PHYTOTOXIC EFFECTS OF ALUMINIUM ON *PARASERIANTHES LOPHANTHA* AND *ACACIA DECURRENS*

M. P. Hughes[1] and J. E. D. Fox[1]
[1]School of Environmental Biology, Curtin University of Technology, GPO Box U1987, Perth WA 6001

SUMMARY

Old abandoned dumps in the Collie coal field have been colonised by both native and introduced species and these indicate a potential for rehabilitation. The coal waste dumps are more acidic than the surrounding forest soils. Soil pH of less than 5.0 indicates a potentially high toxic presence of aluminium. Tolerance to aluminium is a feature of some plant species, and these may accumulate, avoid or exclude aluminium. Excluders are reported to include legumes, such as *Acacia* and *Paraserianthes*, by restricting translocation of the metal which is bound in root cells. Excluders may have reduced plant nitrogen levels generally but nitrogen is also higher in roots than shoots. Evolution of tolerance to metal stress may be comparatively rapid and although aluminium has many adverse affects on growth, plants tolerant of its presence also tend to be drought tolerant, an advantage for survival on old dumps which may be very dry in summer.

Seedlings of *Paraserianthes lophantha* and *Acacia decurrens* from dump and non-dump seed sources were subjected to varied levels of aluminium solution in pot culture. Hydrated aluminium chloride was dissolved in de-ionized water to provide concentrations of 200, 500, 700, 900, 1000 and 1100 ppm. Aluminium solutions were applied three times a week and a nutrient solution once a week. Surviving plants were harvested at ten weeks.

A. decurrens appeared better able to tolerate low levels of applied aluminium than *P. lophantha*, but at 500 ppm both species exhibited severe effects on growth. *P. lophantha* had a number of deaths in aluminium treatments. Plants of dump origin were not able to produce greater plant biomass over the trial period than non-dump plants. However there were distinct effects of treatment in relation to aluminium distribution within plants. Dump origin plants contained less total aluminium than non-dump plants indicating a possible avoidance mechanism. Dump progeny held more aluminium within the roots, transporting lower quantities to the foliage, suggesting an exclusion mechanism. Non-dump plants were more susceptible to wilting and it is hypothesised that dump progeny may have a denser structure than plants from non-dump sites. With increased aluminium applied plant nutrient contents decreased : calcium decline was the most severe. Trace element and manganese concentrations increased with added aluminium, and differences were noted in foliar levels between plants of dump and non-dump origin.

It is possible that the degree of apparent adaptation to aluminium levels may be related to the history of prior exposure. Whereas the *P. lophantha* seed came from plants established three years earlier by direct seeding onto a restored dump, the seed of *A. decurrens* was taken from self - sown trees established many years earlier on an un-restored waste dump. The trial conditions should be viewed as extreme cases of exposure. It is recommended that collection of seed for use on coal-mine rehabilitation sites should be made from plants growing on dumps already colonised successfully.

INTRODUCTION

The Collie coal field, located in the south west of Western Australia, has been exploited for nearly a century, but large scale operations commenced only forty years ago. Rehabilitation was not undertaken on the waste dumps, which were located in sparsely populated areas, until relatively recently. Unrestored dumps became severely eroded and were compacted in places. Some ingress of both native and introduced species is evident on old dumps and these provide a guide to species suitability for rehabilitation. The majority of the materials that make up coal waste dumps were originally below the water table and had not been previously exposed to surface conditions before excavation. When exposed to the atmosphere, such materials undergo rapid oxidation. Because of a high presence of pyrites (FeS_2), sulphuric acid is generated and coal waste dumps have low pH relative to the surrounding surface soils. Of eighty two samples taken at the Western Collieries No. 5 mine, pH ranged from 2.2 to 5.6 with a median value of 4.1 (Bartle & Ritches, 1978). In addition dumps are typically of low moisture and fertility and heavy metals may be present.

Rehabilitation operations can be costly due to correction of pH, ripping the surface and generally preparing the dump spoil for planting. Costs of rehabilitation can be reduced if certain procedures are eliminated. Revegetating with plants able to grow in the conditions provided by the dump is such a technique (Fox *et al.*, 1988). If the mechanisms by which the major constituents of the coal spoil dump prevent healthy growth of plants are understood, steps can be taken to reduce these effects instead of treating the symptoms. A wide range of materials, including clay sub-soil, crushed sand-stone, ripped rock and fine quarry wastes has been shown to be satisfactory for the growth of plants (Temple & Bungey, 1979). Natural succession on abandoned mine dump sites accounts for less than 10 percent of the area and significant cover occurs only where the pH is greater than 5.0 (Bartle & Ritches, 1978). Legumes possess advantages for establishment on these sites (Koch & Bell, 1985). A screening technique for acidity tolerance indicated that dump progeny of *Acacia extensa* are more tolerant of acidic conditions than non-dump progeny (Fox *et al.*, 1988). Soil pH of less than 5.0 is associated with a high toxic presence of aluminium (Brady, 1991).

Aluminium affects the length of the primary root and, at toxic levels, alters root system architecture. Lateral branching is increased whereas root hair density and length of the root hair zone are decreased. A more compact and dense root system results (Barcelo & Poschenreider, 1990; Paganelli *et al.*, 1987; Thornton *et al.*, 1987; Wong & Bradshaw, 1982). Aluminium phytotoxicity has not been studied extensively. Tolerance to this metal would require an extremely adaptable plant species or a complex system of inheritance. Whereas Aniol & Gustafson (1990) suggest that in grasses a single recessive gene may be responsible for the inheritance of aluminium tolerance, Barcelo & Poschenreider (1990) argue that because the effects of aluminium are wide ranging, the inheritance of tolerance to aluminium stress would be more likely to be controlled by more than one recessive gene as it is rare for a useful trait to be controlled by a single recessive gene. Evolution of tolerance to metal stress can be very rapid. Although aluminium has many adverse affects, plants tolerant of its presence are also drought tolerant but do not produce as many seed or fruit (Smith & Bradshaw, 1979).

Rehabilitation is essential for abandoned coal mine sites or spoil dumps as the accumulation of unusable waste land is not acceptable in the current political and social climate (Bartle & Ritches, 1978). Use of plants tolerant to conditions on coal mine waste dumps for revegetation would save costs associated with changing the waste dump environment. If plant species are found that can tolerate the harsh conditions, there would be less need for liming, fertilisation and time and energy could be saved. The work reported here examined the effects of aluminium on two legume species, *Paraserianthes lophantha* and *Acacia decurrens*, with respect to growth, nutrient uptake and concentration in plant tissue. *P. lophantha* is a West Australian native, tall shrub or tree growing to 10 m in winter-wet depressions near creeks or swamps (Marchant *et al.*, 1987). *A. decurrens* is an eastern states native tree reaching 10 m with a 5 m crown (Blomberry, 1971), and now commonly seen in south-west Western Australia as semi-naturalised. Both species can tolerate drought and frost at maturity and can adapt to most soil conditions. Both species grow well on coal-mine dumps at Collie. These traits of hardiness render *P. lophantha* and *A. decurrens* prime candidates for mine dump rehabilitation.

METHODS

Seeds were obtained of *Paraserianthes lophantha* and *Acacia decurrens* from two locations for each, representing a dump source (Marron Pool, Muja and Stockton, Collie) and a non-dump source (Curtin University campus and Westralia Block, Collie). 300 seeds were randomly chosen from each of the dump and non-dump sources and weighed. Seeds were scarified and sown into separate trays. Tray bases were lined with moistened paper towels then covered with a layer of moist, coarse, sterile sand. Seeds were distributed over the moist sand then covered with a thin layer of dry, coarse, sterile sand.

Seedlings were held in the trays until the first leaf opened and then transferred to 150 mm tall, cylindrical pots containing coarse, sterile sand as a growth medium. Seedlings from each of the four groups, *i.e. A. decurrens* dump, *A. decurrens* non-dump, *P. lophantha* dump and *P. lophantha* non-dump, were then divided into seven treatments consisting of one control and six aluminium treatments. The aluminium treatments were applied to the plants by dissolving hydrated aluminium chloride into 20 litre quantities of de-ionized water. Concentrations of 200, 500, 700, 900, 1000 and 1100 ppm were used with fifteen replicates per treatment. Aluminium solutions were applied three times a week, using 50 - 100 ml per application per plant in hot months and 30 - 50 ml during cold months. Hoagland's nutrient solution No. 2 (Jones, 1983) was added once a week to all treatments on a day that aluminium was not supplied. The control group received only the nutrient solution. This nutrient level was used to ensure that plants did not suffer from lack of any essential nutrients, and that any effects on growth could be attributed solely to aluminium treatments. The pot trials were run for ten weeks.

At harvest each plant was divided into roots, shoots and leaves and dried at 105°C in an oven for 24 hours. After drying, the plants were weighed by parts. This enabled root shoot ratios to be calculated and compared between treatments and groups. The experiment was designed to examine the effects of aluminium treatment level on dry matter production for each of the four accessions separately. Analysis of variance was used for each accession and the Duncan test was used to distinguish significant differences between treatment means.

After weighing, plant parts, including seed samples, were ground into fine powder using a rotary mill with a 40 micron mesh. Available phosphorus was extracted from the samples using a Na_2CO_3 solution. The concentration of phosphate was measured colorimetrically (Colwell, 1965). 1M KCL was used to extract ammonium nitrogen and concentration was measured colorimetrically using the indol-phenol blue reaction (Cawse, 1967). Al was determined by dissolving samples in concentrated HNO_3 and oxidising with H_2O_2. Filtrates were directly used for Al determination without further processing. Al concentrations in the prepared samples were measured by AAS (Zhang & Taylor, 1990). Potassium and trace metal concentrations were determined using flame AAS.

Insufficient plant material allowed incomplete nutrient determinations as follows:

Dump plants	*roots*	no analysis at 1000 & 1100 ppm Al, no Al at 200 & 700 ppm;
	shoots	no analysis at 900 - 1100 ppm Al, also no Al at 700;
	leaves	only N at 1000 ppm Al, also no Al at 900 - 1100;
Non - dump	*roots*	no Al at 900 - 1100 ppm Al;
	shoots	no analysis at 900 & 1100 ppm Al, also no Al at 700 & 1000;
	leaves	no analysis at 1100 ppm Al, also no Al at 900 & 1000.

RESULTS

Acacia decurrens

There were no deaths over the trial period. Non-dump plants appeared to show signs of wilting sooner than dump plants between watering events. Wilting on the latter was only observed just prior to watering. Plants of dump origin did not grow as well as those of forest origin in the absence of added aluminium. Dump plants in the control treatment yielded 81% of the dry weight of non-dump plants but were only 2% shorter in height. After 10 weeks *A. decurrens* plants from both dump and non-dump origins differed in mean heights and dry weights (Table 1). Control plants were significantly taller and heavier than all Al treatments, except for dump origin plants at the lowest aluminium level. 200 ppm did not significantly reduce growth in plants of dump origin, but there was a 10% weight reduction. At 500 ppm dump plants attained only 49% of the height and 42% of the weight of the control. Non-dump sets between 200 and 700 ppm were significantly shorter and lighter than the control, and taller and heavier than plants subjected to 900 - 1100 ppm.

Non-dump plants were heavier than dump plants over the range 500 - 900 ppm. The lowest level of aluminium depressed height and weight to two thirds of the control in non-dump plants. Dump plants were consistently taller than non-dump plants at 900 ppm and more; non-dump plants were not exceeded in total weight by dump plants until 1000 ppm.

The contribution to dry weight by leaves was consistently more than that of roots and shoots in both sets (Figure 1). Foliage was in the range of 55 - 64% of total dry weight. Each component generally declined with increased Al in a similar pattern to that of total dry weight. Roots of dump plants were heavier than non-dump plants at 900 ppm and greater, but leaves and shoots did not become heavier in the dump set until 1000 ppm. The % of dry weight in roots declined in non-dump plants from 26% in control to 15% at 900 - 1000 ppm Al. Dump plant roots increased from 21% of total dry weight to 27% at 500 and 900 ppm and then declined to 20%.

TABLE 1. Mean heights and plant dry weights after 10 weeks growth of *Acacia decurrens* by treatment.

Aluminium (ppm)	Height (mm)		Dry weight (g)	
	Dump	Non - dump	Dump	Non - dump
0	360 a	383 a	6.33 a	7.57 a
200	361 a	260 b	5.68 a	4.97 b
500	175 b	220 b	2.69 b	4.59 b
700	160 bc	217 b	2.30 bc	4.09 bc
900	120 bc	73 c	1.57 c	2.06 cd
1000	93 c	75 c	2.06 bc	1.86 cd
1100	94 c	67 c	1.89 c	1.44 d

Values in columns with the same letter are not significantly different at $p<0.05$ using the Duncan test.

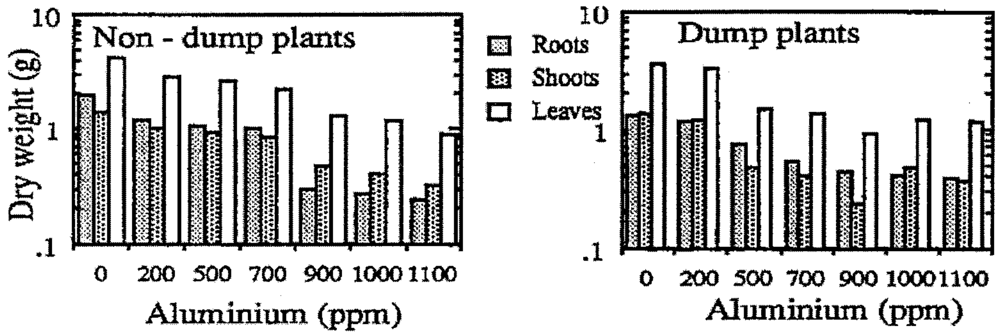

FIGURE 1. *Acacia decurrens* harvest dry weights by plant parts.

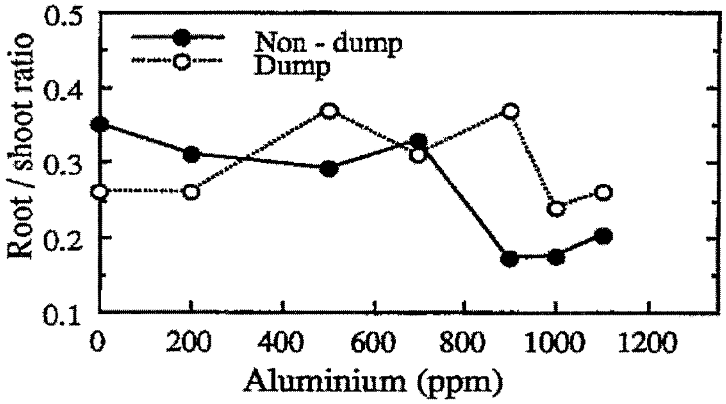

FIGURE 2. *Acacia decurrens* root/shoot ratios.

In the non-dump set the decline in weight as a proportion of control was steady and consistent with increasing aluminium. Root growth declined most rapidly and was only 15% of control at 900 ppm, when leaves and shoots were still more than 30%. At 1100 ppm non-dump root weight had declined to 12% of the control and leaves and roots were 21% and 23% respectively.

Plants of dump origin did not differ as markedly as dump plants between control and the first aluminium level. Dump plant dry weights fell markedly between 200 and 500 ppm with shoot and leaf contributions to dry weight at 35 and 41% of control weights respectively. Roots declined less as a proportion of control weight such that they were 55% of control at 500 ppm. At 1100 ppm the roots were 29% of the control, more than twice the proportion in non-dump plants. Dump plant root dry weights declined consistently and were at higher proportions of control than non-dump plants over the range 900 - 1100 ppm. Dump plant shoots and leaves did not differ over the range of 700 - 1100 from that attained at 500 ppm. At 900 ppm both shoots and leaves in the dump set were of lower weight than at the two more severe levels of aluminium.

Root - shoot ratios for dump plants varied inconsistently with the amount of Al applied (Figure 2), whereas the ratio for non-dump *A. decurrens* generally decreased with increasing concentrations of Al.

In the absence of aluminium both dump and non-dump *A. decurrens* had similar elemental composition in each of roots, shoots and leaves for nitrogen, phosphorus and calcium. Calcium and nitrogen were highest in leaves and lowest in shoots (Table 2). Phosphorus was highest in roots and least in shoots. Root contents of manganese, iron and copper were similar for dump and non-dump plants. However, dump plant roots had higher concentrations of zinc and aluminium than non-dump plants.

Dump plants had lower shoot and leaf concentrations than non-dump plants for all metallic elements, except aluminium. Higher quantities of manganese, iron, zinc and copper entered tissues of non-dump plants and formed a higher percentage of plant dry weight than in dump plants (Table 3). Dump plants retained more in roots and had less in the leaves for all elements than non-dump plants. The difference was most dramatic in respect of zinc where non-dump plants held only 6% of all zinc in the roots.

TABLE 2. Elemental concentration in *Acacia decurrens* not subjected to aluminium treatments.

Source	Tissue	Element							
		%			ppm				
		N	P	Ca	Mn	Fe	Zn	Cu	Al
Dump									
	Root	2.21	0.60	0.45	205	1065	57	66	2071
	Shoot	1.37	0.34	0.28	19	91	13	9	165
	Leaf	2.93	0.41	0.56	26	52	6	6	267
Non - dump									
	Root	2.20	0.63	0.41	219	1126	6	63	1000
	Shoot	1.42	0.30	0.32	126	424	95	51	101
	Leaf	2.80	0.45	0.57	54	100	14	20	279

TABLE 3. Allocation of elemental uptake in *Acacia decurrens* not subjected to aluminium treatments.

Element / Source	Uptake (mg)	% dry weight	% distribution		
			Root	Shoot	Leaf
Zinc					
Dump	0.117	0.0018	65	15	20
Non - dump	0.207	0.0027	6	65	29
Aluminium					
Dump	3.949	0.0624	69	6	25
Non - dump	3.236	0.0427	60	4	36
Manganese					
Dump	0.395	0.0062	69	7	24
Non - dump	0.829	0.0109	51	22	27
Iron					
Dump	1.725	0.0272	82	7	11
Non - dump	3.201	0.0423	68	19	13
Copper					
Dump	0.122	0.0019	71	10	19
Non - dump	0.277	0.0037	44	26	30

Dump plants held more aluminium in total, with more in roots and less in the leaves than the non-dump plants. This indicates that less aluminium was translocated within the dump plants.

Elemental uptake in experimental plants may be compared with the "normal" ranges for plants. Allen (1974) gives the following:

	%		ppm
N	1 - 3	Al	100 - 10,000
P	0.05 - 0.3	Mn	50 - 1,000
Ca	0.3 - 2.5	Fe	40 - 500
		Zn	15 - 100
		Cu	2 - 25

None of the values of Table 3 lay outside these limits except for copper in the non-dump set. Iron and copper levels, in both accessions, were higher in roots (Table 2) than the normal limits.

Aluminium treatments were associated with a reduction in *Acacia decurrens* nitrogen concentration (Figures 3a & 4a). Nitrogen levels were < 1% in shoots of dump plants treated at 200 - 500 ppm aluminium. All other levels for dump plants were > 1%, and roots and leaves were never < 1% N. In contrast non-dump plants had very low leaf nitrogen contents at all levels of aluminium. Roots were not deficient but shoots were so at the two highest aluminium levels. Collectively, nitrogen levels rose slightly over the range of aluminium applied to dump plants but the pattern was more obscure with non-dump plants.

Phosphorus and calcium levels declined with increased aluminium. P - levels were not below the critical level for any tissues in either accession. The greatest change in P content was from zero to 200 ppm aluminium (Figures 3b & 4b). Non-dump plants had a two stage decline and after 500 ppm aluminium P concentrations stabilised at around 0.2%. Foliar P in dump plants progressively declined over the range of added aluminium. In dump plants calcium levels were below the critical limit at all alu-

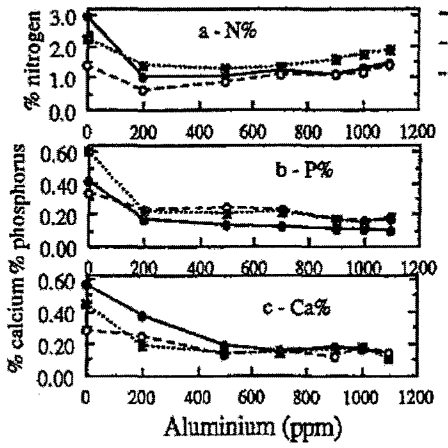

FIGURE 3. Nutrient % in dump plants of *Acacia decurrens.*

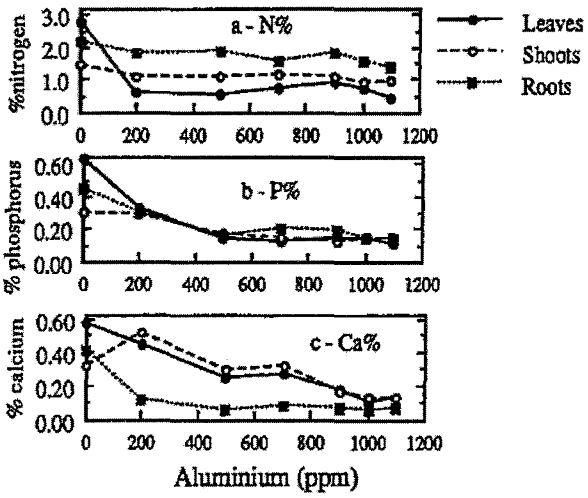

FIGURE 4. Nutrient % in non-dump plants of *Acacia decurrens.*

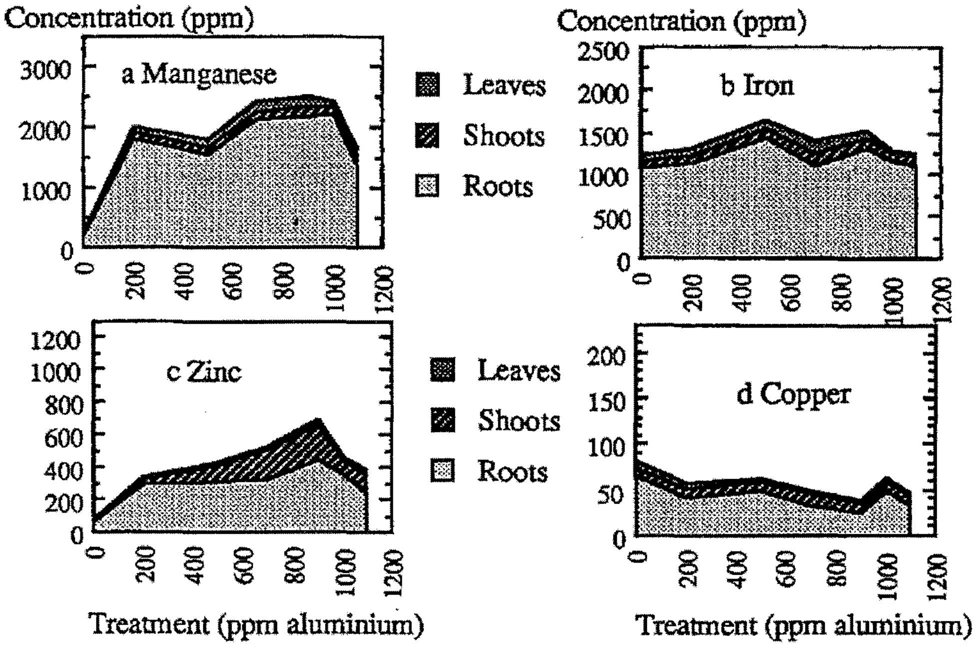

FIGURE 5. Trace elements in tissues of *Acacia decurrens* (dump origin).

minium levels for all tissues (Figure 3c). Levels stabilised after 500 ppm aluminium. Calcium levels were low at all levels in the non-dump plant roots (Figure 4c). Leaf and shoot calcium levels were adequate until after the 700 ppm aluminium level.

Most of the trace elements taken up by dump plants remained in the roots (Figure 5). The levels of copper and iron in roots were higher than normal (Allen, 1974), reflecting the high levels of nutrients supplied, although these levels were not affected by aluminium treatment. For each of roots, shoots and leaves, concentrations of copper and iron were similar to the zero aluminium levels, or less (in the case of copper in roots). Aluminium treatment was associated with increased levels of manganese and zinc in all plant parts. Manganese levels in roots were well over the normal limit of 1000 ppm, but in shoots and leaves, where levels also increased with aluminium, compared to the control, concentrations were between 100 and 160 ppm. Zinc levels were high in all roots (>250 ppm) and shoots (> 100 ppm) at aluminium concentrations of 500 ppm or more.

Non-dump plants of *A. decurrens* (Figure 6) took up greater quantities of trace elements with the addition of aluminium, but retained smaller proportions in the roots, than did dump plants. Whereas root levels of manganese, iron and copper were similar between accessions each of these and zinc had much higher shoot and leaf concentrations in non-dump compared with dump plants. For both manganese and copper non-dump plants had similar root and shoot concentrations. Higher than normal levels (Allen, 1974) of manganese, iron and copper were present in non-dump roots and shoots. Very little zinc was retained in roots of non-dump plants (Figure 6c) with highest concentration in shoots (above normal levels), and leaf levels also generally higher than in roots. Shoot zinc peaked at 1300 ppm in the 700 ppm aluminium treatment along with aluminium (Figure 7). Insufficient plant material was available to analyse aluminium content of dump plant roots at 700 and 1100 ppm Al and shoots at 900 - 1100 ppm Al. Similarly no values are available for non-dump plant roots at 1100 ppm and shoots at 900 - 1100 ppm.

Roots of both dump and non-dump plants had increased levels of Al (Figure 7) with increased aluminium. Root aluminium contents in dump plants tended to stabilise after the 500 ppm treatment at around 6000 ppm aluminium. Dump plant foliar aluminium increased with

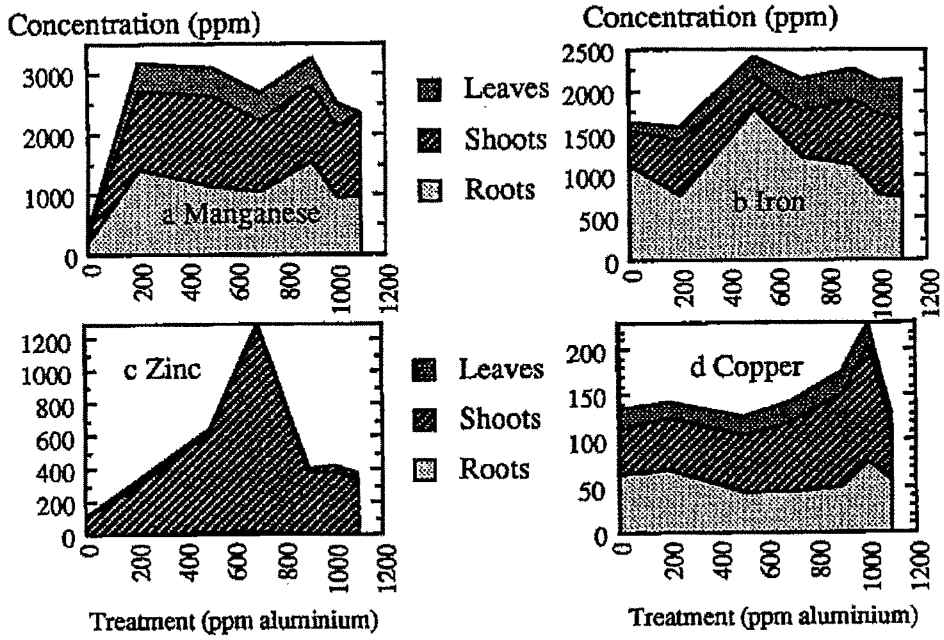

FIGURE 6. Trace elements in tissues of *Acacia decurrens* (non-dump origin).

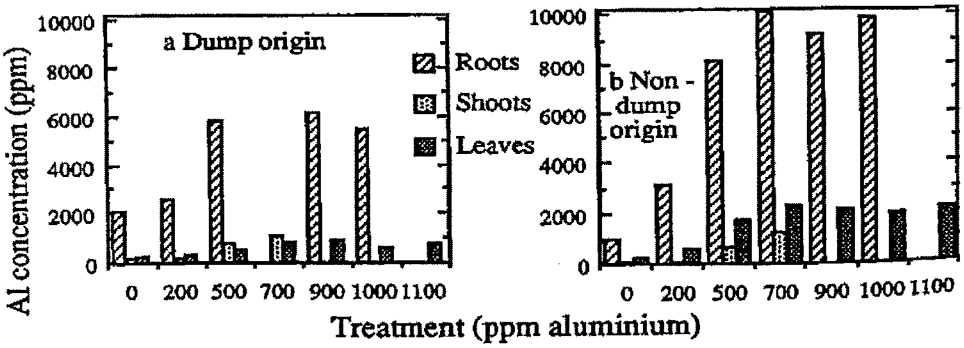

FIGURE 7. Aluminium concentration in tissues of *Acacia decurrens*.

treatment level from 300 ppm at the lowest Al treatment to > 900 at 900 ppm Al, and then fell away to 6 - 700 at higher treatment levels. Both roots and leaves of non-dump plants had much higher concentrations than dump plants, with root levels between 8000 and 10000 ppm after the 500 ppm treatment. Non-dump plant foliar aluminium rose from 600 ppm at the lowest Al treatment to around 2000 ppm for all other treatment levels.

Absolute levels of aluminium in tissues (mg Al) were higher in non-dump plants (Table 4).

With no supply dump plants accumulated more Al in total (4.0 mg versus 3.2 mg). With aluminium supplied both sets took up increased amounts with increased treatment levels, at least to the 700 pm treatment, but non-dump plants absorbed considerably more than dump plants, reaching in excess of 10 mg at the 700 ppm treatment (Table 4). The non-dump plants consistently translocated a higher proportion to foliage, in excess of 30%. Non-dump plants had higher contained total aluminium (mg) in foliage. Dump plants consistently retained a higher proportion of contained aluminium within roots, but for all treatments dump roots had lower amounts of total aluminium than non-dump plants.

Paraserianthes lophantha

In contrast with *Acacia decurrens* where there were no losses, a number of *Paraserianthes lophantha* seedlings died in higher aluminium treatments during the trial. By 10 weeks non-dump plants subjected to the three higher treatment levels had lost three plants each. Dump plant progeny at 700 and 900 ppm had one death each during the fifth week and the 1000 and 1100 ppm treatments both had six deaths in total.

Plants of dump and non-dump origin reached similar mean heights in the absence of aluminium. The dump batch had 16% heavier yield at harvest than plants of non-dump origin (Table 5). Control plants were significantly taller and heavier than all Al treatments : even the lowest Al level significantly reduced both height and weight in both accessions.

P. lophantha plants of dump origin were consistently lighter in weight (but taller) than non-dump plants over the range of applied aluminium level from 200 - 700 ppm. Yields at 200 ppm aluminium were 58% and 70% of the controls for dump and non-dump origin plants respectively. Dry weight yields fell away with more aluminium more quickly in plants of dump origin. At 500 ppm dump plants attained only 30% of the control weight, whereas non-dump plants reached 50%. Plant heights fell away more rapidly in the non-dump treatment plants. Differences associated with treatment levels were significant between treatments at up to 500 ppm aluminium. Between 900 and 1100 ppm aluminium, dry weights had fallen to <20% of the control in both batches of seedlings. Differences between height and yields were not significant at these levels.

Shoot weights as a proportion of control declined more rapidly in *P. lophantha* than in *A. decurrens*. The most dramatic change in resource allocation was a decline in non-dump shoot weight from 24% of total weight in control to 6% at 1100 ppm aluminium. In contrast dump plants had 28% of mass in control shoots and 23% at 1100 ppm. Of the three components measured, leaves generally contributed most to dry weight (Figure 8). However the non-dump set had heavier roots than leaves between 900 and 1100 ppm aluminium. This coincided with a major decline in both shoot and leaf weight.

TABLE 4. Absolute levels of aluminium in tissues of *Acacia decurrens* (* = no data available) and percentage distribution of aluminium between the plant parts for those treatments with sufficient material for analysis.

Treatment ppm Al	Uptake	Dump Root	Dump Shoot	Dump Leaf	Non - dump Root	Non - dump Shoot	Non - dump Leaf
0	mg Al	2.75	0.22	0.98	1.94	0.14	1.15
	% Al	69	6	25	60	4	36
200	mg Al	3.05	0.19	1.04	3.63	0.12	1.72
	% Al	71	5	24	66	2	32
500	mg Al	4.24	0.34	0.70	8.52	0.59	4.53
	% Al	80	6.5	13.5	63	4	33
700	mg Al	*	0.43	2.00	10.26	1.03	5.09
	% Al	-	-	-	63	6	31
900	mg Al	2.64	*	1.43	2.76	*	2.77
1000	mg Al	2.21	*	1.26	2.71	*	2.34
1100	mg Al	*	*	1.37	*	*	1.86

TABLE 5. Mean heights and plant dry weights after 10 weeks growth of *Paraserianthes lophantha*.

Aluminium ppm	Height (mm)		Dry weight (g)	
	Dump	Non - dump	Dump	Non - dump
0	383 a	390 a	6.81 a	5.85 a
200	294 b	217 b	3.94 b	4.12 b
500	183 c	130 c	2.03 c	2.94 c
700	122 d	106 c	1.19 cd	2.47 cd
900	78 e	60 d	1.10 d	0.71 d
1000	60 e	57 d	0.64 de	0.72 d
1100	60 e	53 d	0.56 e	0.48 d

Values in columns with the same letter are not significantly different at p<0.05 using the Duncan test.

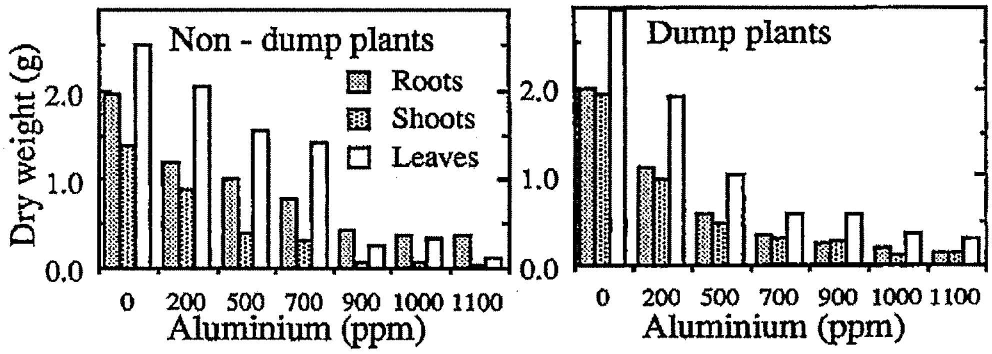

FIGURE 8. ***Paraserianthes lophantha*** **harvest dry weights by plant parts.**

The proportion of dry weight in leaves increased consistently in dump plants from 42% in control to 54% at the highest Al level. Non-dump progeny foliage increased to 56% of biomass at 700 ppm and then fell away to 21% of mass at 1100 ppm aluminium. The proportion of dry weight in roots of non-dump plants increased with increasing aluminium to a high of 73% at 1100 ppm whereas the dump root proportion was similar for all treatments at between 23% and 29%.

Root-shoot ratios were higher for all Al levels in *P. lophantha* plant sets than for *A. decurrens*, except for dump progeny at 900 ppm. Dump plants had a consistently lower, and more constant (0.42 - 0.29), root-shoot ratio than the non-dump sets (Figure 9). Non-dump plants in treatments greater than 700 ppm Al had very high root-shoot ratios reflecting the major declines in leaf and shoot contributions to dry weight.

In *Paraserianthes lophantha* plants grown with nutrient solution only, calcium and nitrogen concentrations were highest in leaves and least in shoots for plants of both sources (Table 6). Leaf nitrogen was above the normal range (Allen, 1974). Root phosphorus content was slightly higher than other tissues.

Non-dump plants had higher concentrations of these three elements in all tissues, with the exception of shoot calcium for which there was no difference between accessions. Dump plant roots had higher concentrations for each of Mn, Fe, Zn, Cu and Al. Non-dump leaves had higher Fe, Zn and Al than dump plants, and non-dump shoots had slightly more zinc. Apart from these all other shoot and leaf samples had higher levels of metallic elements in the plants of dump origin.

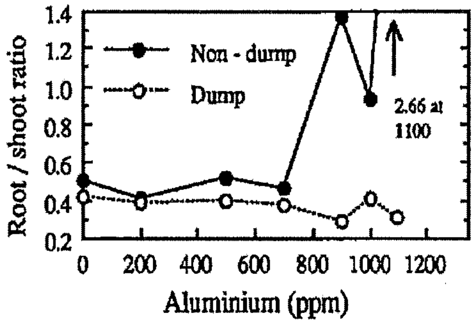

FIGURE 9. ***Paraserianthes lophantha*** **root/shoot ratio.**

The absolute quantities absorbed for each of zinc, aluminium, manganese, iron and copper in controls were higher in dump plants. Except for zinc, each of these elements made a higher contribution to percent dry weight in dump plants (Table 7). Dump plants retained higher percentages in roots and translocated less to foliage. Non-dump plants translocated in excess of 80% of the absorbed zinc and manganese to leaves with only 4 - 5% retained in roots. Dump plants retained 92% of aluminium in roots and sent only 4% to leaves. Both iron and copper in dump plants were above the normal range (Allen, 1974) and this was due to the elevated root levels (Table 6).

Leaf nitrogen content in dump plants remained at > 3% with increased aluminium until the highest treatment level (Figure 10a). The nitrogen content of roots increased with increasing Al addition. In non-dump plants foliar nitrogen remained high at the lowest aluminium level but then declined to < 3% (Figure 11a); root nitrogen generally declined with increased aluminium. Shoot nitrogen was consistently higher in the non-dump plants.

Phosphorus in the leaves of dump plants remained above 0.2% up to an addition of 500 ppm Al and then declined steadily to a minimum of 0.12% in the 1100 ppm treatment (Figure 10b). Both shoots and roots had increased phosphorus percentage with more Al. Phosphorus concentration in non-dump plants decreased with Al (Figure 11b). The decline of phosphorus in the leaves was not as great as in the roots, and both were irregular beyond 500 ppm Al.

The percentage of calcium decreased in the roots, shoots and leaves of the *P. lophantha* dump progeny (Figure 10c). In both sets the greatest change was at the first level of aluminium for root calcium, which was generally lower

TABLE 6. Elemental concentration in *Paraserianthes lophantha* not subjected to aluminium treatments.

Source		Element							
	Tissue	%			ppm				
		N	P	Ca	Mn	Fe	Zn	Cu	Al
Dump									
	Root	1.46	0.24	0.58	188	1406	32	94	3650
	Shoot	1.25	0.21	0.21	37	429	14	11	152
	Leaf	3.87	0.22	0.83	203	125	47	25	108
Non - dump									
	Root	2.38	0.33	0.69	8	101	4	7	1229
	Shoot	1.93	0.28	0.20	16	115	17	8	105
	Leaf	4.05	0.24	0.98	113	225	67	22	160

TABLE 7.
Allocation of elemental uptake in *Paraserianthes lophantha* not subjected to aluminium treatments.

Element / Source	Uptake (mg)	% dry weight	% distribution Root	Shoot	Leaf
Zinc					
Dump	0.228	0.0034	28	12	60
Non - dump	0.199	0.0034	4	12	84
Aluminium					
Dump	7.905	0.1161	92	4	4
Non - dump	2.950	0.0505	81	5	14
Manganese					
Dump	1.032	0.0152	36	7	57
Non - dump	0.319	0.0055	5	7	88
Iron					
Dump	3.999	0.0587	70	21	9
Non - dump	0.921	0.0158	22	17	61
Copper					
Dump	0.280	0.0041	67	7	26
Non - dump	0.078	0.0013	17	13	70

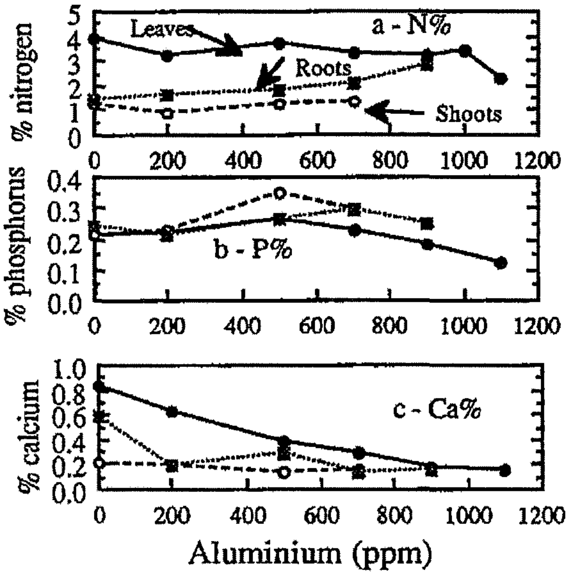

FIGURE 10. Nutrient (%) in dump plants of *Paraserianthes lophantha.*

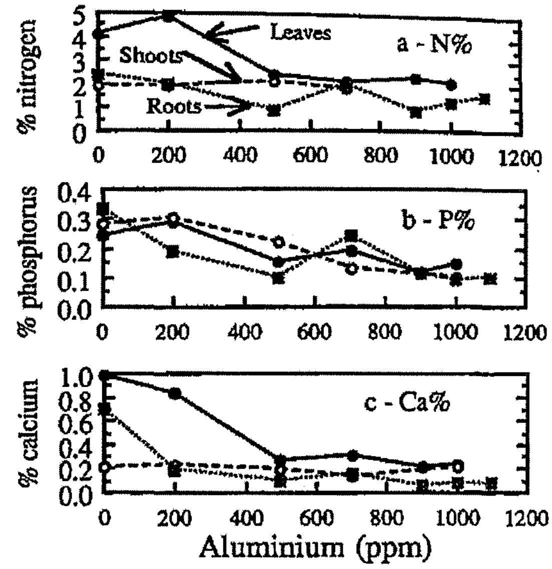

FIGURE 11. Nutrient (%) in non-dump plants *Paraserianthes lophantha.*

than 0.2% with any aluminium level. Leaf calcium remained comparatively high (> 0.6 %) at the first level of aluminium.

Aluminium addition increased the uptake of manganese and zinc in dump plants (Figure 12). Iron content, however, declined steadily and copper uptake was somewhat irregular, with highest root concentration (106 ppm) attained at 500 ppm Al. Most of the uptake of these elements was confined to the roots, and except for zinc, leaf contents were higher than shoots.

All root concentrations for each of manganese, iron, zinc and copper were beyond the normal levels of Allen (1974). Leaf and shoot levels were within normal limits, with the exceptions of stem zinc (128 ppm) at 700 ppm Al, leaf iron at 1100 ppm Al and leaf copper at 500 ppm Al.

In the non-dump set much less manganese, zinc and copper was absorbed than in dump plants. Root zinc levels were in fact below the accepted range with a low in control of 3.8 ppm and a high at 700 ppm Al of 23 ppm Zn. In no treatment were any of manganese, iron, zinc or copper levels in roots higher than the normal range of Allen (1974). Iron levels were similar in both sets of plants at higher aluminium concentrations. Very little remained in the roots and leaves held the bulk of these elements in non-dump plants (Figure 13). Leaf iron levels were above the normal range with aluminium treatments of 500 ppm and greater. Shoot and leaf zinc (150 ppm) and copper (30 and 40 ppm) exceeded the normal upper limits in the 200 ppm Al treatment. Leaf iron content exceeded 800 ppm from 500 ppm Al onwards.

Despite the lack of data points for aluminium contents of tissues there was a trend for higher uptake by non-dump plants when aluminium was supplied, and for these to have higher leaf levels (Figure 14). Non-dump roots in the 500 ppm treatment had 7000 ppm Al and 8000 ppm in the 700 ppm treatment. Foliar aluminium at the 500 ppm Al treatment exceeded 2000 ppm. Dump progeny foliar and root aluminium was lower than in non-dump progeny for each comparable treatment level. Dump root content reached 4000 ppm and foliar content was 700 ppm at the 500 ppm Al treatment level. Shoot Al was similar for both sets.

The ability of dump plants to accumulate aluminium in the control may not have been consistent in the presence of added aluminium (Table 8). Non-dump plants contained more aluminium with increased application and translocated higher proportions to the foliage. Both sources translocated considerably more Al to foliage than remained in shoots.

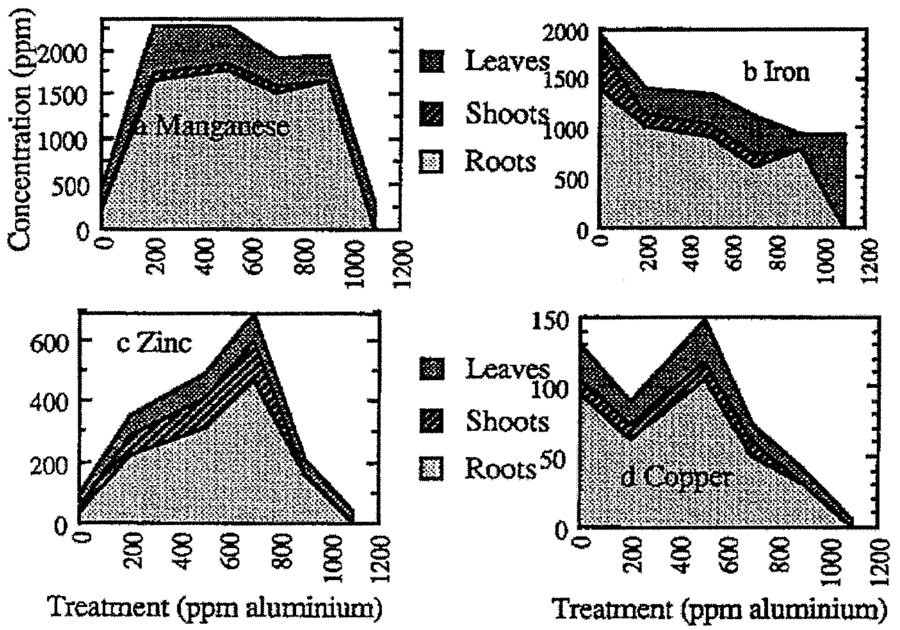

FIGURE 12. Trace elements in tissues of *Paraserianthes lophantha* (dump origin).

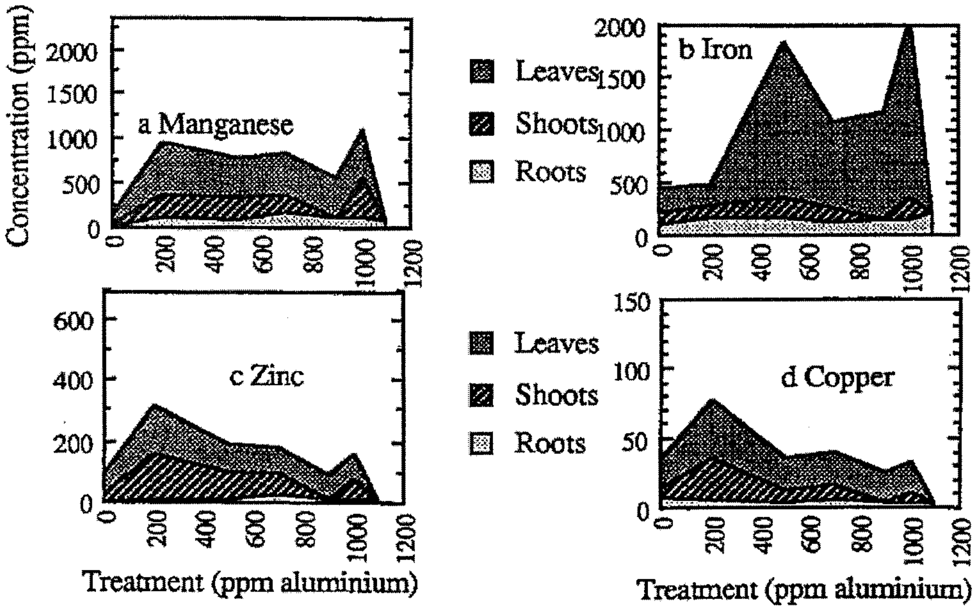

FIGURE 13. Trace elements in tissues of *Paraserianthes lophantha* (non-dump origin).

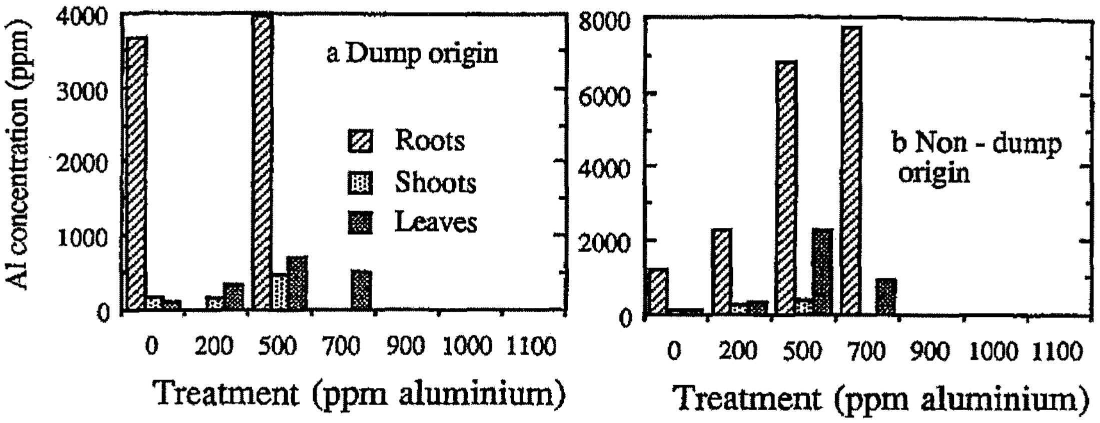

FIGURE 14. Aluminium concentration in tissues of *Paraserianthes lophantha.*

Despite the fact that addition of aluminium decreased growth and increased the death rate, plants subjected to aluminium treatments did not wilt as quickly as control plants. Two days after watering it was noted that all control plants showed signs of wilting while only three to four plants in the 200 ppm treatment appeared affected. At 500 ppm and above, none of the plants appeared to wilt but the leaves and stems were not as succulent as those of the control group.

Seed Analysis

For both species dump seed was significantly heavier than non-dump seed (Table 9). Dump seed had higher levels of nitrogen, phosphorus, manganese, zinc and aluminium whereas non-dump seed had more iron and copper. Non-dump seed of *Acacia decurrens* had most calcium and calcium levels in the two *Paraserianthes* accessions were similar. In terms of the normal ranges *Paraserianthes lophantha* dump seed had high nitrogen, and non-dump seed high copper. Manganese seed levels were an order of magnitude higher in *Acacia decurrens*. All seed was low in calcium.

DISCUSSION

The expected outcome was for an advantage in growth by plants from dump sources when exposed to aluminium, as well as decreased yield (Schaeffer & Walton, 1990) with aluminium concentration. An earlier study indicated a growth advantage for *Acacia extensa* with heavier dump seed, when plants were grown in acidic solution culture (Fox *et al.*, 1988). In the present trial no clear dry weight production advantages were obtained for plants of dump origin over those of non-dump origin. Whereas dump origin *A. decurrens* plants at the lowest level of aluminium had greater total dry weight than non-dump plants, this did not occur again until the two higher levels of application. Greater root mass was responsible. Growth in *P. lophantha* of dump origin was generally less than that of non-dump origin at all levels of aluminium, for both total biomass and root weights.

In nature plant roots would avoid locally high concentrations of potentially toxic elements so that the trial conditions must be viewed as extreme cases of exposure. Growth and life spans are considered important factors which may explain the observed differences. *A. extensa* is a species of relatively fast growth and short life - span, the seed used earlier (Fox *et al.*, 1988), were taken from plants long established on an old, naturally colonised dump and would represent a number of generations of growth on that dump. It is postulated that the degree of apparent adaptation to aluminium levels would be related to the history of prior exposure. Whereas the *P. lophantha* seed came from plants established three years earlier by direct seeding onto a restored dump, the seed of *A. decurrens* was taken from self-sown trees established many years earlier on an un-restored waste dump.

Acidity levels within treatments were not monitored. Al would have affected pH and this in turn influences the availability of nutrients to the plant, independently of aluminium concentration (Temple & Bungey, 1979). The comparatively strong nutrient solution applied sought to avoid any nutrient constraints to growth. In future work it would be useful to examine acidity levels to distinguish between pH and Al effects on growth.

In the absence of aluminium both sets of dump progeny took up more aluminium than the non-dump sets. With aluminium supplied the dump sets took up less than the non-dump sets. Within the plants aluminium was mainly held in roots with lower proportions in aerial parts suggesting an exclusion mechanism (Aniol & Gustafson, 1990). Non-dump *A. decurrens* plants accumulated more aluminium than dump progeny. Roots in dump plants took up to 6000 ppm Al and had < 1000 ppm in foliage, whereas non-dump plants accumulated up to 10,000 ppm Al in roots and > 2000 ppm in foliage after ten weeks of Al treatment. Although there was a lack of sufficient Al

TABLE 8. Absolute levels of aluminium in tissues of *Paraserianthes lophantha* (* = no data available) and percentage distribution of aluminium between the plant parts for those treatments with sufficient material for analysis.

Treatment ppm Al	Uptake	Dump Root	Dump Shoot	Dump Leaf	Non - dump Root	Non - dump Shoot	Non - dump Leaf
0	mg Al	7.30	0.29	0.31	2.40	0.15	0.40
	% Al	92	4	4	81	5	14
200	mg Al	*	0.15	0.61	2.75	0.22	0.67
	% Al	-	-	-	76	6	18
500	mg Al	2.27	0.22	0.72	6.80	0.16	3.52
	% Al	71	7	22	65	2	33
700	mg Al	*	*	*	6.00	*	1.31

TABLE 9. Seed characteristics of *Acacia decurrens* and *Paraserianthes lophantha*.

Elemental contents		*Acacia decurrens* Dump	*Acacia decurrens* Non - dump	*P. lophantha* Dump	*P. lophantha* Non - dump
Nitrogen	%	3.08	2.15	3.64	2.63
Phosphorus	%	0.30	0.14	0.21	0.09
Calcium	%	0.19	0.24	0.16	0.17
Manganese	ppm	467	369	38	31
Iron	ppm	108	210	120	229
Zinc	ppm	29	22	43	38
Copper	ppm	7.6	9.5	9.5	23.5
Aluminium	ppm	138	95	103	81
Seed weight	mg	19.09	12.64	67.60	54.49
S. D.	mg	4.02	5.35	6.64	11.58
Significance		$F = 942$, $p < 0.0001$		$F = 2190$, $p < 0.0001$	

analysis data for *P. lophantha*, similar levels were demonstrated with dump roots at c. 4000 ppm Al and foliage 700 ppm compared with non-dump plants at up to 8000 ppm Al and foliage > 2000 ppm Al. Above ~ 500 - 700 ppm application concentrations, tissue levels of Al remained constant or fell.

Within roots, aluminium may be precipitated in free space or bound in nucleic acids, cell walls and the calcium binding protein calmodulin, as well as binding directly with phosphorus and enzyme mediators of nitrogen (Clarkson, 1969; Webb & Sheehy, 1982; Gomes *et al.*, 1985; Taylor & Foy, 1985; Barcelo & Poschenreider, 1990; Schaeffer & Walton, 1990). Passive uptake of Al could depend on a concentration gradient and the electrical potential across the plasma membrane. If the pathways by which aluminium is absorbed are saturated, then the rate of binding in the roots will not increase. Passive uptake would not be effective once the concentration of Al bound in the roots is at equilibrium with the concentration in the rhizosphere. Uptake is then dependant on metabolic processes (Zhang & Taylor, 1990). These are likely to be the predominant method of uptake and binding of Al. Plants tolerant of aluminium may have the ability to maintain ion fluxes across the plasma membranes of root cells at an acceptable level (Miyasaka *et al.*, 1989). If plants from dump sites are tolerant to aluminium, they would also undergo other changes in physiology and morphology likely to improve their ability to survive and reproduce in the presence of Al (Baker, 1987).

Control plants of both species from dump sites were of shorter height at the end of the ten week trial than plants from non-dump sites. It is hypothesised that the heavier dry weights attained suggest that dump progeny may have a denser structure than plants from non-dump sites. Non-dump plants were the first to wilt and the last to recover turgidity. Dump plants did not wilt until two days without watering. Denser structure may be advantageous under water stressed conditions, whether this is related to either water retention *per se* or to mechanical support, presumably less water would be required to maintain turgidity. Aluminium within plants affects root pressure due to changes in distribution of potassium and hydrogen ions. Interaction between Al and root cell surfaces results in the production of metal - sulphydryl bonds which determine the rate of leakage of ions from the cells. An increase in ion concentration in the rhizosphere alters the potential gradient between the root and soil by increasing the resistance to water entering the root. Root pressure is diminished, causing less water to be transported to the upper parts of the plant (Robinson, 1989; Barcelo & Poschenreider, 1990; Zhang & Taylor, 1990).

Root growth was inhibited in all plants exposed to Al treatments. Roots of dump plants exposed to Al, for both species, had inconsistent changes in the proportion of growth going to roots compared with shoots and leaves. Root - shoot ratios were not correlated with the concentration of Al. Non-dump progeny of *P. lophantha* had a proportional increase in the dry weight of the roots, as Al concentration increased, while *A. decurrens* non-dump progeny had a proportional decrease in dry weights relative to the shoots and leaves. Dry weight production in *A. decurrens* was not as severely affected by Al which suggests that this species has a greater ability to suppress the effects of Al by excluding it from upper parts of the plant. Dump origin plants of *A. decurrens* were consistently heavier than those of *P. lophantha*. *A. decurrens* (dump) accumulated approximately 500 ppm foliar Al after ten weeks of application of a 500 ppm solution, while *P. lophantha* (dump) had accumulated approximately 700 ppm over the same trial period of the same solution. With a higher concentration of Al in the upper parts of the plant than *A. decurrens*, Al would have more harsh inhibitory effects on the growth of the upper parts of *P. lophantha* (dump). This was reflected in lower leaf dry weight production by *P. lophantha*.

Manganese was not supplied to plants via the nutrient solution and presumably came from the sand medium used (not analysed) and seed. *A. decurrens* seed had a high level of manganese, whereas *P. lophantha* seed had relatively insignificant levels. Mn accumulated in high concentrations in roots of both *P. lophantha* and *A. decurrens*. Proportionally insignificant quantities of Mn were in shoots and leaves of *A. decurrens*, equivalent to the concentrations in the seeds collected from the dump site. A high concentration of Mn in the seeds of *A. decurrens* relative to *P. lophantha* suggests that the *A. decurrens* parent plants do not restrict this element as successfully as *P. lophantha*. Pot trials also indicated the inefficient binding of Mn by *A. decurrens*. Mn concentrations in the shoots and leaves of *A. decurrens* (dump) were twice those of *P. lophantha*. Al deteriorates cell membranes and alters enzyme activity (Barcelo & Poschenreider, 1990). This may also result in a partial breakdown of the passive ion control system. The ability of *P. lophantha* to maintain ion fluxes may indicate it is better adapted than *A. decurrens* to tolerate high levels of heavy metals.

Calcium deficiency is caused by aluminium (Schaeffer & Walton, 1990; Zhang & Taylor, 1990). Both non-dump and dump progeny exhibited dramatic falls in calcium, the most severely affected of the macronutrients. Al binds with the calcium binding protein, calmodulin which inhibits uptake of calcium into the plant. Calcium levels in the leaves dropped more than in the roots probably due to a decrease in root pressure which, in turn, would diminish the rate of translocation of nutrients from the roots to the upper sections of the plant. Therefore, any decrease in nutrient levels of the roots would be magnified in the leaves. Nitrogen and phosphorus levels, although significantly affected, were not as diminished as calcium in the roots. The high levels of P supplied in nutrient solution would have reduced the inhibitory activity of aluminium in the root environment. Al binds directly with phosphorus at the root surface and in the intercellular spaces, thus, it may still be absorbed into the roots but translocation is inhibited (Clarkson, 1969). Nitrogen levels in the roots were the least effected as Al does not bind directly with nitrogen but with an enzyme mediator of nitrogen absorption. Al also interferes with amino acid inter - conversion, changing the nitrogen forms translocated in the sap (Gomes *et al.*, 1985; Miyasaka *et al.*, 1989). Thus, while nitrogen content of the roots may increase slightly, as was the case with the *A. decurrens* and *P. lophantha* dump progeny, leaf nitrogen content was reduced significantly as a result of reduced root pressure and Al interference in translocation (Peterson, 1983; Gomes *et al.*, 1985).

Nodulation was not observed in either *A. decurrens* or *P. lophantha* seedlings in any of the Al treatments or control groups, despite the general occurrence of nodulation observed in other pot trials with leguminous species. Al inhibits nodulation because root hair numbers and density are reduced. At a concentration of 0.1 ppm, root hair density is reduced by 50% (Brady, 1991). Very little root hair growth would have occurred in this trial, preventing the establishment of rhizobial nodules.

Dump plant seeds were of heavier weight compared with seed from the non-dump plants, confirming trends noted earlier with species of *Acacia* (Fox *et al.*, 1988). Dump seeds have higher nutrient contents than seeds collected from non-dump sites. It has not been determined whether increased weight is related to seed coat thickness. It is hypothesised that dump plants may be subject to selective forces favouring increased seed coat thickness, this would enhance the regeneration potential of these species in the harsh environment of the dumps.

CONCLUSIONS

Addition of Al to the soil solution had significant inhibitory effects on the plants, as demonstrated by changes in morphology and physiology. Whether the symptoms displayed by the treated plants were a direct result of Al interaction with physiological activity or resulted from secondary toxic effects would require further, more detailed, investigation. Al tended to accumulate in higher concentrations within the roots of all exposed plants while relatively small concentrations were translocated to the shoots and leaves. This supports the theory that legumes, such as *Acacia* and *Paraserianthes*, bind heavy metals in the roots, inhibiting translocation to the shoots and leaves (Aniol & Gustafson, 1990). Understanding the dynamics of Al toxicity and methods by which tolerance is achieved and inherited may lead to more efficient methods of revegetating environments, such as coal mines and waste dumps, in which heavy metals proliferate. Instead of treating the secondary effects of metal toxicity, such as nutrient deficiencies, steps can be taken to treat the problem at the source. Time, money and energy would be saved allowing resources to be distributed over a larger area of rehabilitation work, by the relatively simple expedient of collecting seed for use on coal-mine rehabilitation sites from those dumps already colonised successfully.

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