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Comparative Tolerance of Tropical Green Manures to Toxicity in Acid Sulfate Soils

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ABSTRACT

To determine the growth rates and tolerance of four green manures to Al toxicity, a greenhouse study was conducted.Two acid sulfate soils (Bg : Typic Sulfaquepts; Ra : Sulfic Tropaquepts), high in "Aluminum", were adjusted to four pH levels : 3.8 and 4.0 (original soil pH), 4.5, 5.5, and 6.5. Top dry weight was determined at the 49 day growth stage. Nitrogen, P, K, Ca, Mg, Al, Mn, and Fe concentrations were also determined in top samples.

Soil pH levels affected the dry weight production of each green manures. Tolerant species showed a higher or similar dry weight yield at unamended soil pH or pH 4.5 compared to pH 6.5. The best dry weight yields at pH 6.5 were obtained with those species that were the most acid tolerance with *Cajanus cajan and Sesbania aculeata,* whereas *Sesbania rostrata* and *Sesbania speciosa* were the poorest.

Top analysis was used to determine total nutrient uptake and tolerances of a range of some green manures to excess Al. For all combinations of two soils, poor growth could largely be attributed to problem with Al and Ca uptake. However, high levels of tissue Al were probably the principal limitation to green manure growth at pH 4.5. Tolerance, based on the critical Al concentration at 90% of maximum growth (parentheses in mg kg^{-1}), was of the order in Bg soil : *C. cajan* (60) *S. aculeata (45)* *S. rostrata (39)* *S. speciosa* (29); and in Ra soil : *C. cajan (63)* *S. aculeata (54)* *S. rostrata (49)* *S. speciosa* (40). These results are discussed in terms of current understanding of the nature and potential of Al tolerance in various green manure species.

Keywords : Tropical green manures, acid sulfate soils, acid tolerance, Al toxicity.

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Introduction

There was an increasing interest in the use of green manures as multipurpose plants under the relatively acid conditions which were often found in tropical and subtropical climates (Yost and Evans 1987). It was well known that there were marked differences between plant species, often even between cultivars, in their ability to grow under adverse conditions (Marschner 1986). So called 'acid tolerance' was an example of this (Munns 1986). In general, green manures have been the most successful species in the tropics but does not thrive on acid soils. Effort has been directed to introducing the tolerance of various green manures to acid soils (Hutton 1989), but there is a need to identify other species that are specifically tolerant to acid conditions.

Some tropical green manures were used for annual crop rotations within an increasing large area of clay-textured acid sulfate soils in Central Plain of Thailand. These soils are strongly acidic, and Al toxicity existed when the soils were aerated by oxidation of predominant jarosite, located at lower depth (30-70 cm) in the profile (Parkpian *et al.* 1991). In addition, green manures were important crops in many areas where productivity was limited by acidity. In a recent comprehensive review of crop tolerance, *Sesbania rostrata* was the only tropical and subtropical legume for which data were available *(Joshua et al.* 1989), although more recently, the tolerance of pigeonpea (*Cajanus cajan*) had been studied (Thai 1990, Kerridge 1991). *S. aculeata had the reputation of being reasonably tolerant to salinity in Northeast Thailand (Arunin et al.* 1989), but there is no critical examination in acid soils. had been reported.

As mentioned previously, a vast area of acid sulfate soils could be more productive if Al toxicity is reduced. Acid sulfate soils were frequently at phytotoxic levels as soluble and exchangeable Aluminum Liming was the most common practice to alleviate or to reduce Al toxicity. However, adverse effects on plant growth followed liming of acid soils had been extensively reported. For example, depression of legume growth by liming was investigated by Barnard and Folscher (1988). It was reported that on acid, brown sandy clay loam of Oxisols, the effect was only found above pH 6.0 and was largely temporary and transient. This stressed the care in a short-term growth observation, There were obvious differences in the response to lime by different legumes on different soil pHs. Holford 1985 especially on clover in 15 acid soils.

Since genetic variability of Al tolerance exists in plants, perhaps the best solution for overcoming Al toxicity would be a combination of both liming and selection of Al tolerant plant species, or cultivars within a species. To investigate this approach, four species of green manures were compared for their growth potentials and tolerances to excess Al content in acid sulfate soils.

Materials and Methods

Plant selection from a field survey

Initial phase of the study was involved with a field survey in the Bangkok Plain to investigate the species x soil family (genotype x environment) interaction of tropical legumes with potentials for the use as green manures. Studied plants were included leguminous species of *Cajanus, Crotalaria, Desmodium, Leucaena, Vigna,* and *Sesbania.* Each location was visited several times in order to question farmers on growth behaviors, biomass production and ability to survive under adverse conditions. After evaluation, the most promising species were selected for further greenhouse studies as the representatives of green manures. They were *S. rostrata, S. aculeata, S. speciosa* and *C. cajan.*

Soil sampling and analysis

Two soil series use to grow rice and tropical fruit trees which were currently were collected from paddy field. Acid sulfate soils were taken from two locations as Bang Pakong (Bg) and Rangsit (Ra) which was a very-acid phase series. The Bg was characterized by a pre-oxidized potential acid sulfate soils (Typic Sulfaquepts) while the Ra was actual acid sulfate soils (Sulfic Tropaquepts). Selection of sample site was based on newly revised map of the acid sulfate soils of Thailand (Van der Kevie and Yenmanus 1972).

Soil samples for pot experiment were collected from Ap horizons and jarositic layers. Both samples were air-dried, ground, sieved through a 2-mm screen and kept for chemical analysis. Relevant chemical properties of the studied soils are given in Table 1.

Soil pH (1:1) of the fresh and air-dried samples were determined after 1 hr of intermittent shaking. Electrical conductivity (EC) of the air-dried samples was measured in a suspension of soil at a 1:1 ratio after 1 hr. of intermittent shaking. Cation exchange capcity (CEC) was determined by M NH_4OAc buffered at pH 7 (Chapman 1965). Available P was determined by the Bray 11 method (Bray and Kurtz 1945). Calcium (Ca) and Magnesium (Mg) from the NH_4OAc extract were determined by atomic emission spectro metry, while K and Na were determined by flame photametry. Aluminum (Al) was extracted by M KCl and determined colorimetrically (Barnhisel and Bertsch 1982). Free iron was determined by the method of Mehra and Jackson (1960). Water-soluble sulfate-s was extracted by shaking 10 g soil with 50 ml water for 30 minutes, followed by 5 minutes centrifugation at 1600 rpm. The supernatant was filtered and analyzed. Extractable SO_4 was extracted with 0.01 M Ca $(H_2PO_4)_2$ (Hue *et al* 1985). Sulfate in both extracts was measured turbidimetrically, using a spectrophotometer at 420 rm (Freeney 1986). Texture analysis was determined by the pipette method of Gee and Bauder (1982).

Pot experiment

Experimental treatments consisted of factorial combinations of four species of green manures and four adjusted pH levels (unamended, 4.5, 5.5 and 6.5). Hence, there were 16 treatment combinations in three replicates laid out in a randomized complete block design. The soil pH was adjusted with $Ca(OH)_2$ on the basis of a lime titration curve, to achieve a spread of targeted pH values. All essential macro- and micro-nutrients were added to ensure that growth was not inhibited by an inherent deficiency of any element. The following basic nutrient solution was applied at mg kg^{-1} soil : $Na_2MoO_4.2H_2O$, 0.67; H_3BO_3 0.83; $CUSO_4 . 5H_2O$, 5; $ZnSO_4 . 7H_2O$, 10; $MnSO_4 . H_2O$, 15 and KH_2PO_4, 176. In all these calculations, the soil analysis values were recorded (Table 1). Nitrogen (N) was only given at the beginning of the experiment to stimulate growth initiation in the form of NH_4NO_3 at the rate of 24 mgN kg^{-1} with NH_4NO_3. The P rate was selected from a previous P response experiment to produce 90% maximum growth in each soil in order to minimize the ameliorating effects that at any higher rates of P could have on Al toxicity in the acidified treatment (Jugsujinda *et al.* 1978). The nutrient were mixed through the soils which were then incubated for 14 days at field capacity before planting. Seed was inoculated with appropriate rhizobia strain prior to planting.

Five Seeds of green manures were grown in 15 cm diameter pots filled with 2 kg of both soils. After germination, they were thinned to 2 plants per pot. The pots were watered daily with deionized water, initially to 80% field capacity and later to field capacity as the demand increased. The experiment was carried out in a greenhouse at King Mongkut's Institute of Technology Bangkok, during September-November 1991. Day tem-

Table 1. Some selected physical and chemical Properties of the Two Acid Culfate Soils.

Soil properties	Soil Series			
	Bg		Ra	
	Mean[1]	± Sd	Mean[1]	± Sd
pH (1:1; soil : water)	3.8	0.2	4.0	0.7
EC (1:1; ds m^{-1})	0.7	0.2	0.2	0.1
OM (%)[2]	4.1	0.8	3.8	0.9
Available P (mg kg^{-1})[3]	8.4	3.5	9.0	2.4
CEC (cmol (+) kg^{-1})[4]	21.4	5.4	23.8	3.1
Exchangeable K (cmol (+) kg^{-1})[5]	0.1	0.1	0.2	0.1
Exchangeable Ca (cmol (+) kg^{-1})[5]	2.2	0.7	1.0	1.1
Exchangeable Mg (cmol (+) kg^{-1})[5]	6.7	2.1	1.8	2.1
Exchangeable Na (cmol (+) kg^{-1})[5]	2.1	0.9	0.1	0.1
Exchangeable acidity (cmol (+) kg^{-1})[6]	14.3	3.5	8.1	2.8
Extractable Al (cmol (+) kg^{-1})[7]	7.5	1.7	4.3	1.5
Extractable Fe (mg kg^{-1})[8]	1440.9	511.1	711.1	84.0
Extractable Mn (mg kg^{-1})[8]	36.8	17.1	22.6	17.6
Water soluble $SO4^{=}$ (mg kg^{-1})[9]	502.4	594.4	134.4	202.8
Particle size distribution[10]				
Sand (%)	1.0	0.8	2.6	1.7
Silt (%)	39.7	8.2	35.6	2.8
Clay (%)	55.7	7.0	61.7	1.7

(1) air-dried samples
(2) Walkley-Black method (Black 1965)
(3) Bray II method (Bray-and Kurtz 1945)
(4) M NH_4OAc pH 7.0 (Chapman, 1965)
(5) Exchangeable cations by M NH_4OAc (Black 1965)
(6) M KCl extraction (Mclean 1965)
(7) M KCL extraction and titrate with 0.1 N HCl (Mclean 1965)
(8) 0.005 M DTPA pH 7.3
(9) Water soluble (Hue *et al.* 1985)
(10) Pipette method (Gee and Bauder 1982)

perature fell below 20°C.

At 49 days after sowing, plants were removed from the pots and aerial biomass (dry weight of tops) was obtained from oven dried samples (70-80° C for 48 h). A subsample from each replicates was digested in a 4:1 nitric : perchloric acid mixture and analyzed for K, Ca, Mg by flame emission spectrometry and for P by the Vanado molybdate method. The % N in plant parts was estimated by Kjeldahl digestion. Total Al, Mn, Fe in the plant sample were measured by atomic absorption spectrometer

Simple analysis of variance was used to test the effect of treatments on selected growth components and nutrient uptake of green manures. The relationship between relative dry weight and Al contents in plant tissues were evaluated by simple regression analysis in order to approximate the critical Al concentration above which toxicity is induced.

Results and Discussion

The two acid sulfate soils selected for this study represented at least two potential problems for the growth of green manures Al toxicity and Ca deficiency. The problems were all predictable from the soil analyses data in Table 1. Phosphorus deficiency, another potential problem, has been discounted in this study because the soils was fertilized with adequate amounts of P prior to the experiment. The data on extractable Al and Fe also indicated the relative importance of these potentially toxic elements. **There were approximately two times of more Al and Fe were extracted from the Bg soil than from the Ra soil. Levels of extractable Mn were high; probably due to releasing of water-soluble Mn from MnS during the drying cycles (oxidized conditions) in the fields (Satawatananont *et al.* 1991).**

Dry matter yield and N accumulation

Figure 1 and 2 showed the total growth of some green manure species in Bg and Ra soils at different pH levels. The increase in dry weights (gm pot^{-1}) and % N accumulation were also tabulated in Table 2. Dry weight production was affected significantly ($P < 0.01$) by various species, and adjusted pH levels ($P < 0.01$), and by all interaction of these two factors. **In general, the weight increase was greater in Ra soil than in Bg soil. The data showed that there were large didderences between species in their ability to grow under the original soil pH of 3.8-4.0 (unlimed conditions). *S. speciosa* and *S. rostrata* were the poorest, whereas. *C. cajan* and *S. aculeata* were the best.** With progression in neutralization by adjusted pH to 6.5, growth improved considerably. When growth was poor in the unlimed soil, growth improvement was one-fold at the highest level of liming, whereas with the more 'acid tolerance' species this increase was double or sometimes triple (Yost et al. 1983). It was interesting to note that the best yields at all levels of neutralization were the most acid-tolerant species, especially *C. cajan* and *S. aculeata.*

For all the green manures, N uptake significantly increased ($P < 0.01$) throughout the range of pHs in both soils. At low pH level, *S. speciosa* produced the less dry matter and also a ccumulated the less N in tops (Table 2). At soil pH > 4.5, *C. dajan* was the best to accumulated N although still in small amount, while at *soil pH* > *5.5, S. aculeata* contained more N in the top than *S. speciosa,*. The tops of *S. rostrata* had highest N. content and this resulted in the highest N $plant^{-1}$ among the *Sesbania* species. It was possible that *S. rostrata* had the advantage of root nodules during the early stages of growth and of stem nodules in the later stage. *S. speciosa* showed the slowest growth rate and lowest N content. This species in spite of its photoperiod insensitivity, it did not appear to be a significant green manure crop from this study (Ramani *et al.* 1990).

At pH of 6.5, *C. cajan* and *S. aculeata* were still the only species containing substantially more N than *S. speciosa*. There was a stronger response to soil pH in N yield than in dry matter yield. This suggested that the plant rhizobium symbionts which inoculated at the beginning of experiment, rather than the plant *per se*, responded to high soil pH. The increased N accumulation might have reflected a more efficient symbiosis which might occur in these acid sulfate soils (Personal communication with Dr. P. Prabuddham).

Nutrient concentration in plant tissues

Plant materials from the different treatments were analyzed for all elements of nutritional importance. The data were averaged over the soils in order to avoid the presentation of voluminous data which tended to follow a similar pattern (Table 3). The data also provided information that was more useful in explaining growth differences under acid conditions. This occurred especially where dry matter yield and plant composition were fairly related, as with these green manures, and translocation to seed or storage organs did not apply.

Generally speaking, the total amounts of different elements taken up increased with improved growth of the individual green manures, as might be expected. In all cases, amounts of Al, Mn, Fe in tops decreased and Ca increased with increasing pH. Increasing level of adjusted pH to 6.5 increased the uptake of P, K and Mg above the original soil pH. The most pronounced influence of increasing soil pH on top nutrient content was to decrease Al content and attained statistical significance (P 0.01) at pH 6.5. The reduction in nutrient uptake suggested that Al toxicity affected both tops and root growth. Barber (1985) had demonstrated, through a mechanistic model to describe nutrient uptake, that the rate of root growth was most sensitive in nutrient uptake by plants. Though root growth was not measured in this study, it was fairly well documented that Al Toxicity contributed to root degeneration (Foy 1988).

Estimating critical levels of Al toxicity in green manure tissue

Regression analyses were most useful in approximating critical levels of nutrient concentration in plant tissue (Macnicol and Beckett 1985). If a straight line was draen between points representing sero growth with the attainable maximum growth, a response ceiling was defined. This kind of relationship had been used to successfully interpret the influence of excess Al on the growth and N fixation of soybeans (Munns *et al.* 1981), and excess Mn on cowpea (Kang and Fox 1980).

The data relating Al concentration and relative dry matter yield of tops (shoots) of each green manures in both soils were described by fitting (by least squares) a quadratic polynomial function. Figure 3 and 4, illustrated the above relationships. To be able to assess an optimum Al concentration above which Al toxicity was likely to occur, it was arbitrarily decided that a relative yield below 90% was unsatisfactory, and the Al concentration correspondence to 90% of relative yield was the toxicity threshold above which the plants would suffer some the injury. In the Bg soil, a 90% relative yield corresponded to 60, 45, 39, and 29 mg kg^{-1} Al in plant tissues of *C. cajan, S. aculeata, S.rostrata* and *S. speciosa*, respectively (Table 4.) In such tissue Al critica level could be estimated also in the Ra soil (Table 4). A short extrapolation of the line in Fig.3 and 4, suggested that growth rate of *C. cajan was declined when tops had accumulated 60 mg kg^{-1} Al, which was the highest tolerance level in green manure species. Likewise, extrapolation of the critical Al ranges for S. rostrata, S. speciosa* to 40 and 30 mg kg^{-1} Al suggested that the species were highly sensitive to Al toxicity especially *S. speciosa*

The differences between soils in the tissue of critical Al concentration could be explained by the different amounts of Al content of both soils. Wornanutwatanakul (1986) reported that there was greater change in Al concentration plant tissue per unit change in extractable Al for Bg soils than in Ra soils. This may be related to differential capacities of the soils to sorb Al which would be higher in Bg soil than in Ra soil. Levels of KCl-extractable Al of 7.5 cmol (+) kg^{-1} in Bg soil or higher were also associatew with high and probably toxic tissue levels of Al and could reduce plant weight of the green manures.

To our knowledge, the Al toxicity threshold concentration had not been published for various green manure species at least for the variety used in this study. However, reports on a related crop such as the soybean, and cowpea (*Vigna spp.*) seemed to suggest that the tissue Al levels reported in this study might be in the toxic range for the crop. For instance, Hilyar (1978), related Al toxicity symptoms to concentrations exceeding 40 mg kg^{-1} in soybeans at 43 days after planting, which fell below the Al concentration in plant tissue in most of the treatments in this investigation. Furthermore, it would seem that the average plant tissue had Al levels in both soils exceeding 60 mg kg^{-1}. Al fall within the toxic threshold values for the crops, suggesting that the green manure required Al less than that by most other legumes. The present results agreed with the data reported by Ghai et al. (1985) which indicated that *Sesbania* may be more sensitive to Al toxicity than *C. cajan*.

The data presented here supported the videa that differences in acid tolerance of green manures could be related to differences in both the dry matter production and the ability in Al uptake. Moreover, these results suggested that the critical Al concentration provided a useful diagnostic indication of the reduction in growth associated with low soil pH level less than 4.5. It was not known whether these relationships would exist when there were more variables such as soil types, water regime and nutritional management.

Conclusion

This study evaluated the relative response in dry weight production and tolerance of four green manure species in two acid sulfate soils with factors associated with soil acidity, low pH and Al toxicity. **Adjusted soil pH levels from 3.8-4.0 (original soil pH) to 6.5 (amended with $Ca(OH_2)$ affected dry weight yields of the various green manures significantly Acid-tolerant species (*C.cajan* and *S. aculeata*) showed a higher or similar dry weight yield at pH 4.5 compared with at pH 6.5 Sensitive species yields as *S. speciosa* were lowered at pH 4.5 than at pH 6.5.**

The present results showed that in the two soils studied, Al toxicity was most likely to be a factor limiting the growth of green manures. This had two implications to consider. Firstly, each species would be adversely affected by high Al content in plant tissues. In general, Al in tops was higher at original soil pH or pH 4.5 than at pH 6.5. Secondly, attempts to use the concept of critical Al levels at 90% reduced relative top yields imposed by a limited nutrient, produced an estimate tolerance limit of green manure species. In particular, these data suggested that *C. cajan* and *S. aculeata* were tolerant to Al toxicity in a range of critical Al levels between 60 and 45 mg kg^{-1} of both soils, whereas *S. speciosa* and *S. rostrata*. were sensitive to Al toxicity determined by low levels of critical Al of 30 and 40 mg kg^{-1} respectively.

Table 2. Total Top Yield and N Accumulation of 4 Species Green Manures in Relation to Soil Series and adjusted pH Levels

Soil Series	pH	Dry weight (gm pot^{-1}) (1)				N concentration (%) (1)			
		SRO (2)	SAC	SSP	CCA	SRO (2)	SAC	SSP	CCA
Bg	3.8	1.27^{d}	1.56^{d}	0.93^{d}	1.64^{d}	1.81^{b}	2.15^{b}	1.38^{c}	1.82^{c}
	4.5	2.40^{c}	2.61^{c}	1.95^{c}	3.08^{c}	2.55^{a}	3.27^{a}	1.78^{bc}	3.18^{ab}
	5.5	3.29^{b}	3.96^{b}	2.36^{b}	4.99^{b}	2.90^{a}	3.45^{a}	2.19^{b}	3.51^{a}
	6.5	4.50^{a}	4.61^{a}	2.64^{b}	5.55^{b}	3.04^{a}	3.38^{a}	2.43^{ab}	3.67^{a}
Ra	4.0	1.70^{cd}	1.86^{d}	1.17^{d}	1.97^{d}	2.50^{ab}	2.34^{b}	1.99^{b}	2.52^{bc}
	4.5	2.31^{c}	2.77^{c}	2.32^{bc}	5.56^{b}	2.58^{a}	3.61^{a}	2.05^{b}	3.76^{a}
	5.5	4.17^{a}	4.55^{ab}	3.54^{a}	6.82^{a}	3.13^{a}	3.65^{a}	2.79^{a}	3.59^{a}
	6.5	4.18^{a}	4.76^{a}	3.00^{ab}	6.58^{a}	3.11^{a}	3.77^{a}	3.06^{a}	3.73^{a}

(1) Mean of 3 replications. Means followed by common letters in a column are not significantly different (P = 0.05).

(2) Species abbreviation as : SRO = *S. rostrata*. SAC = *S. aculeata*
SSP = *S. speciosa*. CCA = *C. cajan*

Table 3. Main Effects of adjusted pH Levels and Greem Manure Species on Nutrient Concentration(1) in top tissue

Treatment	P	K (%)	Ca	Mg	Al	Mn (mg kg^{-1})	Fe
pH levels							
3.8	0.16	1.81	0.23	0.45	155	199	443
4.5	0.21	1.98	0.68	0.70	114	172	356
5.5	0.28	1.93	1.05	0.97	68	149	246
6.5	0.31	2.04	1.18	1.17	53	116	133
Species							
S. rostrata	0.23	1.94	0.58	0.80	101	168	376
S. aculeata	0.25	2.12	0.94	0.93	100	131	271
S. speciosa	0.23	1.56	0.48	0.55	76	224	378
C. cajan	0.26	2.13	1.14	1.01	114	113	152
LSD (P = 0.05)	0.06	0.48	0.30	0.27	24	29	58

(1)Mean of 6 repplications averaged over two acid sulfate soils

Table 4. Regression equation Parameters[(1)] and r^2 Values relating Relative Dry Matter yield (Y)[(2)] to Al Concentration (X) in Green Manures, and the Mean Critical Al Levels which reduced yield by 90%

Soil Series	Species	Parameter[(1)] a	b_1	b_2	$r^{(3)}$ (n=12)	Critical Al[(4)] ($mg\ kg^{-1}$)
Bg	*S. rostrata*	104.43	-0.3647	-0.00034	0.97**	39
	S. aculeata	114.47	-0.4979	-0.00087	0.98**	45
	S. speciosa	99.82	-0.3275	-0.00060	0.98**	29
	C. cajan	114.18	-0.3513	-0.00079	0.97**	60
Ra	*S. rostrata*	125.36	-0.7411	-0.00062	0.98**	49
	S. acuteata	107.15	-0.2413	-0.00143	0.98**	54
	S. speciosa	111.65	-0.5058	-0.00099	0.97**	40
	C. cajan	103.58	-0.0832	-0.00208	0.97**	63

(1) $Y = a + b_1X + b_2X^2$

(2) Relative yield = Yield at any pH levels/Maximum yield × 100

(3) Significant al P = 0.01

(4) Critical Al levels correspond to Al in plant tops

Figure 1. Top growth of *C. cajan* in a) Bg and b) Ra soils at four adjusted pH levels.

Figure 2. Top growth of *S. aculeata* in *a) Bg* and *b) Ra* *soils at four adjusted pH levels.*

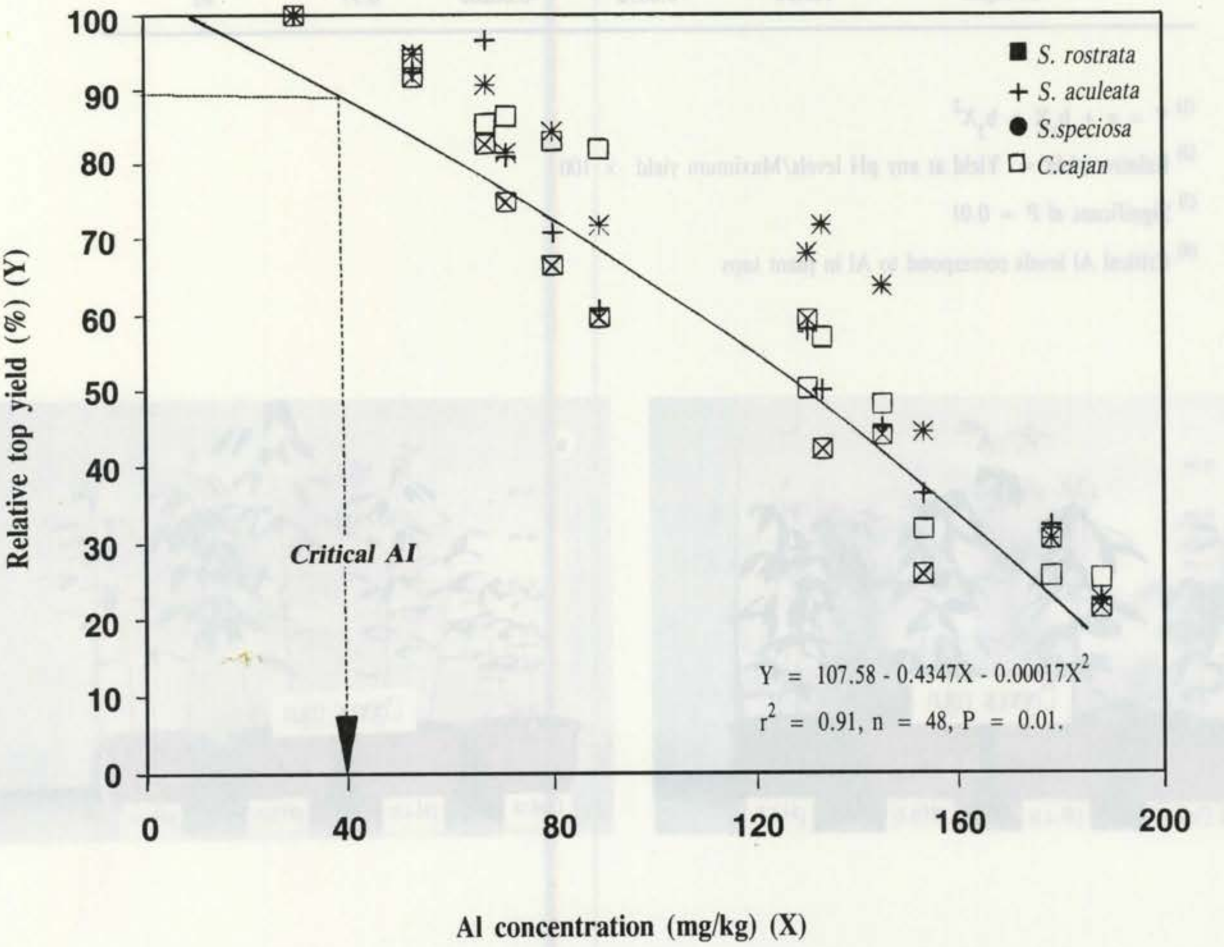

Figure 3. Relationship between Al concentration in top tissue (X) of four green manures and relative dry weight yield (Y) in Bg soil.

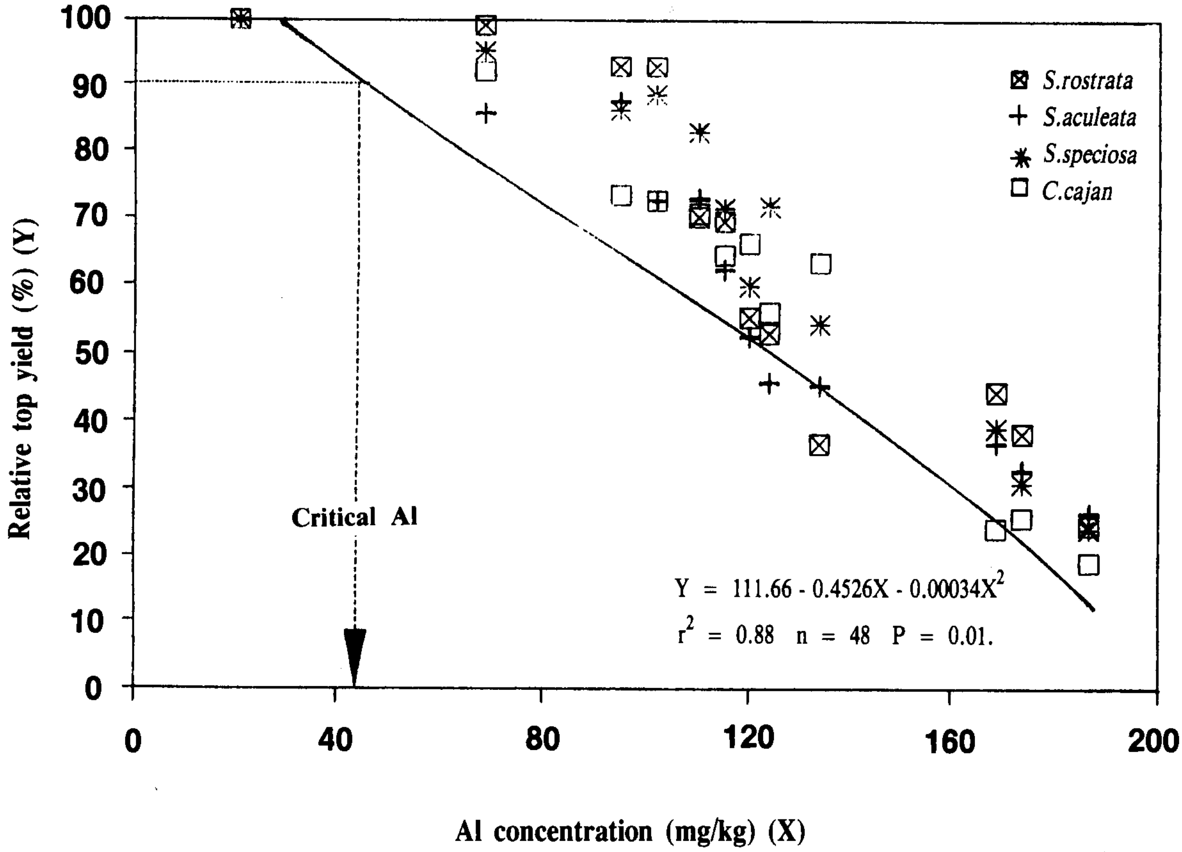

Figure 4. Relationship between Al concentration in top tissue (X) of four green manures and relative dry weight yield (Y) in Ra soil.

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