

11-1. REEXAMINATION OF ANALYTICAL METHODS FOR Al AND Mo IN PLANT MATERIAL

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11-1. REEXAMINATION OF ANALYTICAL METHODS FOR AL AND Mo IN PLANT MATERIAL

A. Analysis for Al in plant material

Aluminon is a kind of pigment which easily dissolves into water and shows weak acidity. Under weak acid or neutral condition, it combines with a trace amount of aluminium and develops red color. Every aluminon colorimetric method has the disadvantage that the Al-aluminon complex is unstable. Some researchers intend to improve the method. Authors carried out reexaminations of Al analysis according to "Saibai Shokubutsu Bunseki Sokuteiho" (2) and checked the following points

1. Heating time;
2. Time course of discoloring;

3. Recovery of Al added to plant material.

Heating time

Color development of aluminon-Al complex is promoted by heating. Excessive heating may result in discoloring. In order to investigate the relation between heating time and color intensity, the volumetric flasks containing the reaction mixture were put into boiling water for 2, 4, 6, 8, 10 and 15 min, and the absorbances were measured 120 min after the heating began. From the results shown in Table 1, it is concluded that the reaction mixture should be heated for 6 min.

Table 1. Relation between color intensity and heating time.

N ^o of measure	heating time (min.)	absorbance (520 nm)					
	2	4	6	8	10	15	
1	0.195	0.199	0.201	0.203	0.200	0.195	
2	0.192	0.193	0.201	0.202	0.189	0.203	
average	0.194	0.196	0.203	0.203	0.195	0.199	

Time course of discoloring

1 g of the standard sample of soybean was ashed in a muffle furnace and extracted with 50 ml of N HCl. Mixtures of 5 ml of the extract and the reagents were heated for 6 min, and the changes in color intensities were investigated.

As shown in Fig. 1., the rate of discoloring is very high up to 60 min after color development and is decreased with increasing of time. The rate 120 min after color development is less than 1 percent per hour. Therefore, it is concluded that the

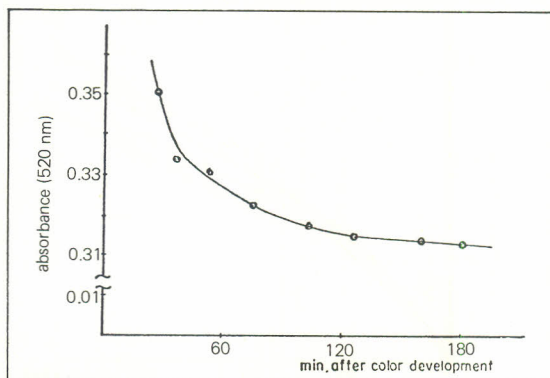


FIG. 1. Relation between color intensity of aluminon-Al complex and time after color development.

absorbance should be measured 120 minutes after color development.

Recovery test

In order to check the recovery of Al added to a standard sample of soybean, authors determined Al concentrations in samples, to which were added 1 mg of Al or not.

From the results shown in Fig. 2 and Table 2, the average recoveries are 97 - 99%, and thus it is concluded that this method is applicable for analyzing Al concentration in soybean plant material.

From the facts described above; it was decided to use the following method:

Table 2. Results of recovery test (Al)

N ^o of measure	Concentration of Al (mg/g) at 520 nm			
	Standard sample		Standard sample + 1 mg of Al	
	120 min.*	150 min.*	120 min.*	150 min.*
1	1.34	1.30	2.29	2.28
2	1.28	1.29	2.28	2.26
3	1.32	1.28	2.29	2.31
4	1.30	1.35	2.25	2.27
5	1.29	1.24	2.28	2.27
average	1.31	1.29	2.28	2.28
C.V.(%)	1.6	2.7	0.7	0.8

* Time after color development

Recovery

$$120 \text{ min.} * 2.28 - 1.31 = 0.97 \dots \dots \dots 97\%$$

$$150 \text{ min.} * 2.28 - 1.29 = 0.99 \dots \dots \dots 99\%$$

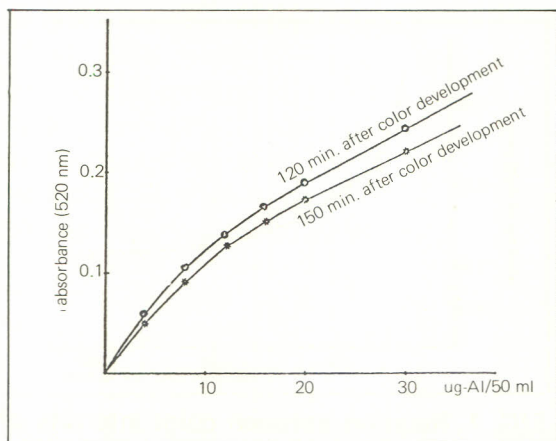


FIG. 2. Calibration curve

Reagent

1. 0.22% aluminon solution (in distilled water)
2. 1% mercaptoacetic acid solution (in distilled water)
3. 20% ammonium acetate buffer pH 4.8
4. Al standard solution
2 ml of Al stock solution (1000 ppm) is transferred to 1 liter-volumetric flask and made up to volume with distilled water.

Procedure

Put 1 g of dried ground sample into a 30 ml pyrex beaker and ash in a muffle

furnace at 500°C for 5 hours. After cooling, transfer 5 ml of 5N HCl or 5N H₂SO₄ to the beaker and boil for 2 min on a hot plate. Transfer the extract to 50 ml – volumetric flask, make up to volume and shake. Put 1 ml of the sample solution into a 50 ml – volumetric flask, add 10 ml of ammonium acetate buffer, 20 ml of distilled water and 1 ml of mercaptoacetic acid solution and shake. Heat in boiling water for 6 min., and make up to volume with distilled water. Measure absorbance at 520 nm and compare with the absorbances of Al standard.

Prepare each Al standard by placing the amount of 2 ppm solution and water indicated below, 1 ml of 5N HCl or 5N HCl or H₂SO₄, 10 ml of ammonium acetate buffer and 1 ml of mercaptoacetic acid solution in a 50 ml, volumetric flask.

Al standard hg/50ml	Al solution ml (2 ppm)	distilled water ml
0	0	30
4	2	28
8	4	26
12	6	24
16	8	22
20	10	20
30	15	15

B. Analysis for Mo in plant material

Mo in plant material was analyzed routinely with the dithiol method by one of the authors in Japan. However, the experimental condition in CPAC (water, reagents and instruments etc.) might possibly differ from NIAS⁽¹⁾ in Japan. In particular, 50 ml-volumetric flask had to be used in place of separating funnels due to a careless oversight by one of the authors and the method was modified as follows:

Reagents

1. dithiol reagent: Transfer 1 ml of dithiol to a brown colored bottle

containing 500 ml of 0.5N NaOH solution warmed to about 30°C. Agitate using glass rod. When most of dithiol is dissolved, add mercaptoacetic acid until a slight precipitation appears. Store the bottle in a refrigerator.

2. 50% sulfuric acid (V/V)
 3. ferric ammonium sulfate solution: Dissolve 9.1 g of NH₄Fe(SO₄)₂ in 100 ml of 2% H₂SO₄ solution.
 4. 50% tartaric acid (W/V)
 5. 50% potassium iodide solution (W/V)
 6. 50% ascorbic acid solution
 7. 10% thiourea solution
- reagents 5, 6, 7 should be prepared just before the determination.
8. Mo standard solution (1 ppm): Dilute the stock solution (100 ppm) to 1 ppm with distilled water.

Procedure

Put 1 - 5 g of dried ground plant material into a 30 ml-pyrex beaker. Ash in a muffle furnace at 500°C for 5 hours. After cooling, transfer about 2 ml of distilled water and 10 ml of 50% sulfuric acid to the beaker. Boil on a hot plate for 3 min. Transfer the extract to a 50 ml-volumetric flask and add distilled water until the volume of solution reaches about 30 ml. Transfer 0.3 ml of ferric ammonium sulfate solution and 0.3 ml of KI solution to each flask. Shake occasionally for 10 - 15 minutes. Add 4 - 5 drops of ascorbic acid solution. The brown color disappears completely. Transfer 0.3 ml of tartaric acid solution and 2 ml of thiourea solution, mix, and add 4 ml of dithiol reagents. Stopper and shake vigorously and then let stand for 30 minutes. Then transfer 5 ml of isoamyl acetate to the flask. Shake vigorously and let stand for 20 minutes. Add distilled water until the interface

between the water and the isoamyl acetate reaches to the neck of flask. Transfer the isoamyl acetate phase to a centrifuge tube with a pipette and centrifuge at 2000 r.p.m. for 20 minutes. Measure the absorbance of the isoamyl acetate layer at 680 nm and compare with the absorbance of the Mo standard.

Prepare each of the Mo standards by placing the amount of 1 ppm solution and water indicated below and 10 ml of 50% H₂SO₄ in a 50 ml-volumetric flask.

Mo standard ug/50ml	Mo solution (1 ppm) ml	Water ml
0	0	20
2	02	18
4	4	16
6	6	14
8	8	12
10	10	10

Recovery Test

In order to confirm that the modified method is applicable to estimate Mo concentration in plant material, the recovery was examined. The calibration curve shown in Fig. 3 seems to be almost linear. The average recovery summarized in Table 3 is 99% and almost complete. Therefore, the authors conclude that the method described above is suitable to determine Mo concentration in plants.

Reference

- 1) ANNUAL Report of the National Institute of Agricultural Sciences. Division of Plant Nutrition. Tokyo, 1972.
- 2) HIRAMINE, J. Aluminium. In: **Saibai Shokubutsu Bunseki Sokuteiho**. Tokyo, Yokendo, 1976. p. 146-8.

Table 3. Results of recovery test (Mo)

No of measure	Content of Mo g/2g at 680 nm	
	Standard sample	Standard sample + 6 g of Mo
1	0.26	6.14
2	0.28	6.24
3	0.22	6.18
4	0.22	6.19
5	0.27	6.20
average	0.25	6.19

Recovery

$$6.19 - 0.25 = 5.94$$

$$5.94 - 6.00 \times 100 = 0.99\%$$

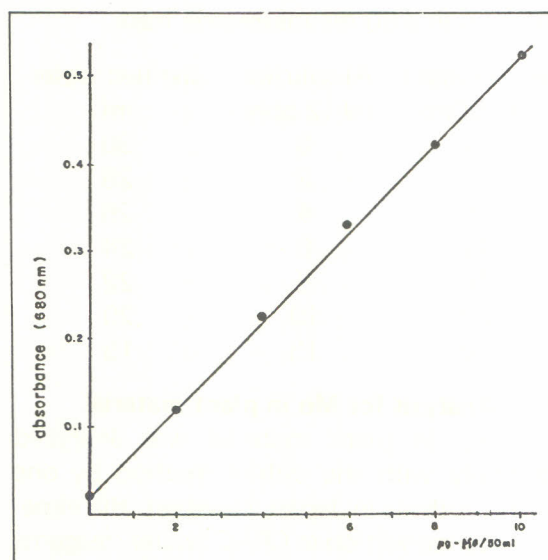


Fig. 3. Calibration curve (Mo)

Final report

Junji Ishizuka

April 2, 1980

- I — Reexamination of analytical methods for Al and Mo in plant material.
 - a. Aluminum

According to "Saibai Shokubutsu Bunseki Sokuteiho", analytical method for Al was carried out and the following pint were checked.

Results

1. Heating time

The reaction mixture should be heated for 6 min.

2. Time course of discoloring

The rate of discoloring was very high up to 60 min after color development and was decreased with increasing of time. The rate 120 min. after color development was less than 1% per hour. Therefore, it was concluded that the absorbance should be measured 120 minutes after color developments.

3. Recovery of Al added to plant material

Average recoveries were 97 - 99%, and thus it was concluded that this method was applicable for analusing Al concentration in soybean plant material.

From the facts shown above, it was decided to use the method described in the other sheets in detail.

According to annual report of division of plant nutrition (NIAS), dithiol colorimetric method was examined.

The results of recovery test showed that the modified method was applicable to analysis for Mo in plant material. The method was described in the other sheets in detail.

II — Effects of liming and phosphorus fertilization on status of mineral nutrients in soybean plants grown in the cerrado soil.

In order to investigate the effects of liming and P- fertilization on the macro - and micronutrients absorp-

tion and their mobilities within the soybean plants grown in experimental field, concentrations of Ca, Mg, Fe, Zn, Mn, P, Mo and Al were determined and the results were summarized as follows:

1. Plant growth

Liming and P-fertilization influenced beneficial effects on plant growth.

2. Ca

The concentrations were increased with increasing of both lime and P-application.

3. Mg

The concentrations were increased by liming, but effect of P was not clear.

4. Fe

The concentrations were decreased with increasing of liming and P-fertilization.

5. Zn

The concentrations in stems and roots were decreased by liming and P-fertilization, but in leave, their effects on Zn were not clear.

6. Cu

Liming and P-fertilization do not affect on Cu concentration.

7. Mn

The concentrations were decreased by liming and P-fertilization except in leaves, and the effects of P-fertilization were not clear.

8. P

P concentrations were increased with increasing of P-fertilization however the effects of liming is obscure.

9. Mo
When 4^t/ha of lime was applied, the Mo concentrations were increased by P-fertilization. But when 1,5^t/ha and 0,5^t/ha of lime were applied, they were decreased. Liming was seem to increased the ability of soil to supply Mo.

10. Al
The concentrations were decreased by liming and P-fertilization. The plant growth negatively correlate with the Al concentration in leaves and stems.

This work was described in the other sheets in detail.

Acknowledgement

The author stayed in Brasilia from Feb. 7 to April 2, 1980, and worked on the subjects described above. This work was greatly assisted by continued efforts of M.V. Mesquita. During his stay, he received guidance and encouragement from Dr. W. J. Goedert and got close relationship with all members of CPAC, especially Dr. E. Wagner. This work could not have been performed without helpfull discussion with the Japanese experts. He would like to thank all of them.

RELATIONSHIP BETWEEN NUTRITIONAL DISORDERS CAUSED BY Al AND MICRONUTRIENT STATUS.

(Junji Ishizuka)

Nutritional disorder should not considered to be caused by excess or deficiency of a sole nutrient, but influenced by interaction of various nutrients. For example, Zn deficiency is stimulated by P-fertilization; Mo uptake by plants is reduced by sulfate addition and increased by liming and P-fertilization; Cu of Mn induce Fe deficiency. In my experiments with rice plants, I found that disorder by heavy metals is promoted by N-deficiency. In relation to Al, we should be interested in the interaction among other nutrients such as P, N, Mn, and Mo, etc.

Mesquita et al intend to clarify the relationship amogn Al tolerance of 15 soybean varieties, P-fertilization and liming.

Recently, I observed the soyvean plants in his experimental field, and I recognized the effects os P-fertilization and liming on plant growth and differences among varieties. On the other hand, I was interested in nodule formation.

Generally, root nodules appear up to first trifoliage-leaf emergence date. The soybean plants grown by Dr. Milton were nodulated, but the main root were not. From these phenomena, I supposed that the factor repressed nodulation in early growth stage in cerrado soil. Therefore, the effect of various elements other than Al on nodulation should be examined, but I shall not be able to solve the problem because of my short-term stay.

RELATIONSHIP BETWEEN
NUTRITIONAL DISORDERS CAUSED
BY AI AND MICRONUTRIENT STATUS.

(Junji Ishizuka)

(Feb. 21-22)

Analysis for nutrients in soybean plants grown in Mesquit's experimental field.

Sampling:

- varieties. . . . 3 (tolerant, moderate, sensitive)
- liming level3 (500kg/ha, 1500kg/ha, 4000kg/ha)
- P-fertilization level . . .3 (160kg/ha, 778kg/ha, 1374kg/ha)
- Plant part3 (leaf, stem, root)
- Total3 x 3 x 3 x 3 = 81

(Feb. 25-26)

Sample preparation

(Mar. 3-4)

Reexamination of analytical methods for Al and Mo.

- Al - Aluminon colorimetric method
- Mo - Dithyol colorimetric method (as soon as reagents and implements arrive from Japan)

(Mar. 5-21)

Analysis:

N. P. K. Ca. Mg. Al
Fe. Mn. Zn. Mo. Cu. Zu.

- A) Visit to Araxá Experiments and farm in São Paulo
- B) Preparation of Report.