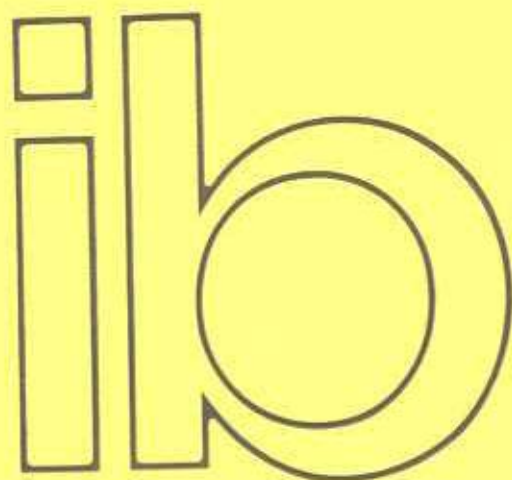


NOTA 244

DETERMINATION OF INORGANIC MONOMERIC ALUMINIUM WITH THE 60 S
PYROCATECHOL VIOLET TECHNIQUE

door

KURNIATUN HAIRIAH, MEINE VAN NOORDWIJK & INEKE STULEN



NOTA 244

DETERMINATION OF INORGANIC MONOMERIC ALUMINIUM WITH THE 60 S
PYROCATECHOL VIOLET TECHNIQUE

door

KURNIATUN HAIRIAH^{*}, MEINE VAN NOORDWIJK^{**} & INEKE STULEN^{***}

* *Brawijaya University, Faculty of Agriculture, Malang 65145,
Indonesia*

** *DLO-Institute for Soil Fertility Research, Haren*

*** *Department of Plant Physiology, University of Groningen, Haren*

Nota's van het Instituut voor Bodemvruchtbaarheid zijn in principe interne communicatiemiddelen en worden derhalve niet als officiële publikaties beschouwd. Zij zullen veelal resultaten van niet afgesloten onderzoek bevatten en/of als discussiestuk dienen. Eventuele conclusies mogen niet als definitief worden beschouwd. Deze nota's worden slechts in beperkte mate of in het geheel niet buiten het Instituut verspreid.

1991

Instituut voor Bodemvruchtbaarheid, Oosterweg 92, Postbus 30003, 9750 RA
Haren (Gr.)

ABSTRACT

A method for measuring the concentration of inorganic, monomeric Al in fluid samples was tested. The technique was found suitable as Al-organic acid complexes (malate) and polymeric Al (as calculated on the basis of sample pH and total Al concentration) did not interfere. A much simpler calibration procedure than in the original description by *Kerven et al. (1989)* was found satisfactory. Application of the technique to samples from a nutrient solution experiment show the importance of a tight pH control in such experiments.

CONTENTS

1. Introduction	2
2. Material and methods	3
3. Results	
3.1 Calibration line	6
3.2 Interference	11
3.3 Analysis of nutrient solution samples	12
4. Discussion	14
Acknowledgements	16
References	16

Appendices:

1. Preparation of solutions for calibration series B.
2. Absorbance data used for the construction of calibration lines of series A.
3. Absorbance data used for calibration lines of series B.
4. Effect on absorbance of adding HNO₃ to AlCl₃ solutions.
5. Parameters used and example of the output of a TITRATOR programme.
6. Solution pH and calculated distribution of Al over various monomeric and polymeric Al-forms for calibration series B.
7. Monomeric Al concentrations in nutrient solution samples of IB 5141 (1991).

1. INTRODUCTION

Only inorganic, monomeric forms of Al - that is Al^{3+} , $\text{Al}(\text{OH})_x$ and $\text{Al}(\text{SO}_4)^+$ - are usually considered to be toxic to plants. Polymerization of Al can occur at pH values above about 4.5. In soils organic acids can complex Al and make it less toxic to the plant. Methods used for the determination of Al in soils or nutrient solutions should therefore discriminate between inorganic monomeric Al on the one hand and inorganic polymeric forms and organically complexed Al on the other hand.

One of the possible Al-tolerance mechanisms of plants is detoxification of Al_3^+ in the rhizosphere through exudation by the roots of chelating agents e.g. organic acids binding Al. Another possibility is local increase of pH in the rhizosphere leading to polymerization of Al. A measurement technique specific for monomeric inorganic Al is required to test how effective these mechanisms are.

Colorimetric methods using short reaction times have been developed to discriminate between inorganic monomeric and organically complexed forms of Al. Short reaction times reduce the risk of release of polymeric or organically bound Al during the measurement. For the concentration range of 0-25 μM Al a method based on pyrocatechol violet (PCV) with a reaction time of 60 s was found to be the best (Kerven et al, 1989). These authors also found a good relationship between inorganic monomeric Al in soil solutions and the severity of Al-toxicity based on relative yield data of peanuts. Toxic levels in nutrient solution studies and in soil solution appear to be similar, when based on this method.

In this report we describe the method in detail and discuss the calculations based on the calibration line. We found difficulty in following the calculations of Kerven and suggest a more simple approach here. A number of checks on the sensitivity of the method to errors and interference of other ions was made. Application of the method is described to the first series of samples of nutrient solutions from experiments with *Mucuna sp.* (Velvet beans).

MATERIALS AND METHODS

Two standard calibrations line were used:

- A. Inorganic Al standard solutions,
- B. Standard solutions with various amounts of organically complexed Al.

Preparations

A. Inorganic Standard solutions:

1. 1 M AlCl_3 : Dissolve 24.145 g $\text{AlCl}_3 \cdot 6 \text{H}_2\text{O}$ into 100 ml demineralized water.
2. 1 M HNO_3 : Dilute 6.924 ml of 65% HNO_3 to 100 ml of water.
3. Inorganic Al standard solutions in the range 0 - 25 μM are prepared by serial dilution of the 1 M AlCl_3 into 100 ml of water.

Conc. inorg. Al (μM)	1 M AlCl_3 (ml)
2	0.2
5	0.5
10	1.0
15	1.5
20	2.0
25	2.5

4. Add 0.1 ml of 1 M HNO_3 to every solution, to avoid polymerization of Al.
5. Dilute to 100 ml.

B. Standard solution containing an Al-malic acid complex:

1. 1 M KOH: Dissolve 5.611 g KOH into 100 ml demineralized water.
2. 10,000 μM Al-malate ($\text{C}_4\text{H}_6\text{O}_5$):
 - Dissolve 134 mg L (-) malic acid into 60 ml demi water,
 - Add 1 ml of 1 M AlCl_3 (without HNO_3), and stir it,
 - Adjust the solution' pH slowly to 4.5 by adding 1 M KOH,
 - Stir constantly for another 24 hours,
 - Adjust the solution's pH to 4.5 once more,
 - Dilute to 100 ml.
3. Standard solutions are prepared by mixing of 100 μM AlCl_3 (0.1

ml of 1 M AlCl_3 into 1 l of water) and 100 μM Al-malate (1 ml of 10,000 μM Al-malate into 100 ml of water), to make Al concentrations of 5, 10, 15, 20, 25 μM , with 0, 25, 50, 75 and 100% of the total Al in inorganic form. Prepare the standard solution series by series:

- Prepare five 100 ml volumetric flasks for each series of standard solutions.

Series of 5 μM concentration:

% inorg. Al	conc. inorg-Al (μM)	conc. org-Al (μM)	100 μM AlCl_3 (ml)	100 μM Al-malate (ml)
0	0	5.00	0	5.00
25	1.25	3.75	0.125	3.75
50	2.50	2.50	0.250	2.50
75	3.75	1.25	0.375	1.25
100	5.00	0	0.500	0

- Dilute each solution to 100 ml.
- Immediately after this preparation add the reagent and measure the absorbance (see analytical procedures).
- Other series of standard solutions are prepared in a similar way, to obtain 10, 15, 20 or 25 μM of Al (Appendix 1).

Reagents for PCV colour reaction:

1. Iron interference reagent:

0.2 % of 1,10 Phenanthroline chloride (monohydrat) ($\text{C}_{12} \text{H}_9 \text{Cl N}_2 \cdot \text{H}_2\text{O}$, Merck) + 0.5 % L-ascorbic acid, by dissolving 0.2 g of 1,10 phenanthroline and 0.5 g ascorbic acid into 100 ml of water. This reagent should be daily prepared. pH = 2.5.

2. Indicator reagent:

Pyrocatechol violet (PCV) 0.0375 % is prepared by dissolving 0.0937 g PCV ($\text{C}_{19} \text{H}_{14} \text{O}_7 \text{S}$, Merck) into 250 ml of water. pH = 3.5.

3. 5 M HCl: 103.5 ml of 37% HCl into 250 ml of water.

4. 15 % hexamine buffer:

- Dissolve 75 g hexamethylentetramin ($C_6 H_{12} N_4$, Merck) into 375 ml of water,
- Add 16 ml of concentrated ammonia (NH_3) solution,
- Stir it and adjust the solution's pH to 6.2 by adding 5 M HCl drop by drop.
- Dilute to 500 ml by adding water.

*Analytical procedures*a. Inorganic Al calibration line (0-25 μM):

- Pipet 3 ml of every standard solution into a 10 ml vial,
- Add 0.5 ml iron interference reagent,
- Add 0.2 ml PCV reagent (the colour becomes yellow)
- Add 1 ml of hexamine buffer (dark blue, the higher the inorganic-Al concentrations, the solutions colour becomes darker),
- Mix properly by using a tube shaker (vortex), and leave it for 20 minutes,
- Read the Absorbance at 578 nm (585 nm would give a slightly larger difference with the blank according to Dougan and Wilson, 1974).

b. Organically-complexed-Al standard solutions:

- Prepare a series of standard solutions,
- Pipet 3 ml of every standard solution into a 10 ml vial,
- To one tube is added:
0.5 ml iron interference reagent + 0.2 ml PCV + 1 ml of hexamine buffer;
this takes about 15 s,
- Mix properly by using a tube shaker (3 s),
- After the reagents have been added to one tube and the sample has been mixed, a second tube can be treated the same way,
- Exactly 1 minute after the hexamine buffer was added, the absorbance at 578 nm is measured for each sample, (within 1 minute 2 samples can be treated and measured).

Every determination should be done in duplicate. Blank samples (demine-ralized water + reagents) have to be prepared and measured each day.

c. Analysis of nutrient solution samples and soil extracts:

The procedure for analysis of solution samples and soil extracts is the same as described above for the standard line of organically-complexed Al.

RESULTS

Calibration line

A first calibration line was prepared for standard series A by plotting the Absorbance after a reaction time of 20 minutes, corrected for the blank, against the inorganic-Al concentration (Figure 1). Figure 1 also gives a calibration line for a reaction time of 1 minute. The calibration lines did not differ between dates. Both lines had an intercept with the x-axis of about 2 μM . The absorbance measured after 1 min was about 85 % of the absorbance for a reaction time of 20 minutes, for solutions $> 5 \mu\text{M}$.

Figure 2 shows the absorbance values of a 1 minute PCV reaction for various mixture of inorganic-Al and Al-malate (standard series B). All series can be described by straight lines. The slope of the lines decreased proportionally with an decreasing percentage of inorganic Al. A pure Al-malate solution (0 % inorganic Al) gave hardly any absorbance even at a concentration of 25 μM . As shown in Fig. 3 the absorbance of all solutions of series B can be adequately

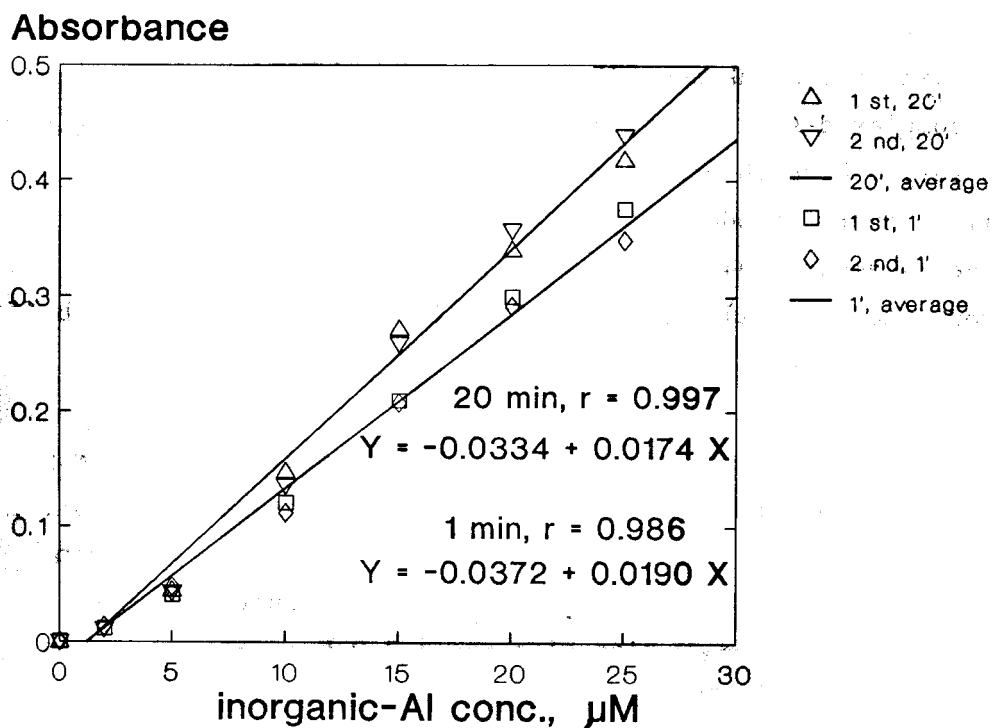


Figure 1. Calibration line of absorbance versus Al concentration for series A (only inorganic Al present) for a reaction time of 1' and 20'. For both reaction times calibration lines were measured on two occasions.

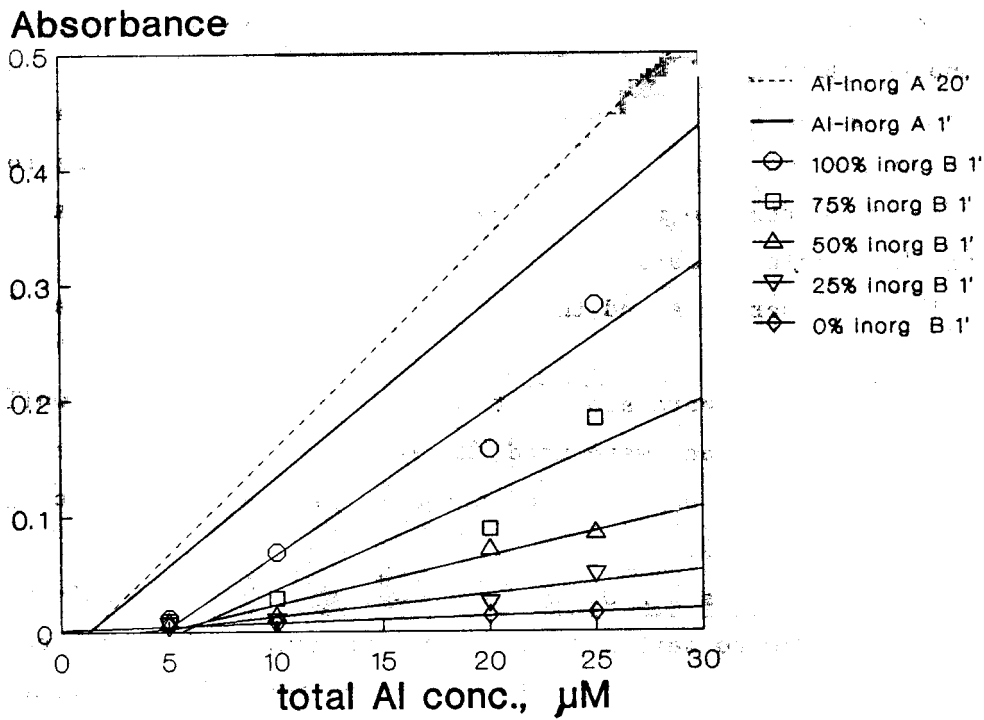


Figure 2. Calibration line of absorbance versus total Al concentration for series B, consisting of various mixtures of inorganic Al and Al-malate; for reference the lines of Fig. 1 are included.

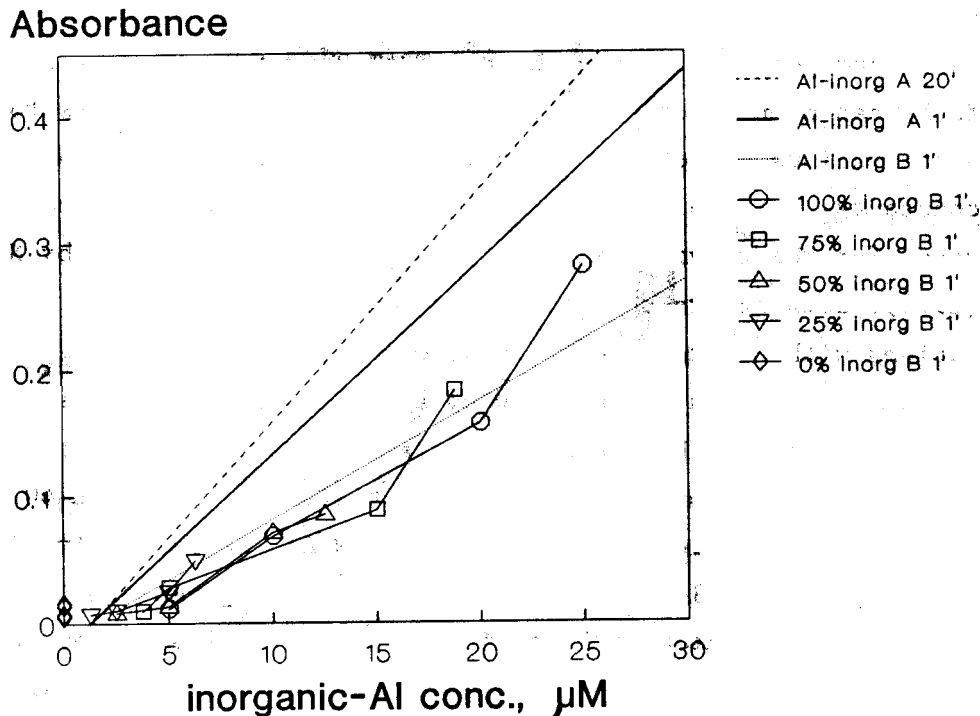


Figure 3. The same data as in Fig. 2, but plotted against the inorganic Al concentration in the mixture.

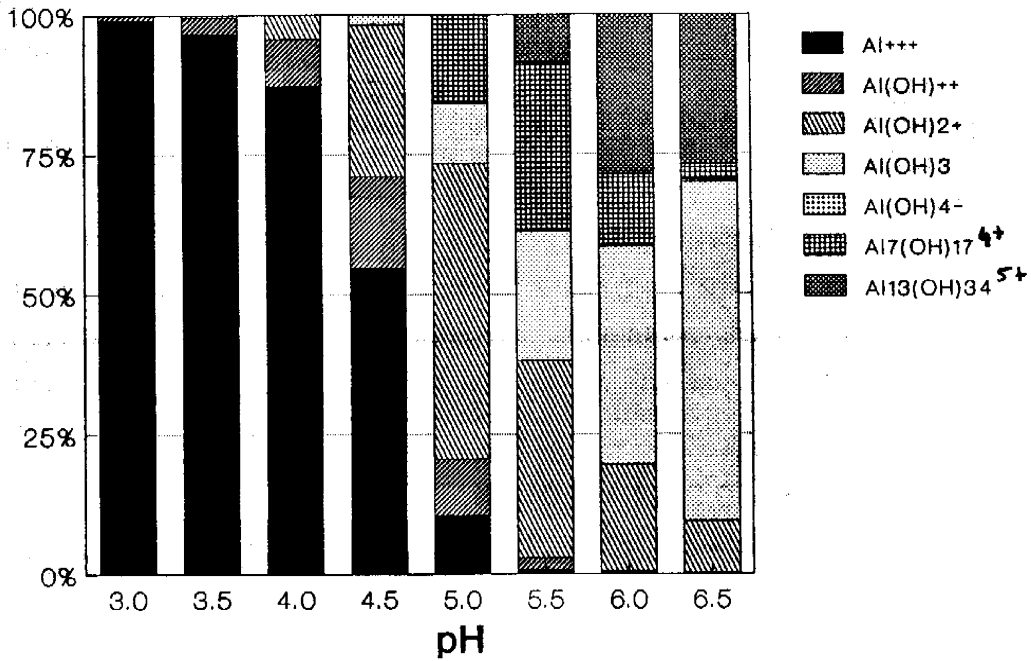
predicted on the basis of the inorganic Al concentration of the solution; the Al-malate component does not interfere. All the data for series B are clearly below the 1' calibration line for series A, however. The line for the 100 % inorganic Al (Fig. 2) runs almost parallel to the 1' calibration line for series A, but it is shifted to the right by approximately 3 mM and has an intercept with the x-axis at about 5 μ M.

Between calibration series A and the 100% inorganic solutions of series B two differences exist:

1. The standard solutions A were stored at the final concentration, while for series B freshly made solutions were used. However, no differences were found between old and freshly made solutions of series A, so storage time could not explain the differences found.
2. To the solutions of series A HNO_3 was added while in series B this was not done. When HNO_3 was added to series B, the 100% inorganic solution became equal to that of series A. By adding HNO_3 , however, the solutions containing Al-malate gave a much higher absorbance.

As differences in pH between were probably responsible for the differences in absorbance between series A and B, the pH of each solution was measured. Using this pH and the total inorganic Al concentration as input, the programme TITRATOR (Cabaniss, 1987) was used to predict the fraction of inorganic Al in various monomeric and polymeric forms. With TITRATOR distribution of Al over the various forms can be predicted for equilibrium conditions. Inputs to the programme are the various hydrolysis and polymerization reactions and their equilibrium constants. For the hydrolysis reactions parameters of Lindsay (1979) were used, for the polymerization reactions parameters given by Nair and Prenzel (1978). Details are described in appendix 3. As shown in Fig. 4A and B for solutions with a total inorganic Al concentration of 5 and 25 μ M, respectively, the Al_3^+ form is dominant up to a pH of 4.0. Between a pH of 4 and 5 monomeric Al-hydroxides become more abundant and polymerization starts, especially at higher Al concentrations. If the pH is above 5.0 about 40 and 80% of the Al is in a polymeric form for 5 and 25 μ M Al, respectively. In appendix 6 the pH values of all solutions of series B are given and the predicted Al forms. In Fig. 5 the absorbance is plotted for all solutions of series A and B versus the calculated concentration of monomeric inorganic Al.

A. Al-forms



B.

Al-forms

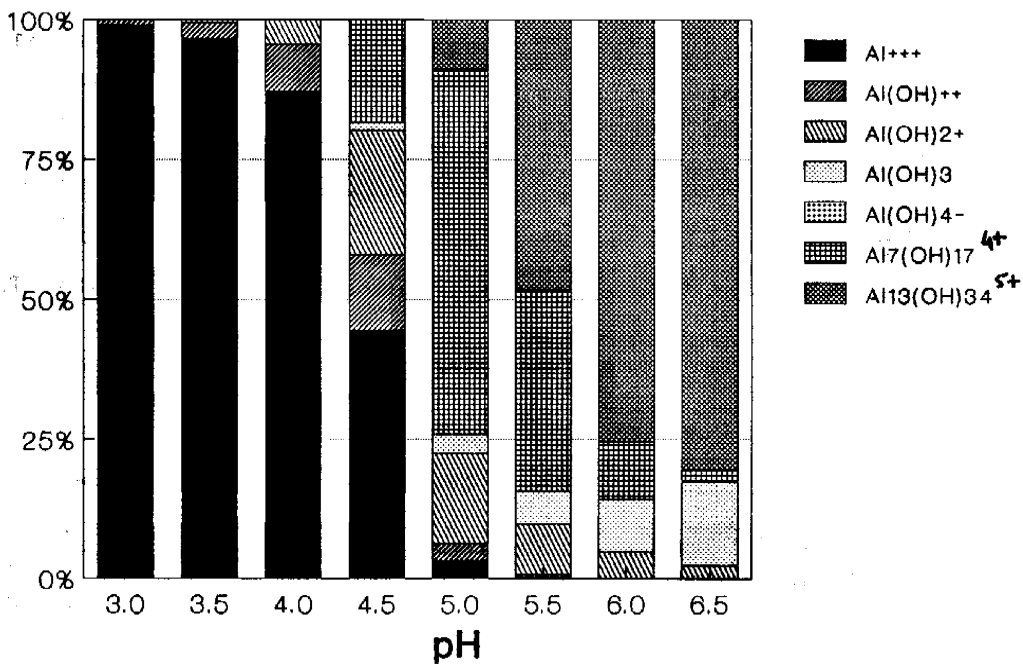


Figure 4. Calculated distribution of inorganic Al over various monomeric and polymeric forms as a function of pH. A. for 5 μM , B. for 25 μM . Calculations were made with the TITRATOR programme; parameters used are given in appendix 6.

Absorbance

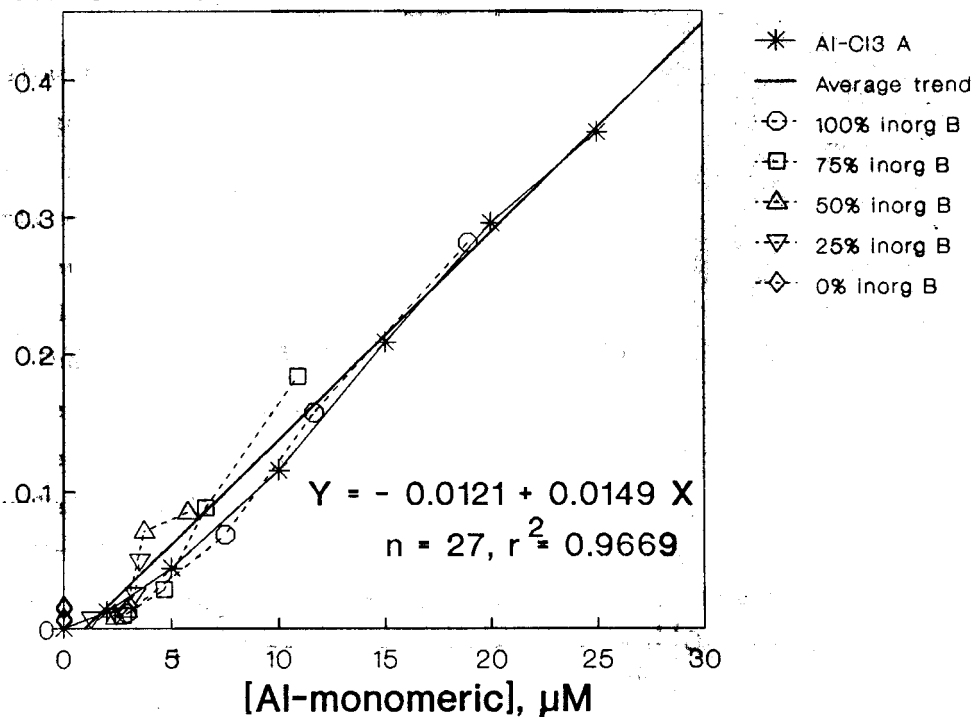


Figure 5. Calibration line for series A and B, using as an x-axis the calculated concentrations of monomeric Al, based on pH and inorganic Al concentration of each sample.

The difference between series A and B has disappeared completely and a single straight calibration line gives an adequate description of the data. The intercept with the x-axis now is $0.8 \mu\text{M}$.

We therefore conclude that the 1' PCV reaction is valid in the range $1 - 25 \mu\text{M}$ as measuring technique for monomeric, inorganic Al. The presence of polymeric forms occurring at higher pH values or Al-malic acid complexes does not interfere with the measurement.

Interference

Some further tests were performed to check on the possible interference with the measurement of the presence of Cl^- or NO_3^- ions. No interference was found by adding up to 1 M KCl or 1 M KNO_3 .

As reaction time appears to be critical, absorbance was measured as a function of reaction time between 15" and 20'. The results in Fig. 6 show a gradual increase of colour intensity with time. Small deviations around a reaction time have only a small effect on the absorbance.

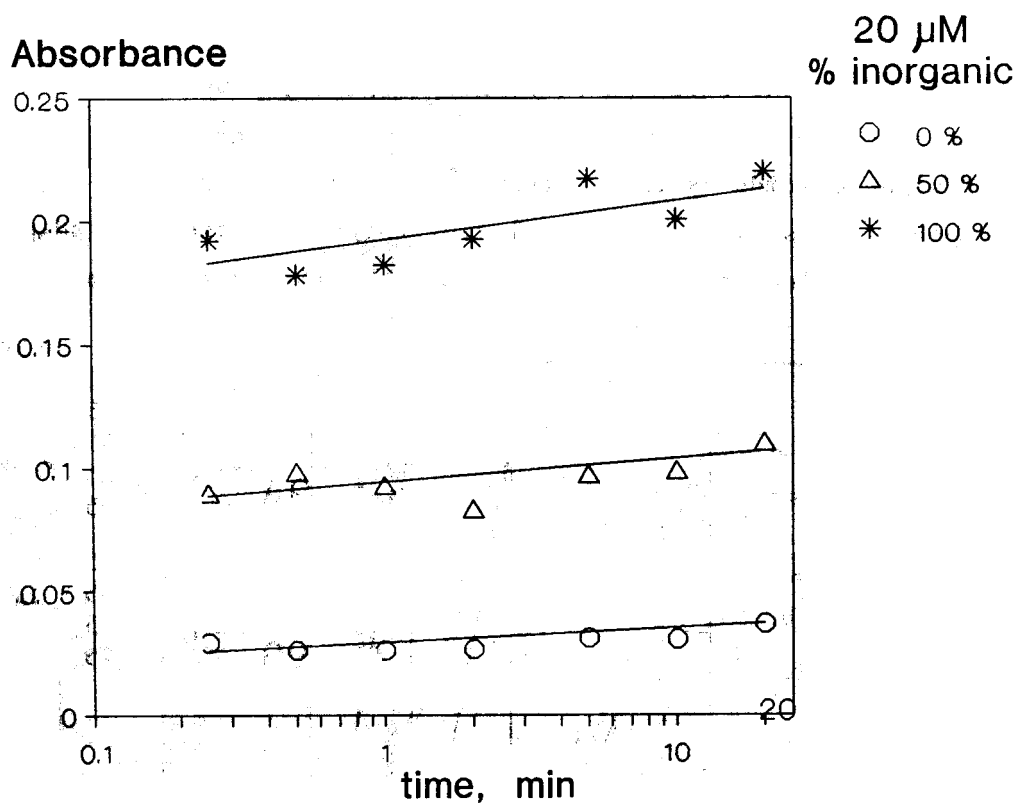


Figure 6. Effect of reaction time on absorbance for three solutions.

Analysis of nutrient solution samples

In Fig. 7 results of the analysis of a first series of nutrient solution samples is shown. The samples were taken from a solution culture experiment in May and June 1990 with *Mucuna pruriens utilis*. Four types of solution were used (two Al-concentrations * two Ca-concentrations). The solution pH was daily measured and corrected to a value of 4.2. The solution was renewed once a week and solution samples were stored at -20 °C in the dark. The 1' PCV test shows that at the time of renewing the solution very little monomeric, inorganic Al remained. From previous experiments we expect that the total Al content of the solution showed little change during the week (Hairiah et al., 1990), so probably the form in which Al occurs has changed.

Two possibilities which should be investigated further are:

- polymerization during the daily pH-rise, not compensated by correcting the pH,
- excretion of organic acids by the roots.

If the first mechanism is important, simulating the actual sequence pH changes of the solution should lead to a similar decrease in 1'-PCV reactive Al.

monomeric-Al, μM

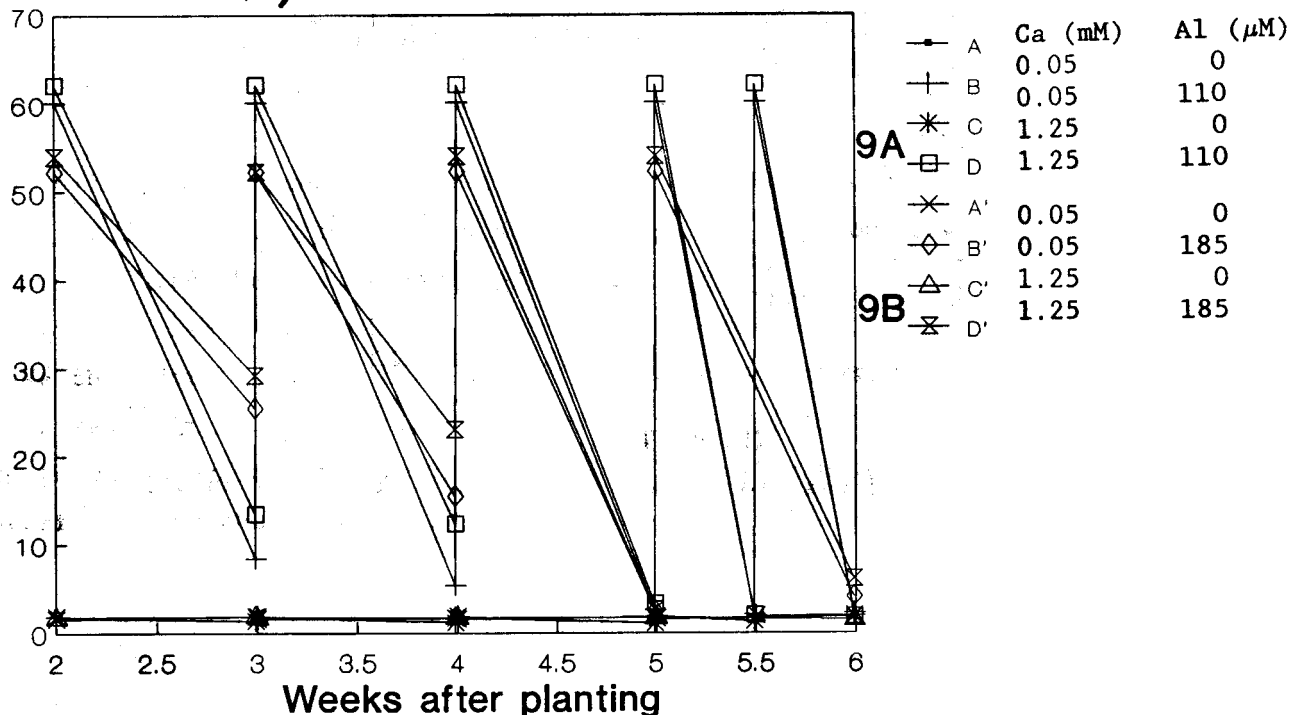


Figure 7. Inorganic monomeric Al concentration in the course of a solution culture experiment, with weekly refreshing of the solution.

A simple experiment was carried out to study the effect of pH fluctuations on the monomeric Al concentration. A nutrient solution of pH 4.2 was prepared, as used in experiment 9B (0.05 mM Ca, 185 μ M Al). By adding KOH 8 series were made with a pH of 4.2, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5, respectively. Samples were stored at room temperature. After 18 hours at the increased pH, monomeric Al was measured in all samples. Subsequently, pH was restored to 4.2 by adding HNO₃ and after 1 and 18 h the concentration of monomeric Al was again determined. In a second cycle pH was again raised and after 1 and 18 h the concentration of monomeric Al was determined. Figure 8 shows the results of this experiment.

Increasing the pH to a value of 4.5 and higher drastically reduced the monomeric Al concentration. Restoring pH to 4.2 gave an increase of monomeric Al, but to only about 20% of the original level. From this result we conclude that the observed reduction in monomeric Al in the nutrient solutions could easily have been caused by the daily increase in pH. In future experiments a strict pH control is required to test whether organic acid excretion may have some additional effects.

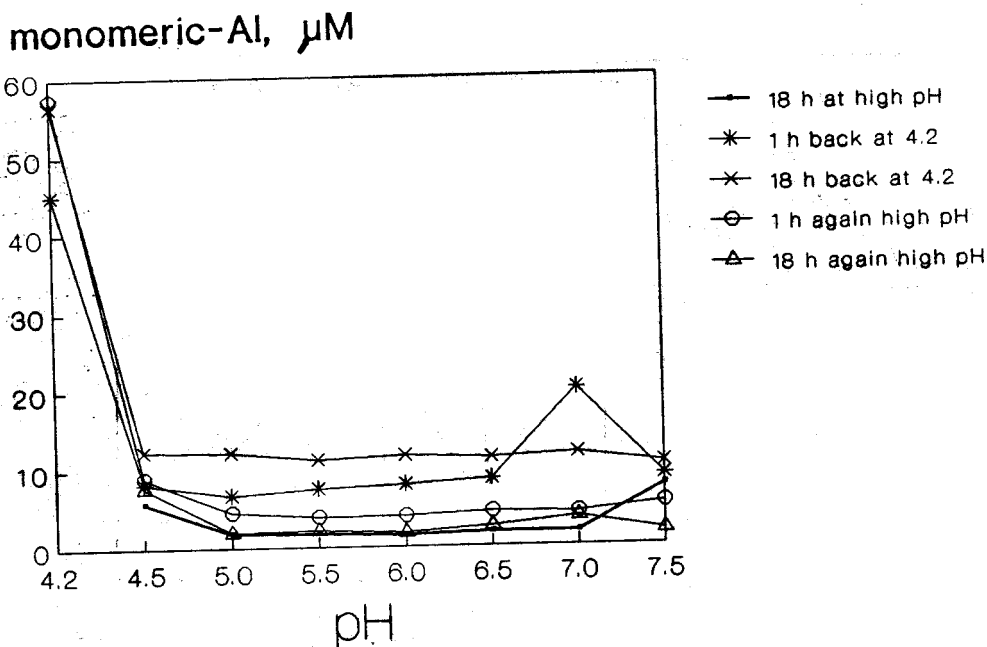


Figure 8. Monomeric Al concentration of a nutrient solution during shifts in solution pH.

DISCUSSION

The 1'-PCV technique is apparently suitable for measuring inorganic, monomeric Al-forms. An unsolved problem is the difference between our results and the calibration lines published by Kerven *et al.* (1989). They found straight calibration lines for calibration series B when the fraction, f , of total Al in inorganic form was plotted against the fraction of total Al which reacted. For the X-axis they calculated an apparent Al concentration for the 1' colour reaction, read from calibration line for the 20' reaction time.

From the data in figure 9A they conclude that a calibration line of the following form can be used:

$$f = -0.2747 - 0.009819 C + 1.696 X, r^2 = 0.9783$$

When our data are presented in the same way (Fig. 9B), the lines for the various concentrations are not straight, their slope is less and even the relative position of the various concentrations is different. A fundamental

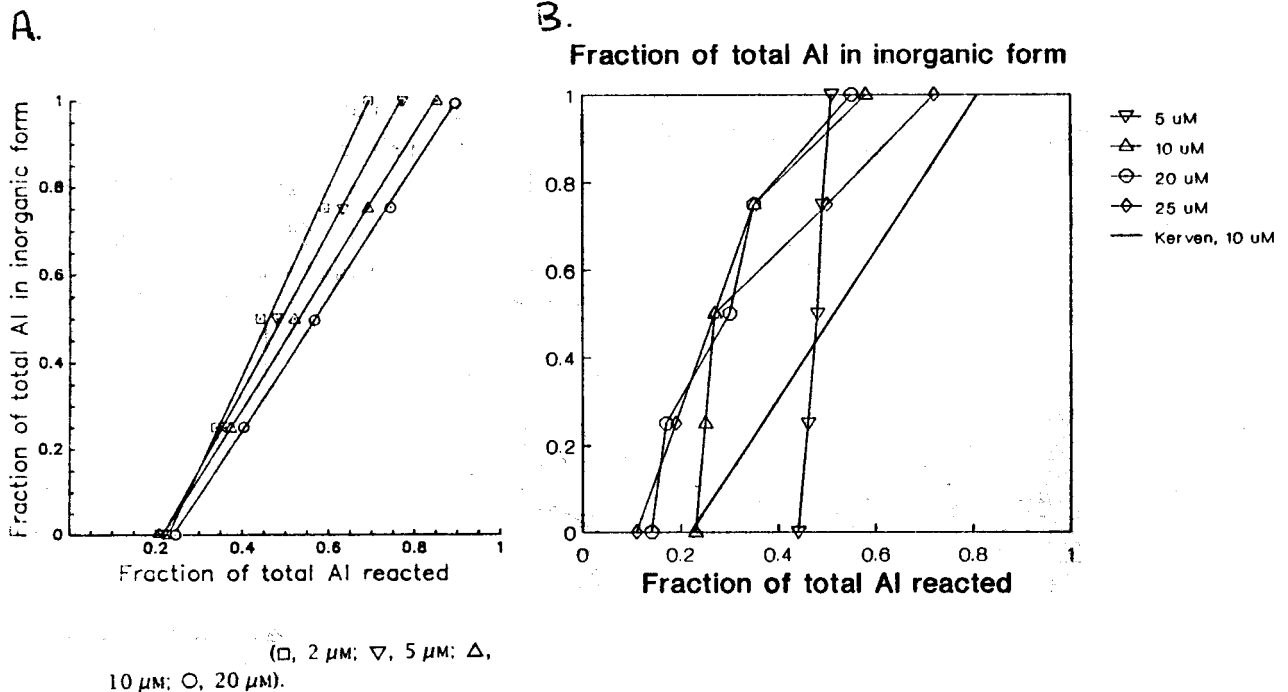


Figure 9 A. Calibration line as published by Kerven *et al.* (1989); B. our data for series B plotted in the same way.

difference between our results, based on Fig. 6 and the calibration procedure of Kerven *c.s.* is, that in their approach no allowance is made for the possible occurrence of polymeric Al forms in the solutions for the standard line.

The measurements of monomeric Al in solution samples indicate the need for a strict pH-control in Al-experiments. Daily control of pH is not enough for NO_3 -fed plants, as any increase to a pH of 4.5 or higher leads to loss of monomeric Al. The small experiment with pH changes shows that the loss of monomeric Al at higher pH is only partially reversible when pH is restored to 4.2. This observation indicates that not only thermodynamic equilibria, as considered in the Titrator programme, are important, but also kinetic processes. If polymerization occurs at higher pH values, this can be considered as an almost *irreversible* process during a solution culture experiment. Apart from polymerization, Al-phosphates might be formed which either precipitate or stay in solution in poorly reversible form. To avoid such complications future experiments should be performed with an automatic pH-stat system.

ACKNOWLEDGEMENTS

Thanks are due to W.J. Chardon and J. Brill for discussions on Al speciation.

REFERENCES

- Cabaniss S E 1987 TITRATOR: an interactive program for aquatic equilibrium calculations. *Environ. Sci. Technol.* 21: 209-210.
- Dougan W K and A L Wilson 1974 The absorptiometric determination of aluminium in water. A comparison of some chromogenic reagents and the development of an improved method. *Analyst* 99: 413-430.
- Hairiah K, I stulen and P J C Kuiper 1990 Aluminium tolerance of the velvet beans *Mucuna pruriens* var. *utilis* and *Mucuna deeringiana*. I. Effects of aluminium on growth and mineral composition. in: M.L. van Beusichem (ed.) *Plant Nutrition - Physiology and Applications*. Kluwer Academic Publishers. pp 365-374.
- Kerven G L, D G Edwards, C J Asher, P S Hallman and S Kokot 1989 Aluminium determination in soil solution. II. Short-term colorimetric procedures for the measurement of inorganic monomeric Aluminium in the presence of organic acid ligands. *Austr. J. Soil Res.* 27: 91-102.
- Lindsay W L 1979 *Chemical equilibria in soils*. New York, John Wiley & Sons. 449 pp
- Nair V D and J Prenzel 1978 Calculations of equilibrium concentration of mono- and polynuclear hydroxyaluminium species at different pH and total aluminium concentrations. *Z. Pflanzenernähr. Bodenk.* 141: 741-751.

APPENDIX 1. Preparation of solutions for calibration series B.

Series of 10 μM concentration:

% inorg. Al	conc. inorg-Al (μM)	conc. org-Al (μM)	100 μM AlCl ₃ (ml)	100 μM Al-malate (ml)
0	0	10.00	0	10.00
25	2.50	7.50	0.250	7.50
50	5.00	5.00	0.500	5.00
75	7.50	2.50	0.750	2.50
100	10.00	0	1.000	0

Series of 15 μM concentration:

% inorg. Al	conc. inorg-Al (μM)	conc. org-Al (μM)	100 μM AlCl ₃ (ml)	100 μM Al-malate (ml)
0	0	15.00	0	15.00
25	3.75	11.25	0.375	11.25
50	7.50	7.50	0.750	7.50
75	11.25	3.75	1.125	3.75
100	15.00	0	1.500	0

Series of 20 μM concentration:

% inorg. Al	conc. inorg-Al (μM)	conc. org-Al (μM)	100 μM AlCl ₃ (ml)	100 μM Al-malate (ml)
0	0	20.00	0	20.00
25	5.00	15.00	0.500	15.00
50	10.00	10.00	1.000	10.00
75	15.00	5.00	1.500	5.00
100	20.00	0	2.000	0

Series of 25 μM concentration:

% inorg. Al	conc. inorg-Al (μM)	conc. org-Al (μM)	100 μM AlCl ₃ (ml)	100 μM Al-malate (ml)
0	0	25.00	0	25.00
25	6.25	18.75	0.625	18.75
50	12.50	12.50	1.250	12.50
75	18.75	6.25	1.875	6.25
100	25.00	0	2.500	0

APPENDIX 2. Absorbance data used for the construction of calibration lines of series A.

20 minutes reaction:

inorg-Al conc(μM)	AlCl_3		avg.
	abs. -bl.	abs. -bl	
	I	II	
Blank	0.032	0.032	0.032
2	-	0.011	0.011
5	0.044	0.043	0.044
10	0.147	0.136	0.141
15	0.272	0.260	0.266
20	0.340	0.357	0.349
25	0.418	0.439	0.428

1 minute reaction time:

Al-inorg conc(μM)	AlCl_3		avg
	abs. -bl.	abs. -bl	
	I	II	
Blank	0.035	0.029	-
2	0.011	0.013	0.012
5	0.041	0.048	0.044
10	0.120	0.112	0.116
15	0.209	0.208	0.208
20	0.300	0.292	0.296
25	0.375	0.348	0.362

APPENDIX 3. Absorbance data used for calibration lines of series B.

Solution B (5, 10, 20, 25 μM):

inorg-Al conc (μM)	inorg-Al conc. (μM)	corrected conc (μM)**	1' reaction abs-bl
100	5.00	2.90	0.011
	10.00	7.50	0.068
	20.00	11.64	0.157
	25.00	18.91	0.281
75	3.75	2.74	0.009
	7.50	4.62	0.028
	15.00	6.53	0.088
	18.75	10.89	0.183
50	2.50	2.40	0.008
	5.00	2.90	0.013
	10.00	3.72	0.071
	12.50	5.76	0.085
25	1.25	1.25	0.007
	2.50	2.41	0.010
	5.00	3.29	0.025
	6.25	3.52	0.049
0	0	0	0.005
	0	0	0.007
	0	0	0.014
	0	0	0.016

**) corrected by calculation of TITRATOR program, based on solution pH (Appendix 6).

APPENDIX 4. Effect on absorbance of adding HNO₃ to AlCl₃ solutions.

Soln.I : 1 day old, + 1 ml of 1 M HNO₃ (series A)
 Soln.II : freshly made, + 1 ml of 1 M HNO₃ (series B')
 Soln.III: freshly made, without HNO₃ (series B)

inorg-Al conc(μ M)	Soln.I abs-bl	Soln.II abs-bl	Soln.III abs-bl	Soln.II: Soln.III
5	0.033	0.035	0.012	2.917
10	0.106	0.110	0.058	1.897
15	0.189	0.194	0.150	1.294
20	0.271	0.275	0.219	1.256
25	0.344	0.338	0.307	1.103

APPENDIX 5. Parameters used and example of the output of a TITRATOR programme.

APPENDIX 6. Solution pH and calculated distribution of Al over various monomeric and polymeric Al-forms for calibration series B. All concentrations are expressed in μM . Al_t = total Al concentration; Al_i = inorganic Al concentration; Σ -mono = sum of monomeric forms; $\text{P}_7 = \text{Al}_7(\text{OH})_{17}$; $\text{P}_{13} = \text{Al}_{13}(\text{OH})_{34}$; Σ -poly = sum of polymeric forms.

Al_t	Al_i	pH	Al_3^+	$\text{Al}(\text{OH})^{++}$	$\text{Al}(\text{OH})_2^+$	$\text{Al}(\text{OH})_3$	$\text{Al}(\text{OH})_4^-$	Σ -mono	P_7	P_{13}	Σ -poly
<i>100% inorganic</i>											
5	5	5.73	0.009	0.048	1.356	1.487	0.002	2.90	1.152	0.942	2.09
10	10	4.79	2.009	1.182	3.826	0.482	0.000	7.50	2.479	0.020	2.50
20	20	4.69	4.169	1.951	5.014	0.501	0.000	11.64	8.250	0.110	8.36
25	25	4.53	9.750	3.158	5.616	0.389	0.000	18.91	6.042	0.025	6.07
<i>75% inorganic</i>											
5	3.8	5.98	0.002	0.018	0.918	1.79	0.005	2.74	0.042	0.592	1.02
10	7.5	5.06	0.451	0.494	2.977	0.698	0.000	4.62	2.764	0.113	2.88
20	15	4.93	1.084	0.882	3.94	0.685	0.000	6.59	8.01	0.394	8.40
25	18.8	4.71	3.698	1.81	4.87	0.510	0.000	10.89	7.74	0.108	7.85
<i>50% inorganic</i>											
5	2.5	6.23	0.004	0.006	0.533	1.849	0.008	2.40	0.053	0.004	0.06
10	5	5.91	0.003	0.025	1.077	1.788	0.004	2.90	0.80	1.303	2.10
20	10	5.39	0.076	0.179	2.305	1.155	0.007	3.72	4.51	1.772	6.28
25	12.5	4.99	0.752	0.701	3.594	0.717	0.002	5.76	6.37	0.360	6.73
<i>25% inorganic</i>											
5	1.3	6.50	0.000	0.001	0.166	1.074	0.009	1.25	0.000	0.000	0.00
10	2.5	6.27	0.000	0.005	0.499	1.897	0.009	2.41	0.044	0.045	0.09
20	5	6.36	0.000	0.005	0.575	2.691	0.016	3.29	0.221	1.49	1.71
25	6.3	5.32	0.103	0.205	2.252	0.961	0.005	3.52	2.36	0.36	2.72

APPENDIX 7. Inorganic-Al PCV, nutr. solution exp 9A & 9B, 1990.

a = absorbance, corrected for blank; c = calculated concentration of inorganic, monomeric Al (μM).

Sol.	1st, 3WAP		2nd, 4WAP		3th, 5WAP		4th, 5.5WAP		5th, 6WAP		FRESH SOLN	
	a	c	a	c	a	c	a	c	a	c	a	c
<i>Exp 9A</i>												
A	0.0085	1.38	0.007	1.28	0.004	1.08	0.013	1.68	0.0155	1.85	0.0135	1.72
B	0.1135	8.43	0.068	5.38	0.0265	2.59	0.019	2.09	0.0215	2.26	0.167	60.10
C	0.008	1.35	0.005	1.15	0.004	1.08	0.007	1.28	0.0165	1.92	0.016	1.89
D	0.1875	13.40	0.1705	12.26	0.0368	3.28	0.017	1.95	0.0145	1.79	0.173	62.11
<i>Exp 9B</i>												
A	0.016	1.89	0.013	1.68	0.0145	1.79	-	-	0.0145	1.79	0.0115	1.58
B	0.064	25.54	0.218	15.44	0.0240	2.42	-	-	0.0485	4.07	0.1435	52.21
C	0.0155	1.85	0.014	1.75	0.0135	1.72	-	-	0.01	1.48	0.0135	1.72
D	0.075	29.23	0.331	23.03	0.0270	2.62	-	-	0.0785	6.08	0.1485	53.89