does have some physiological effect on tea leaves but that it cannot, when applied in leaves make up for adverse soil conditions.

BUFFER INDEX AND ALUMINIUM UPTAKE

The failure of seedlings and eventually of mature bushes in alkaline soils and soils with a reaction above pH 6.0 indicates that a severe upset in the whole metabolism of the plant has taken place. This is certainly related to the buffer capacity or buffer index of the roots both in relation to iron chlorosis and aluminium uptake. In order to verify this, buffer index curves were prepared according to Small's method [30] [31] from titration – pH figures of 10 : 1 macerated water extracts of roots, stems and leaves from healthy

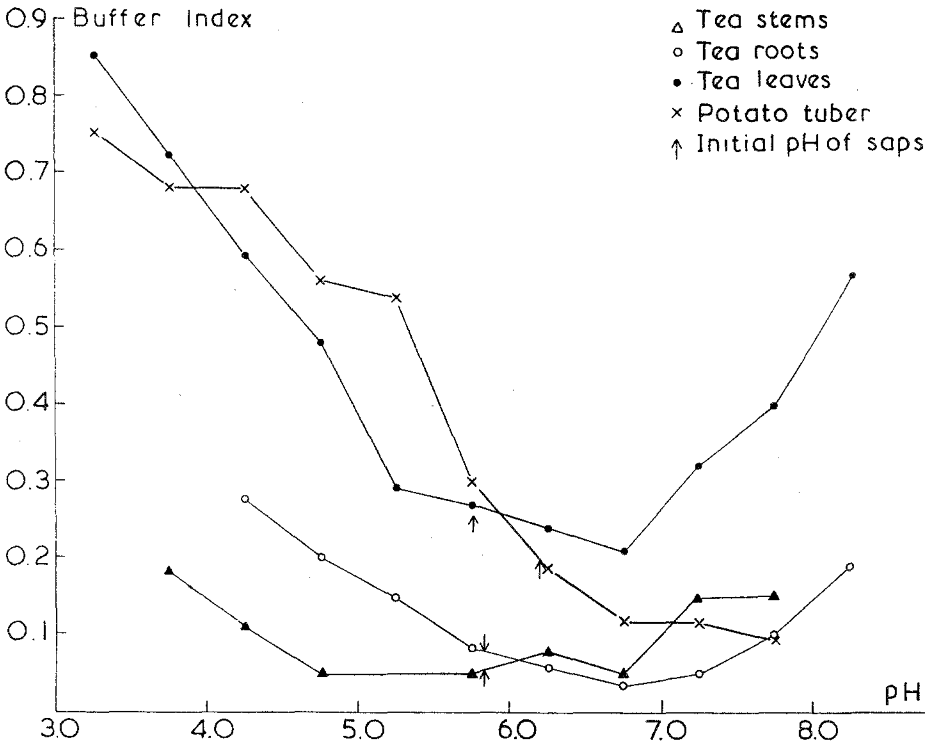

Fig. 2. Buffer index curves of tea bush sap compared with potato sap.

tea seedlings. Small used expressed sap for his determinations but as a press was not available a micromacerator was used and the results calculated on the basis of the sap present before dilution. In Figure 2 is a comparison curve for a potato tuber which is strongly buffered and this emphasises that the buffer capacities of the tea roots and stems are very low but it is somewhat higher in the leaves. This is manifest in the reaction of roots of tea plants grown in the alkaline pot experiment. These are pH 6.90 against pH 5.85 for healthy roots. Iron chlorosis usually occurs when the pH of root and stem tissue rises above pH 6.05. Aluminium intake is also severely restricted; the aluminium content of the leaves of 2 four-year old stunted plants in the alkaline pot experiment was only 42 ppm for a control and 89 ppm for a leaf that had been brushed with aluminium sulphate, and in a very chlorotic one-year old seedling growing in the same soil only 4.2 ppm.

In Ceylon the failure of tea on alkaline soils has long been associated with "bitten – off" disease. This condition is characterised by complete absence of tap root and few discoloured brittle lateral roots between the seed and the "bitten off" stump about one inch below. Internodes are short and leaves small and pale green. Since no pathogens could be found Eden and Gadd [11]) ascribed the disease to soil alkalinity. Whether it is due to some upset in the buffer system of the roots, lack of iron or aluminium is not known but a waterculture experiment is now in progress at Kawanda to study this question.

THE TEA BUSH IN RELATION TO OTHER ALUMINIUM-PLANTS

Now that tea has been shown to be such a constant accumulator of aluminium, even if it be luxury consumption, it should be interesting to examine the position of the tea plant in this respect with the rest of the plant world. The tribe *Gordonieae* to which the tea bush belongs and the tribe *Ternstroemieae* are almost exclusively aluminium accumulators and this feature is not shown in other members of the *Theaceae*. Airy–Shaw [1]) on morphological grounds has advocated the separating of these tribes into distinct families or else include them with another family which is placed in present taxonomic systems about as far as possible from the *Theaceae*. This family is the *Symplocaceae* one of the strongest of all aluminium-plant families, with aluminium contents rising to 70,000 ppm. This relationship has been cited as one of the best examples of reticulate affinity [12]).

Evidence has been adduced [7]) to show that aluminium-plants are primitive; some are indeed, among the most primitive of all plants in their respective classes, *e.g. Andraeaceae* of the mosses and *Marattiaceae* among the ferns. Fossil records of the aluminium-accumulating fern families *Gleicheniaceae, Matoniaceae* and *Dipteraceae* show that these tropical ferns once extended far over Asia, even to Greenland. Today they are restricted to the wettest tropics and the last two families to a small corner of S.E. Asia. Reduction in area covered by plants is related to degree of senescence – such plants if not living fossils are relict forms. Among the flowering aluminium-plants there are several which are definitely on the decline; one, at least, (*Shortia galacifolia*) was thought extinct in the wild state for a considerable time. In this connexion it is interesting to speculate on the status of the tea plant because truly wild specimens have rarely been collected.

According to Sealy [29]) no authentic wild bushes of the broad-leaved Indian variety *assamica* have yet been found despite diligent searching by Kingdon Ward [19]). But the narrow-leaved Chinese variety *sinensis* was discovered by Henry in virgin forests of South Yunnan and by Forrest in West Yunnan. Virgin forests described by collectors have often subsequently proved to be secondary; it would be instructive to have a modern ecologist's opinion on these Yunnan forests. It is not beyond the bounds of possibility that the tea bush, like the Ginkgo, owes its survival to cultivation and that its existence would have been precarious if its stimulating properties had not been discovered.

SUMMARY

The phenomenon of uptake of aluminium by the tea bush has been examined in relation to its constancy as a characteristic feature, age of leaf and tree, genetic constitution, resistance to certain diseases, distribution within the plant, interactions with manganese and phosphorus, soil, essentiality and finally in relation to other aluminium-plants.

Strong aluminium absorption appears to be a constant feature for all healthy bushes of any age, the element is stored in the oldest leaves but it does not impart any resistance to "blister blight" but it occurs to a greater extent than normal in flushes with "tea yellows"; it is gene-controlled, there being three distinct levels of accumulation corresponding with the three major divisions of the species. The presence of abundant available aluminium in the soil will not prevent excessive uptake of manganese accompanied by severe leaf scorch and spotting in bright light. Aluminium tends to diminish leaf phosphorus while manganese tends to increase it. Large amounts of available soil manganese may induce greater uptake of aluminium and *vice versa*. Small quantities of aluminium within tea leaves are associated with degree of greenness, but the large accumulations probably do not serve any useful purpose. Exchangeable soil aluminium may stimulate roots, particularly tap-roots or root-stocks. The tea bush may be a relict plant like so many of other aluminium accumulators.

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APPENDIX

METHODS OF ANALYSIS

Exchangeable aluminium using sodium chloride or calcium acetate

Preparation of samples. About 1 g soil (air dry, 2 mm sieved) is accurately weighed out (to .01 g) into a folded filter-paper, the paper with soil is placed in a funnel inserted in a 100 ml measuring flask; distilled water is added and the soil leached until the 100-ml mark is reached. The leachate contains water-soluble Al which is not detectible above pH 4.3.

The funnel (and its contents) is then inserted into another 100 ml measuring flask and the soil leached with 100 ml 1 Molar NaCl. The leachate contains practically all the exchangeable Al for soils above pH 5.0, a second leaching with 100 ml NaCl may be necessary for more acid clayey soils.

The leachates should be perfectly clear and colourless.

Normal calcium acetate extracts are prepared in the same way.

Reagents*). Aluminon reagent — Ammonium aurine tricarboxylate, 0.75 g; gum acacia, 15 g; ammonium acetate, 200 g; concentrated hydrochloric acid (A.R.), 189 ml; dissolved separately, mixed, filtered and made up to 1500 ml

Thioglycollic acid — One ml diluted to 100 ml.

Aluminium sulphate standard — Stock solution containing 250 ppm of aluminium and containing 4 ml of concentrated nitric acid per litre. Ten ml of this diluted to 250 ml gave the 10-μg aluminium standard, aliquots of which were used in preparing the curves. The stock solution must be analysed gravimetrically for aluminium, especially in the tropics, where loss of water of crystallisation may lead to errors.

Development of red aluminium lakes

2 ml are pipetted into 25 ml numbered measuring flasks, 1 ml (1 : 50 v/v) HNO_3 added to each, then 1 ml (1% thioglycollic acid) and then 5 ml Aluminon reagent. The contents of the flasks are mixed by swirling and made up to the mark. A batch of 15 are prepared at a time and the flasks placed in a perforated zinc basket (perforations 1 cm) 25 cm diam. taking care to wedge them up with test-tubes or lead piping to prevent spilling. The zinc basket is then dropped carefully into a sauce-pan of vigorously boiling water (heated with 2 bunsens) and heated for 15 minutes (the water usually takes about 2 minutes to come to the boil again and should be kept gently boiling for the next 13 minutes). On taking out of the water-bath the flasks are removed from the basket and stood in a shady place to cool down to room temperature. The cooling should last at least 6 hours. So long as all determinations are made at approximately the same temperature, (± 2°C) the actual temperature is not important. The coloured solutions are compared in a photo-electric colorimeter at a *fixed* time between 6 and 30 hours after taking out of the boiling water bath. Rapid cooling under the tap is also feasible but the solutions should be stood for at least an hour at more or less constant temperature afterwards.

Curves are constructed from the standard Al solutions plus 2 ml of the extractant with a range of 0 to 55 mg Al per 25 ml in exactly the above manner, and the concentration of the unknowns read off directly.

Exchangeable aluminium with ammonium acetate as extractant

10 grams of soil are weighed into 250-ml conical flasks and 50 ml of ammonium acetate added to each. The ammonium acetate has the same pH (± .05) as the soil. The contents of the flasks are swirled, allowed to stand overnight, swirled and filtered into a dry flask next morning. Two ml of the leachate are used as above to determine aluminium without previ-

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*) E. M. Chenery, Analyst **73**, 501 (1948).

ously making up the final volume up to 50 ml as equilibrium is completed in the original conical flasks. Quantities of aluminium up to 1 ppm on the soil basis may be determined directly in this way. For soils with less than .5 ppm exchangeable aluminium, 25 ml of the ammonium acetate leachates are pipetted into silica dishes or crucibles and evaporated to dryness overnight. The dishes are then placed in the electric muffle and maintained at 450–500°C for about 6 hours. Ten drops of conc. HNO_3 are carefully added to the residues and then 1 drop of 100 volume H_2O_2. The acid is carefully driven off on a low temperature hot plate and heated until none can be detected by smell. 1 ml of .5% thioglycollic acid (or $\frac{1}{2}$ ml of 1%) are added to each dish which are then warmed for a few minutes, 2 ml of N ammonium acetate at pH 7.0 are added followed by 2 ml of aluminon reagent. The whole is well stirred and transferred to a test-tube graduated at 10 ml and made up to the mark. The red aluminium colour is developed in the usual way. If the soil contains between .5 and 2.0 ppm the above procedure is used but all quantities are multiplied $2\frac{1}{2}$ times and the colour developed in 25 ml graduated flasks.

Standard curves are constructed using 2 ml of *N* ammonium acetate at pH 7.0 in each standard.

Total aluminium in plants

The cleanest possible material is selected and gently rubbed with a swab of cotton wool soaked in 50% alcohol, acidified to about 1% with HNO_3. It is then shaken vigorously 3 times with the warm acidified alcohol in a test-tube or covered beaker. After being placed on a clean filter-paper with platinum-tipped forceps it is securely folded in the paper and dried for 3–6 hours at 105°C if the material was originally air-dry; if fresh 24 hours drying is necessary.

The leaves or roots may then be crushed in the filter-paper to very fine particles without coming into contact with the hands or they may be ground in an agate mortar (reserved for plants only). A mechanical grinder is undesirable as the risk of contamination is too great.

100 to 200 mg of the clean, oven-dry material from non-aluminium accumulating species or 3 to 15 mg of accumulator-plant leaves, are weighed into tared platinum crucibles (1 or 5 ml). A batch of 6 is a convenient number to handle at one time. The crucibles are covered with lids (9/10 coverage) and placed over bunsens adjusted to the lowest possible flame or in an electric furnace, at 400 to 500°C. When all the volatile matter has been driven off the flames are raised very slightly but under no circumstances are they allowed to come into contact with the crucibles nor are these allowed to show the slightest sign of reddening. A small sample takes 3 to 10 minutes and a larger one up to 1 hour to become completely ashed. If an electric furnace is used the final temperature should be set at 500°C.

To the cooled ashes are added 1 drop conc. HNO_3 and 1 drop 5 vol. % H_2O_2 taking care that the crucibles are held in a slant-wise position to avoid spattering. They are then similarly placed in a sand-bath at about

200°C and evaporated to dryness. If much manganese is present the ashes are pale brown to almost black in colour and visible detection of small unburnt carbon particles is impossible. A second gentle heating for 2–3 minutes followed by the above treatment usually suffices to complete the ashing. The final acid solution must be absolutely clear and colourless; rarely are more than 2 evaporations necessary to ensure this. Care should be taken to see that any ash adhering to the sides of the crucibles is attacked by the acid and peroxide.

When the final traces of HNO_3 have been driven off (no detectible smell), the crucibles are cooled slightly and 1 ml 1 : 50 HNO_3 added to each, together with 1–2 ml water. They are heated on the sand-bath for a further 10 minutes and their contents then transferred to the 25 ml measuring flasks. If silica particles are present, they must be filtered off during this transference. Silica always occurs in graminaceous species, beech trees and a few other broad leaved trees but very rarely in aluminium-accumulators.

1 ml 1% thioglycollic acid and 5 ml Aluminon reagent are added to each flask and the red aluminium lake developed as before.

It is often desirable to determine Fe, Mn and P on the same ash extract, in such cases the minimum quantity of leaf material is raised to 50 mg and aliquots taken from the ash solutions previously made up to 25 ml.

An accuracy of better than ± 1% error can be obtained by these methods and a general working error of ± 2% of the actual Al present.

Note. If the platinum crucibles are allowed to show signs of reddening the aluminium is rendered insoluble and can only be estimated by a sodium carbonate fusion.