Annex I.

Candidate Parameters for Catchment Analysis

For description of elemental dynamics, the following items of each material may be candidate parameters. Number of the plots and frequency of surveys depend on the parameter.

Materials	Items of analysis

Water sample:
  Rain(Precipitation)
  Through fall,
  Stem flow
  Soil water
  Ground water
  Stream water
	Amount,
	pH, EC (Λ),
	Cation (NH_4^+, Na^+, K^+, Ca^{2+}, Mg^{2+}),
	Anion (NO_3^-, Cl^-, NO_2^-, PO_4^{3-}, SO_4^{2-})
	Alkalinity

Plant sample :
  Fresh leaf
  Fallen
  litter
  Litter layer
  Root system
	Weight,
	N, P, S, K, Ca, Mg, F, Cl,
	Al, Fe, Mn, Cu, Zn, Pb, Cd

Though the detailed methods for each parameter are not shown in this manual, the procedures of fresh leaf analysis, which was the optional item in the preparatory phase, are only described below.

Ex.) Fresh leaf analysis

1) Introduction

To evaluate the pollution level, dry deposition and nutrient condition of trees, fresh leaves should be analyzed on nutrients, halogens and heavy metals. These elements are absorbed from root or accumulated as the dry deposition. Nutrient condition may be affected by soil acidification.

2) Sampling method

● **Selection of sample tree**

Five of trees needed for the sampling should be selected according to the following criteria:

(a) The trees are spread over the total plot area, or around the plot, if the stand is homogeneous over a larger area;

(b) The trees belong to the predominant and dominant classes (forest with closed canopy) or to the trees with average height plus-minus 20% (forest with open canopy);

(c) The trees are in the vicinity of the soil sampling plots. However, care must be taken that the main roots of the sample trees have not been damaged by soil sampling.

(d) The trees should be representative for the mean defoliation level of the plot (within 5%).

● **Collection of leaf sample**

500 g of fresh leaf samples should be collected from the top of crown or outer canopy of the selected trees. Matured and current year leaves (three or four month after leaf extension) should be collected at sunny side in upper canopy. 2nd or 3rd year leaves can be collected as the reference to current year one when compare the dry deposition. But it needs to classify by the ages and take note the leaf age. Leaves collected from five trees will be mixed. Sampling at continuous raining or long drought days is not preferable.

● **Pretreatment**

The leaf sample should be oven dried in two days at 80 $^{\circ}$C and ground smaller than 0.5mm particle by Willey mill. Get about 100 g and keep in plastic bottle.

3) Chemical Analysis

The following parameter may be useful for estimate of nutrient status or polluted level. The detailed procedures of each analysis are not shown in this manual.

1) Total nitrogen(T-N): by Kjeldahl or Duma methods, %.
2) Total sulfur (T-S): using LECO Sulfur analyzer, or other useful methods (ICP-AES, Turbidimetry, etc.), mg/100 g.

3) Total phosphorus(T-P):

Colorimetric (Vanadomolybdophosphoric yellow) methods after wet digestion using nitric acid and hydrogen perchloride, mg/100 g.

4) Total K, Ca, Mg, Na, and other heavy metals

The leaf samples are digested with acid (and oxidants) and followed by atomic absorption analysis or ICP-AES. Although various acid (and oxidants) are used alone or as a mixture for the digestion, HNO_3, HNO_3 /H_2SO_4, HNO_3 /$HClO_4$ and HNO_3 /H_2O_2 are usually recommended. The digestion procedure can be carried out in open vessel or in closed systems (e.g. sealed bomb digestion or microwave assisted digestion).

5) Fluoride and Chloride

Calcium oxide incineration method for total fluorine, steam distillation method for separation from perchloric acid, and determine using SPADNS method.

Calcium oxide incineration method for total Chlorine and potentiometric titration method with $AgNO_3$.

Annex II.

Statistical procedures for estimation of precision and improvement of data

1. Estimating precision of monitored data: collaborative experiment

1.1. How to determine permissible tolerance
1.1.1. General
In order to determine the permissible tolerance in a certain measurement method, the repeatability, the reproducibility within-laboratory, reproducibility, etc. shall be estimated respectively by collaborative experiment in which as many laboratories as possible take part. The general procedures and the necessary matters for it are described.

Besides; if the obtained repeatability, reproducibility-within-laboratory, reproducibility, etc. are dissatisfied, they shall be pursuant to the 2nd section.

1.1.2. Preparation of collaborative experiment
  1) Requisite for or organizer of collaborative experiment
     The organizer of the collaborative experiment shall be endowed with the following conditions at minimum:
  (1) To have enough technical knowledge for the measurement method to be objective.
  (2) To have enough statistical knowledge necessary for design and analysis of experiment results.
  (3) To have enough knowledge and understanding with regard to the meaning and the practice of collaborative experiment.

       Informative reference: Success or failure of the collaborative experiment depends to a great extent upon whether the organizer is proper or improper as well as on whether the participating laboratories are proper or not.

  2) Requisite for participating laboratory
     The participating laboratories shall ensure the following conditions:
  (1) The facilities, equipment, reagents, etc. specified in the measurement method to be objective are well equipped and, therefore, the operation can be carried out in compliance with the specified method.

(2) They have the experience to a certain degree in the measurement method to be objective.

(3) They have the knowledge and understanding about the meaning of collaborative experiment and the error in measurement method.

3) Requisite for number of laboratories

Eight or more laboratories, which satisfy the requisite of 2), are necessary. If it is not able to keep the necessary number of laboratories by any means, any one of the following ways shall be selected:

(1) Add the laboratory without experience. In this case, have it trained in the measurement method by using in advance a spare test sample and the like.

(2) Consider the within-laboratory-reproducibility condition resulting from the change of workers and facilities within the same laboratory to be the reproducibility condition.

4) Requisite for preparation room of sample for collaborative experiment

The preparation room of the sample for collaborative experiment shall ensure that the following conditions are satisfied:

(1) Unevenness between the sample preparation and the prepared sample and between samples are thoroughly recognized and understood.

(2) The facilities and the apparatus necessary for sample preparation are well equipped and, therefore, it is possible to make fully the quality variation between the prepared samples small.

(3) The age-based variation (by temperature, light, atmosphere, etc.) are fully understood, and countermeasures can be taken against it.

(4) The error control for sample preparation is carried out sufficiently.

5) Preparation and delivery of sample for collaborative experiment

(1) Selection of sample: Three kinds or more samples (the more the better) which satisfy the following conditions are selected.

(a) Kinds of samples in accord with the indication in the scope of application in the measurement method standard or quality standard to be objective.

(b) For several kinds of samples, the aimed characteristic value includes the upper limit and lower limit of the scope of application in the measurement

method standard or quality standard (^{6}).

In addition, the values are distributed in this range at equal intervals as far as possible.

Note (^{6}):
For the raw materials or the products for which standard is not developed, the range of characteristic value of those in market shall be taken into consideration.

(c) The range of characteristic value which influences the measurement of aimed characteristic value accords approximately with the range capable of occurring on the sample shown in the scope of application in the measurement method standard.

(2) Preparation of sample: In preparation of sample , thorough attention shall be paid to the evenness of the sample to be sent to each laboratory after preparation.

Informative references:
i) It happens many times that the failure of collaborative experiment is caused by the unevenness of sample.
ii) A way to study the evenness of sample should be pursuant to ISO Guide 35 (Certification of reference materials- General and statistical principles).

(3) Sending of sample: The prepared samples are distributed to each laboratory after putting into the appropriate container to prevent from determination and loss and marking the necessary information.

(4) Matters to be marked on sample: The following items shall be marked, as a rule.

(a) Classification and No. of sample (^{7}).

Note (^{7}):
Consideration should be given so that the measurer has no preconception about the result of measurement.

(b) Year, month and date of preparation.

6) Preparation and sending of instruction for experiment

   A detailed instruction for experiment shall be sent with sample. The items to be described in the instruction shall be as follows:

(1) Purpose: The purpose of the collaborative experiment is clearly described.

(2) Handling method after receipt of sample: Instruction is given for the following items.
- (a) Confirmation of number and classification of sample.
- (b) Preservation method for sample.
- (c) Method for fractional and method for pre-treatment of sample at the time of test.
- (d) Other necessary matters concerning handling of sample

(3) Instruction for practice　The following matters shall be included:
- (a) The items to be aimed for in collaborative experiment (Characteristic to be object, kind of element to be object, etc.).
- (b) Title of standard or title of draft proposal of standard. In the case of draft proposal of standard, the draft is included therewith.
- (c) Designation of measurement method. If there are plural measurement methods for the same sample or the same characteristic, the method to be used is designated.
- (d) Operational procedure. In both cases of standard and draft proposal of standard the test shall be carried out in accordance with the specified operational procedure.
- (e) Designation of number of times of repetition in collaborative experiment. In the case where the standard is used, the number of times of repetition particular to the collaborative experiment may be designated.
- (f) As to measurement condition, repeatability condition, within-laboratory-reproducibility condition and so on, the measurement conditions adopted in the collaborative experiment are concretely specified.
- (g) Other necessary matters (designation of the term during which the test is carried out, contact point with the organizer of the collaborative test , etc .).

7) Form of report and investigation into experiment condition

The form of report and investigation into test condition in which the following items can be described as detailed as possible shall be prepared for every item to be tested and every method and distributed to each laboratory.

(1) Name of laboratory and name of person for contact

(2) Kind of sample

(3) Item to be tested

(4) Date and time of test (space should be given to report weather, room temperature, humidity atmospheric etc., if necessary.)

(5) All observed values (necessary number of digits are designated)

(6) Average value and range

(7) Details of facilities, apparatus, reagents, etc. used actua1ly

(8) When the procedure was unable to be carried out as shown in the operational procedure, the reason why it was unable and the details of procedure adopted actually.

(9) Quantity of sample taken up, blank test, calibration method, various kinds of factors and other items considered to influence the observed value.

(10) Special note (space should be given to report when repeated due to the actual failure and all other matters)

1.1.3. Carrying out of collaborative experiment

In each laboratory, the measurement shall be carried out as shown in the experiment instruction ([8]) after reading the experiment instruction carefully prior to starting the experiment. If the measurement can not be carried out as shown in the experiment instruction due to the facilities, instruments, etc. ([8]), the method adopted actually shall be described in the report or in the investigation into experiment condition ([9]).

Notes ([8]): If ambiguous points or difficult points are found in the experiment instruction, inquiries shall be made to the organizer of collaborative experiment.

Notes ([9]): Sometimes it is better that the organizer of collaborative experiment collects the information directly from the participants in experiment by holding the meeting immediately before or after the experiment.

Remarks : Making report only on the even observed value well-selected after repeating the experiment more times than specified in the experiment instruction taking care too much of the precision in self laboratory , shall be absolutely

avoided. By reason like this sometime bias will be introduced in .the average value of the laboratory and, moreover, lead to the wrong estimation on the permissible tolerance.

1.1.4. Delivery of experiment result

Each laboratory shall send the report and investigation into experiment condition to the organizer of collaborative experiment within the limit of term of submission after finishing the experiment.

1.1.5. Analysis of experiment result

When the laboratories located at l places carry out the measurement m times respectively under the within-laboratory-reproducibility condition on the identical material by means of the same method, the analysis shall be carried out in accordance with the following procedures:

Procedure 1: Examination of report and investigation sheet into experiment condition

(1) If the experiment is not carried out as shown in the experiment instruction, examination shall be carried out thoroughly from the technical point of view to decide whether or not the observed value of that laboratory may be taken up. If judged that there is a problem from the technical point of view, the observed value shall be rejected, as a rule.

> **Remarks:** When having rejected the observed value, the reason and the information on the rejected observed value shall be recorded. These information help in many ways improve the measurement method.

(2) Arrange the observed values as shown in Table 1.1.

Table 1.1 Result of measurement

Laboratory	L_1	L_2	···	L_i	···	L_l		
Observed value	x_{11}	x_{21}	···	x_{i1}	···	x_{l1}		
	.	.	···	.	···	.		
	x_{1j}	x_{2j}	···	x_{ij}	···	x_{lj}		
	.	.	···	.	···	.		
	x_{1m}	x_{2m}	···	x_{im}	···	x_{lm}		
Total	T_1	T_2		T_i		T_l	Sum total	T_T
Average	x_1	x_2		x_i		x_l	Total average	x

Where, total T_T and total average x are obtained by the following formula (1) and formula (2) respectively:

$$T_T = \sum_{i=1}^{l} \sum_{j=1}^{m} x_{ij} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (1)$$

$$x = \frac{T_T}{lm} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (2)$$

Procedure 2: Preliminary examination of analysis

(1) Examine the evenness of within-laboratory precision by means of 1.3.
(2) Examine the average value in each laboratory by means of 1.3.

Remarks: When judged to be abnormal in preliminary examination, the analyses in Procedures 3 to 5 are made in both cases where the laboratory in question is omitted and is included.

Procedure 3: Analysis of variance   Do the analysis of variance using all observed values of lm from l laboratories $\times$ m pieces.

Remarks: When omitting the laboratory judged to be abnormal, the number of the remaining laboratories shall be l anew.

(1) Obtain correction term CT.

$$\text{CT} = \frac{T_T^2}{lm}$$

(2)Obtain total sum of squares *Sr*.

$$S_r = \sum_{i=1}^{l} \sum_{j=1}^{m} xij^2 - C\,T$$

(3)Obtain within-laboratory sum of squares S_R and within-laboratory-reproducibility sum of squares S_{RW}.

$$S_R = \sum_{i=1}^{l} \left(\frac{Ti^2}{m}\right) - C\,T$$

$$S_{Rw} = S_r - S_R$$

(4) Obtain total degree of freedom $\varnothing_T$, inter-laboratories degree of freedom f_R and within-laboratory-reproducibility degree freedom $\varnothing_{RW}$.

$$\varnothing_T = lm-1$$
$$\varnothing_R = l = 1$$
$$\varnothing_{RW} = \varnothing_r - \varnothing_R = l(m-1)$$

(5) Obtain variance of inter-laboratory V_R and within-laboratory-reproducibility variance V_{RW}.

$$V_{Rw} = \frac{S_{Rw}}{\varnothing_{Rw}}$$

$$V_R = \frac{S_R}{\varnothing_R}$$

(6) Arrange to the table of analysis of variance as Table 1.2.

Table 1.2. Table of analysis of variance

Factor	S	Ø	V	E (V)
Inter-laboratory	S_R	$Ø_R$	V_R	$s_{Rw}^2 + m s_b^2$
Within-laboratory reproducibility	S_{Rw}	$Ø_{Rw}$	V_{Rw}	s_{Rw}^2
Total	S_T	$Ø_T$		

Procedure 4: Estimation of precision

Obtain laboratory component of variance s_b^2, within-laboratory standard deviation s_{Rw} and inter-laboratory standard deviation s_R.

$$s_b^2 = \frac{V_R - V_{Rw}}{m}$$

$$S_{Rw} = \sqrt{V_{Rw}}$$

$$S_R = \sqrt{s_b^2 + \frac{S_{Rw}^2}{n}}$$

n is the number of times of measurement specified in the measurement method standard.

Procedure 5: Examination of precision

Examine as to whether the obtained within-laboratory precision and inter-laboratory precision are sufficient or not in comparison with the required precision.

Remarks: The required precision is determined according to one of the following.

(a) Value required on the basis of material standard, product standard, environmental regulation, etc.

(b) Value expected from the experience the past, and

(c) Value specified in international standards and other countries' standards.

Informative reference:

The inter-laboratories precision (standard deviation) is many times within the degree as large as three times the within-laboratory precision. In the case of the trace analysis and physical test, this ratio becomes sometimes three times or more.

(1) Examination of within-laboratory precision

Examine whether or not the reproducibility-within-laboratory s_{Rw} is sufficient for the required precision s_{Rwo}. This verification is carried out as shown below setting null hypothesis $Ho : s_{Rw} = s_{Rwo}$ against alternative hypothesis $H_1 : s_{Rw}$ s_{Rwo}.

Obtain

$$x_o{}^2 = \frac{S_{Rw}}{s_{Rwo}{}^2}$$

and abolish the null hypothesis when $x_o{}^2 > [x^2\ l(m\text{-}1), a]$.

When the null hypothesis is abolished, the judgment shall be that it is insufficient for the required precision.

Remarks: The interval estimation of confidence coefficient of within-laboratory precision $1-$. is carried out as shown below.

$$\frac{S_{Rw}}{x^2 \left[l(\ m\ -\ 1)\ ,\ \dfrac{a}{2} \right]} = s_{Rw}{}^2 = \frac{S_{Rw}}{x^2 \left[l(\ m\ -\ 1)\ ,\ 1\text{-}\dfrac{a}{2} \right]}$$

(2) Examination of inter-laboratories precision

Examine whether the inter-laboratories precision s_R is sufficient for the required precision s_{Ro}. This verification is carried out setting the null hypothesis $Ho: s_R = s_{R\ o}$ against the alternative hypothesis $H_1 : s_R > s_{RO}$.

Obtain

$$x_o{}^2 = \frac{\varnothing_R * s_{R}{}^{e2}}{s_{Ro}{}^2}$$

The degree of freedom $\varnothing_R*$ is obtained approximately here by the following formula :

$$\text{Ø}_R{}^* = \cfrac{m^2 s_R{}^4}{\cfrac{V_R{}^2}{\text{Ø}_R} + \cfrac{\left(\cfrac{m}{n} - 1\right)^2 V_{Rw}{}^2}{\text{Ø}_{Rw}}}$$

Discard the decimal part of $\text{Ø}_R{}^*$.

Abolish the null hypothesis when $x_0{}^2 > x^2(\text{Ø}_R{}^*, a)$.

When abolished the null hypothesis, the judgment shall be that it is insufficient for the required precision.

Remarks 1: The approximate interval estimation of confidence coefficient of inter-laboratories precision l-a is as follows :

$$\cfrac{\text{Ø}_R{}^* s_R{}^2}{x^2\left(\text{Ø}_R{}^*, \cfrac{a}{2}\right)} = s_R{}^2 = \cfrac{\text{Ø}_R{}^* s_R{}^2}{x^2\left(\text{Ø}_R{}^*, 1 - \cfrac{a}{2}\right)}$$

Remarks 2: In the case of repeatability condition read "within-laboratory-reproducibility" above-mentioned as "repeatability" and replace the suffix $_{Rw}$ with the suffix $_r$ in its analysis.

1.1.6. Calculation and determination of permissible tolerance

1) In the case where outlier is not included in the result of collaborative experiment
In the case where the within-laboratory precision and the inter-laboratories precision are sufficient as a result of the examination in Procedure 5 in 1.1.5, it is calculated by the following each formula according to the classification of permissible tolerance. In this case, the value is calculated to the digit of two places lower than the value to which the permissible tolerance is applied (observed value or measurement result) and rounded off to the digit of one place lower.

(1) In the case of repeatability measurement

$$r(n)=D(n, 0.95)s_r$$

where n: number of times of measurement specified in the measurement method standard. Usually, (n) may be omitted.

(2) In the case of within-laboratory-reproducibility measurement

$$R_w(n)=D(n, 0.95)s_{Rw}$$

(3) In the case of reproducibility measurement

$$R=D(2, 0.95)s_R$$

Table 1.3. Value of D (n, 0.95)

n	2	3	4	5	6	7	8	9	10	11	12
$D(n,0.95)$	2.8	3.3	3.6	3.9	4.0	4.2	4.3	4.4	4.5	4.6	4.6

n	15	20	25	30	35	40	50	60	80	90	100
$D(n,0.95)$	4.8	5.0	5.2	5.3	5.4	5.5	5.6	5.8	5.9	6.0	6.1

2) In the case where outlier is included in the result of collaborative experiment
In this case, two kinds of permissible tolerance, i.e., one which includes the outlier and the other which does not do so, are calculated, and if the larger one of them satisfy the examination in 1.1.5 in Procedure 5, this value is taken up. If it does not satisfy, then the value from which the outlier is removed is treated in accordance with 1).

1.1.7. Expression of permissible tolerance
The permissible tolerance determined in 1.1.6 are arranged in the form of the value corresponding to the quality divisions of characteristic value[10] or the function of observed value or measurement result.
Beside, it is desirable to include s_{Rw} or $_{sr}$, and s_R calculated when determining the

permissible tolerance.

Note ([10]**):** Quality division of characteristic value is made based on any one of the following.

(1) Based on the division of characteristic value specified in the quality standard.

In the case where the quality standard corresponding to the object to be tested is developed, it is desirable to make it correspond to its quality division. An example of this case is shown in Example 1.

(2) Based on the division in practical use.

In the case where there is no quality standard or, if it exists, it is not necessary to divide the characteristic value into every quality division in the term of precision, it is decided taking the relation between the quality of the object to be tested practically and the precision into consideration. An example of this case is shown in Example 1.

Example 1: Example of expression of permissible tolerance corresponding to divisions of characteristic value

In the case of ash content of coal, is expressed in the form as in Table 1.4, corresponding to the divisions of coal for coke making, steam coal and uncleaned coal. Moreover, the stipulation is that number of times of within laboratory measurement is two times, and the observed value and measurement value are reported up to two decimal place and one decimal place respectively.

Table 1.4.　Example of expression of permissible tolerance corresponding to divisions of characteristics value

Characteristic value	Division (%)	Permissible tolerance	
		Within-laboratory- reproducibility (observed value)	Reproducibility (measurement result)
Ash content	10max.	0.200	0.40
	10.1 to 20	0.300	0.60
	20.1min.	0.400	0.80

Informative reference:

The standard deviations at the collaborative experiment carried out by 10 laboratories under the within-laboratory-reproducibility condition were as shown in Table 1.5.

Table 1.5.   Result of collaborative experiment carried out for determination out permissible tolerance

Sample	Number of times of measurement	Total average	Standard deviation	
			Within-laboratory-reproducibility S_{Rw}	Reproducibility S_R
A	2	6.052	0.071	0.15
B	2	14.031	0.107	0.20
C	2	22.808	0.142	0.28
D	2	29.503	0.150	0.33

Example 2: Example of expression of permissible tolerance in the form of linear equation for characteristic value

In the case where the relation between the quantity of component in the sample and the precision as phosphorus in iron and steel, it is expressed in the form of Table 1.6. Provided that it is stipulated that the number of times of within-laboratory measurement is two times, and the observed value and the measurement result are reported up to the decimal three places.

Table 1.6.   Example of expression of permissible tolerance in the form of liner equation for characteristic value

Characteristic value	Analysis method	Permissible tolerance	
		Within-laboratory-reproducibility (observed value)	Reproducibility (measurement result)
Phophorus	Volumetric method	0.002 6P+0.000 8	0.060 6P+0.002 8
	Absorptiomentry	0.037 1P+0.000 3	0.050 4P+0.002 5

For P here, the average of two observed values is used in the case of within-laboratory permissible tolerance and the average of two measurement results in the case of inter-laboratories permissible tolerance.

Informative reference:

The standard deviation of collaborative experiment carried out by 12 laboratories under the within-laboratory-reproducibility condition are as shown in Table 1.7.

Table 1.7. Result of collaborative experiment for determination of permissible tolerance

Analysis method	sample	Number of times of measurement	Total average	Standard deviation	
				Within-laboratory -reproducibility S_{Rw}	Reproducibility S_R
Volumetric method	A	2	0.031 2	0.000 3	0.001 7
	B	2	0.101 4	0.000 4	0.003 0
	C	2	0.198 6	0.000 5	0.005 1
Absorptiometry	A	2	0.030 5	0.000 5	0.001 5
	B	2	0.102 1	0.001 4	0.002 6
	C	2	0.199 3	0.002 6	0.004 3

1.2. Determination of critical limit from certified value

1.2.1. General

In order to determine the trueness in a certain measurement method, the collaborative experiment in which as many laboratories as possible shall carry out on the reference materials the true characteristic value of which is known or which is certified. In order to determine the critical limit from certified value the within-laboratory precision and the inter-laboratories precision estimated from the result of this collaborative experiment are used. In this clause, the general procedures and the necessary matters to be cared for this purpose are specified.

1.2.2. Preparation of collaborative experiment

1) Requisite for preparation room of sample for cooperative experiment
In addition to the requisite shown in 1.1.2. 4), it is necessary to choose the preparation room which has enough experience and understanding with respect to the adjustment and the certification of the reference materials.

2) Preparation and distribution of sample for cooperative experiment

(1) Selection of sample
The sample to be used for experiment to obtain the trueness shall be similar to the daily sample in physical characteristic, chemical characteristic and measurement range, and, in addition to 1.1.2. 5) (1), the characteristic value of the reference material shall be fully defined or certified. In other words, the sample arranged to have the known characteristic value, or the characteristic value of which is determined by the method in which the deviation in test or measurement can be ignored shall be selected.

In addition, the confidence interval of the reference material corresponding to tested object should be small to the extent that can be ignored even if the within-laboratory-reproducibility in the collaborative experiment is taken into account.

Remarks:
The organizer of the collaborative experiment shall preserve the following information with regard to the certified reference material.

(a) Name and address of certification organization

(b) Title and formality of certificate

(c) Date of certification

(d) Characteristic value certified and its confidence interval

(e) Measurement technique used for certification

(f) Explanation about reference material (name, whether pure material or not, shape, size, origin, those who supplied and prepared)

(g) Name of certifier

Informative reference:

With regard to the reference materials, it is recommended that reference is made to

ISO Guide 30: Terms and definitions used in connection with reference materials

ISO Guide 31: Contents of certificates of reference materials

ISO Guide 33: Uses of certified reference materials

ISO Guide 35: Certification of reference materials-General and Statistical principles

(2) Deliver of sample 1.1.2. 5) (3) applies, and it is of importance to make the information about the observed value of the sample given to the measurer .

1.2.3. Carrying out of collaborative experiment

Section 1.1.3 applies hereto.

1.2.4. Delivery of experiment result

Section 1.1.4 applies hetero.

1.2.5. Analysis of experiment result

When the laboratories located at l places measure m times respectively the reference material with certified value of μ by using the same method, the analysis shall be carried out pursuant to the following procedures:

Procedures 1: To analysis is carried out as to the precision pursuant to Procedure 1 to Procedure 4 in 1.1.5.

Procedure 2: The precision is examined pursuant to Procedure 5 in 1.1.5.

Procedure 3: Estimation of trueness.

The trueness is estimated by the following formula:

$$d=x-\mu$$

The variance of estimated of value of trueness is estimated as

$$sd^2 = \frac{V_R}{l\,m}$$

The interval estimation of confidence coefficient 0.95 of trueness is given as

$$d\text{-}2.0s_d \;=\; d \;=\; d\text{+}2.0s_d$$

If this interval includes 0,it can not be considered that there is deviation in the measurement method.

Remarks:

In the case of repeatability condition, the "within-laboratory-reproducibility" aforementioned shall be read as "repeatability" and suffix $_{Rw}$ as suffix $_r$ to analyze.

1.2.6. Calculation and determination of critical limit from certified value

In the case where it can not be considered that there is deviation in the measurement method as a result of the examination of Procedure 3 in 1.2.5, the critical limit from certified value can be calculated. The critical limit from certified value at the time where a certain laboratory carries out within-laboratory-reproducibility measurement n times on the reference material with the certified characteristic value of μ becomes

$$C\,B_{Rw} = 2.0\ S_R = 2.0\ \sqrt{s_b^2 + \frac{s_{Rw}^2}{n}}$$

In the case where the difference from the average of observed value at n times μ is smaller than this critical difference, it can not be considered that there is deviation . In calculation of critical difference, the digits shall be obtained down two

places of the numerical value to be applied (observed value or measurement result) and shall be rounded off to the digit of one place.

Remarks: In the permissible tolerance *CBr*, in the case of repeatability measurement, s_r is used instead of s_{Rw}.

1.3. Statistical method used for preliminary analysis of result of collaborative experiment

This section specifies the statistical method used for the preliminary analysis of the result of collaborative experiment for obtaining the precision.

1.3.1. Method of Grubbs   [see Reference document A)]

1) Purpose

This Grubbs method is used when verifying whether there is outlier or not in the data considered to follow the normal distribution. The outlier mentioned here is to the utmost the outlier in the statistical sense, namely, it means the value isolated from the group. Be aware of the fact that it does not necessarily mean that the abnormality is in the sample or the operation of chemical analysis or the other process of the monitoring.

Therefore, even though a certain data was judged to be the outlier by the statistical method, the data is not discarded simply but the format on monitoring data for each calendar year is examined or the inquiry is made to the laboratory concerned so as to study technically the cause of abnormality.

2) Method for verification

Consider the data here are x_1, x_2, ···, x_n. Verify the maximum value $x_{(n)}$ or the minimum value $x_{(l)}$ by using the $\bar{x}$ average value   and the variance *V* of data.

   Remarks: This method is applicable to the average values x_1, ···, x_n obtained from n laboratories. In this case, analysis is to be made considering the each average value $\bar{x_i}$ as the data.

First, obtain the average value $\bar{x}$ and the variance *V* by the following formula.

$$x = \frac{1}{n}(x_1 + x_2 + \cdots x_n)$$

$$V = \frac{1}{n-1} \sum_{i=1}^{n} (x_i - x)^2$$

For verification of the maximum value $x_{(n)}$

$$T = \frac{x(n) - x}{\sqrt{V}}$$

is used.

And for verification of the minimum value $x_{(1)}$

$$T = \frac{x - x(1)}{\sqrt{V}}$$

is used. When T is compared with the value $G(n ;a)$ in Table 1.8, if $T>G(n ;a)$,it is judged to be the outlier at the significance level of a

Table 1.8. Rejection limit value G (n :a) in the Grubbs method

Number of data	Upper probability(x)		Number of data	Upper probability(x)	
n	0.05	0.01	n	0.05	0.01
3	1.153	1.155	31	2.759	3.119
4	1.463	1.492	32	2.773	3.135
5	1.672	1.749	33	2.786	3.150
			34	2.799	3.164
6	1.822	1.944	35	2.811	3.178
7	1.938	2.097			
8	2.032	2.221	36	2.823	3.191
9	2.110	2.323	37	2.835	3.204
10	2.176	2.41	38	2.846	3.216
			39	2.857	3.228
11	2.234	2.485	40	2.866	3.240
12	2.285	2.55			
13	2.331	2.607	41	2.877	3.251
14	2.371	2.659	42	2.887	3.261
15	2.409	2.705	43	2.896	3.271
			44	2.905	3.282
16	2.443	2.747	45	2.914	3.292
17	2.475	2.785			
18	2.504	2.821	46	2.923	3.302
19	2.532	2.854	47	2.931	3.310
20	2.557	2.884	48	2.940	3.319
			49	2.948	3.329
21	2.580	2.912	50	2.956	3.336
22	2.603	2.939			
23	2.624	2.963	60	3.025	3.411
24	2.644	2.987	70	3.082	3.471
25	2.663	3.009	80	3.130	3.521
			90	3.171	3.563
26	2.681	3.029	100	3.207	3.600
27	2.698	3.049			
28	2.714	3.068			
29	2.730	3.085			
30	2.745	3.103			

Informative reference example:

In order to obtain the inter-laboratories precision of an element content in soil seven laboratories analyzed three times each, then, the following seven average values were obtained:

0.236, 0.310, 0.478, 0.220, 0.284, 0.223, 0.303 (%)

Verify whether the maximum value of 0.478 is abnormal or not. As $\bar{x}$ =0.292.

V=0.008 028 2, $x_{(n)}$=0.478 therefore T=2.076 and G(7 ; 0.05) =1.938, it is judged to be significant at the significance level of 0.05.

1.3.2. Method of Cochran [see Reference Documents B) and F)]

1) Purpose

The method of Cochran is used, when there are l sets of data of largeness of n considered to follow normal distribution, for the verification of the uniformity of variance relating to the variance V_i obtained from each of them.

Remarks:

This method is applicable to the variance V_1, $\cdots$ V_l obtained at l laboratories.

2) Method for verification

Consider the variance to be $V_1, V_2, \cdots, V_l$. Verify the maximum $V_{(l)}$ of variance.

For the verification

$$c = \frac{V(l)}{S \ V_i}$$

is used. When c is compared with the value $C(l,n;a)$ in Table 1.9, if $c>C(l,n;a)$, it is judged not to be uniform variance at the significance level of a.

Table 1.9. Rejection limit value $C(l,n;a)$ of Cochran method

l	$n=2$		$n=3$		$n=4$		$n=5$		$n=6$	
	0.050	0.010	0.050	0.010	0.050	0.010	0.050	0.010	0.050	0.010
2	0.998	0.999	0.975	0.995	0.939	0.979	0.906	0.959	0.877	0.937
3	0.967	0.993	0.871	0.942	0.798	0.883	0.746	0.834	0.707	0.793
4	0.907	0.968	0.768	0.864	0.684	0.781	0.629	0.721	0.589	0.676
5	0.841	0.928	0.684	0.789	0.598	0.696	0.544	0.633	0.506	0.588
6	0.781	0.883	0.616	0.722	0.532	0.626	0.479	0.564	0.444	0.520
7	0.727	0.838	0.561	0.664	0.479	0.569	0.430	0.508	0.397	0.465
8	0.680	0.795	0.476	0.615	0.437	0.521	0.390	0.461	0.359	0.421
9	0.639	0.754	0.444	0.573	0.402	0.479	0.358	0.424	0.328	0.386
10	0.602	0.718	0.416	0.536	0.373	0.445	0.331	0.392	0.302	0.356
11	0.567	0.681	0.392	0.501	0.348	0.416	0.308	0.365	0.281	0.331
12	0.541	0.653	0.370	0.473	0.326	0.390	0.288	0.342	0.262	0.309
13	0.513	0.621	0.351	0.448	0.307	0.368	0.270	0.321	0.246	0.290
14	0.490	0.599	0.334	0.425	0.290	0.348	0.255	0.303	0.232	0.273
15	0.469	0.572	0.319	0.405	0.275	0.331	0.242	0.287	0.219	0.259
16	0.450	0.553	0.305	0.387	0.262	0.315	0.230	0.273	0.208	0.245
17	0.433	0.529	0.292	0.370	0.250	0.300	0.219	0.260	0.198	0.234
18	0.417	0.514	0.281	0.355	0.239	0.287	0.209	0.248	0.189	0.223
19	0.402	0.493	0.270	0.341	0.229	0.275	0.200	0.238	0.181	0.213
20	0.389	0.477	0.261	0.328	0.220	0.264	0.192	0.228	0.173	0.204
21	0.377	0.465	0.000	0.318	0.212	0.255	0.185	0.220	0.167	0.197
22	0.365	0.450	0.252	0.307	0.204	0.246	0.178	0.212	0.160	0.189
23	0.354	0.437	0.243	0.297	0.197	0.238	0.172	0.204	0.155	0.182
24	0.343	0.425	0.235	0.287	0.191	0.230	0.166	0.197	0.149	0.176
25	0.333	0.411	0.228	0.277	0.184	0.221	0.160	0.190	0.144	0.169
26	0.325	0.402	0.221	0.270	0.179	0.215	0.155	0.184	0.140	0.164
27	0.316	0.391	0.215	0.262	0.173	0.209	0.150	0.179	0.135	0.159
28	0.308	0.382	0.209	0.255	0.168	0.202	0.146	0.173	0.131	0.154
29	0.300	0.372	0.203	0.248	0.164	0.196	0.142	0.168	0.127	0.150
30	0.292	0.361	0.198	0.240	0.159	0.191	0.138	0.163	0.123	0.145
31	0.286	0.355	0.193	0.235	0.155	0.181	0.134	0.159	0.120	0.141
32	0.280	0.347	0.188	0.229	0.151	0.186	0.131	0.155	0.117	0.138
33	0.273	0.339	0.184	0.224	0.147	0.177	0.127	0.151	0.114	0.134
34	0.267	0.332	0.179	0.218	0.144	0.172	0.124	0.147	0.111	0.131
35	0.262	0.325	0.175	0.213	0.140	0.168	0.121	0.144	0.108	0.127
36	0.256	0.318	0.172	0.208	0.137	0.165	0.118	0.140	0.106	0.124
37	0.251	0.312	0.168	0.204	0.134	0.161	0.116	0.137	0.103	0.121
38	0.246	0.306	0.164	0.200	0.131	0.157	0.113	0.134	0.101	0.119
39	0.242	0.300	0.161	0.196	0.129	0.154	0.111	0.131	0.099	0.116
40	0.237	0.294	0.158	0.192	0.126	0.151	0.108	0.128	0.097	0.114

1.3.3. Other statistical methods

1) Dixon method [see Reference Document C)]

(1) Purpose

The Dixon method is used when verifying whether there is outlier or not in the data considered to follow the normal distribution.

(2) Method for verification

Rearrange the data to become $x_{(1)} = x_{(2)} = \cdots x_{(n)}$. According to the value of n, any one of the following is used:

> **Remarks:** This method can be used for the same purpose as the Grubbs method specified in 2.

a) In the case of $3 = n = 7$

(a) Verification of the minimum value $x_{(1)}$

$$r_{10} = \frac{x_{(2)} - x_{(l)}}{x_{(n)} - x_{(l)}}$$

is used. When r_{10} is compared with Dix $(n;a)$ in Table1.10, if $r_{10} > \mathrm{Dix}(n;a)$, it is judged to be the outlier at the significance level of a.

(b) Verification of the maximum value $.x(n)$.

$$r_{10} = \frac{x_{(n)} - x_{(n-1)}}{x_{(n)} - x_{(l)}}$$

is used. When if r_{10} is compared with $\mathrm{Dix}(n;a)$ in Table 1.10, if $r_{10} > \mathrm{Dix}(n;a)$, is judged to be the outlier at the significance

b) In the case of $8 = n = 10$

(a) Verification of the minimum value $x_{(1)}$

$$r_{11} = \frac{x_{(2)} - x_{(1)}}{x_{(n-1)} - x_{(1)}}$$

is used. When r_{11} is compared with Dix(n;a) in Table 1.10, if r_{11}>Dix(n;a), it is judged to be the outlier at the significance level of a.

(b) Verification of the maximum value $x_{(n)}$

$$r_{11} = \frac{x_{(n)} - x_{(n-1)}}{x_{(n)} - x_{(2)}}$$

is used. When r_{11} is compared with Dix(n;a) in Table1.10, if r_{11}>Dix(n;a), it is judged to be the outlier at the significance level of a.

c) In the case of $11=n=13$

$$r_{21} = \frac{x_{(3)} - x_{(1)}}{x_{(n-1)} - x_{(1)}}$$

(a) Verification of the minimum value $x_{(1)}$

is used. When r_{21} is compared with Dix(n;a) in Table 1.10., if r_{21}>Dix(n;a), it is judged to be the outlier at the significance level of a.

(b) Verification of the maximum value $x_{(n)}$

$$r_{21} = \frac{x_{(n)} - x_{(n-2)}}{x_{(n)} - x_{(2)}}$$

is used. When r_{21} is compared with Dix(n;a) in Table 1.10., if r_{21}>Dix(n;a),it is judged to be the outlier at the significance level of a.

d) In the case of $n = 14$

(a) Verification of the minimum value $x_{(1)}$

$$r_{22} = \frac{x_{(3)} - x_{(1)}}{x_{(n-2)} - x_{(1)}}$$

is used. When r_{22} is compared with Dix(n;a) in Table 1.10., if r_{22}>Dix(n;a),it is judged to be the outlier at the significance level of a.

(b) Verification of the maximum value $x_{(n)}$

$$r_{22} = \frac{x_{(n)} - x_{(n-2)}}{x_{(n)} - x_{(3)}}$$

is used. When r_{22} is compared with $\text{Dix}(n;a)$ in Table 1.10., if $r_{22} > \text{Dix}(n;a)$, it is judged to be the outlier at the significance level of a.

Table 1.10. Rejection limit value Dix($n;a$) of

the Dixon method

Number of data n	Culculation formula	Upper probability(a)	
		0.05	0.01
3		0.941	0.988
4	$r_{10} = \dfrac{x_{(2)} - x_{(1)}}{x_{(n)} - x_{(1)}}$	0.765	0.889
5		0.642	0.780
6	$r_{10} = \dfrac{x_{(n)} - x_{(n-1)}}{x_{(n)} - x_{(1)}}$	0.560	0.698
7		0.507	0.637
8	$r_{11} = \dfrac{x_{(2)} - x_{(1)}}{x_{(n-1)} - x_{(1)}}$	0.554	0.683
9		0.512	0.635
10	$r_{11} = \dfrac{x_{(n)} - x_{(n-1)}}{x_{(n)} - x_{(2)}}$	0.477	0.597
11	$r_{21} = \dfrac{x_{(3)} - x_{(1)}}{x_{(n-1)} - x_{(1)}}$	0.576	0.679
12		0.546	0.642
13	$r_{21} = \dfrac{x_{(n)} - x_{(n-2)}}{x_{(n)} - x_{(2)}}$	0.521	0.615
14		0.546	0.641
15		0.525	0.616
16		0.507	0.595
17	$r_{22} = \dfrac{x_{(3)} - x_{(1)}}{x_{(n-2)} - x_{(1)}}$	0.490	0.577
18		0.475	0.561
19	$r_{22} = \dfrac{x_{(n)} - x_{(n-2)}}{x_{(n)} - x_{(3)}}$	0.462	0.547
20		0.450	0.535
21		0.440	0.524
22		0.430	0.514
23		0.421	0.505
24		0.413	0.497
25		0.406	0.489

2) Grubbs method for one side two outliers [see Reference Document A)]

(1) Purpose

This Grubbs method is used when verifying the one side two outliers for the data considered to follow the normal distribution.

(2) Method for verification

Rearrange the data and make them $x_{(1)} = x_{(2)} = x_{(n)}$. Verify the maximum value $x_{(n)}$ and the next value $x_{(n-1)}$, or, the minimum value $x_{(1)}$ and the next value $x_{(2)}$.

First, obtain the average value $\bar{x}$ and the sum of square S by the following formula.

$$x = \frac{1}{n}(x_{(1)} + x_{(2)} + \cdots + x_{(n)})$$

$$S = \sum_{i=1}^{n}(x_{(1)} - x)^2$$

For the verification of the minimum value $x_{(1)}$ and the next value $x_{(2)}$, obtain $\bar{x}_{1,2}$ and $S_{1,2}$ by the following formula.

$$S_{1,2} = \sum_{i=3}^{n}(x_{(i)} - x_{1,2})^2$$

$$x_{1,2} = \frac{1}{n-2}(x_{(3)} + x_{(4)} + \cdots + x_{(n-2)})$$

and calculate

$$g = \frac{S_{1,2}}{S}$$

For the verification of the maximum value $x_{(n)}$ and the next value $x_{(n-1)}$, calculate

$$x_{n-1,n} = \frac{1}{n-2}(x_{(1)} + x_{(2)} + \cdots + x_{(n-2)})$$

$$S_{n-1,n} = \sum_{i=1}^{n-2}(x_{(i)} - x_{n-1,n})^2$$

$$g = \frac{S_{n-1,n}}{S}$$

When g is compared with the value $G_2(n;a)$ in Table 1.11, if $g < G_2(n;a)$, it is judged to be the outlier at the significance level of a.

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Table 1.11.   Rejection limit value $G_2(n;a)$ of the Grubbs Method

Number of data n	Upper probability (a)		Number of data n	Upper probability (a)	
	0.05	0.01		0.05	0.01
4	0.000 8	0.000 0	31	0.609 5	0.536 9
5	0.018 3	0.003 5	32	0.617 8	0.546 5
			33	0.625 7	0.555 7
6	0.056 4	0.018 6	34	0.633 3	0.564 6
7	0.102 0	0.044 0	35	0.640 5	0.573 0
8	0.147 8	0.075 0			
9	0.190 9	0.108 2	36	0.647 4	0.581 1
10	0.230 5	0.141 4	37	0.654 1	0.588 9
			38	0.660 4	0.596 3
11	0.266 7	0.173 6	39	0.666 5	0.603 5
12	0.299 6	0.204 3	40	0.372 4	0.610 4
13	0.329 5	0.233 3			
14	0.356 8	0.260 5	41	0.678 0	0.617 0
15	0.381 8	0.285 9	42	0.683 4	0.623 4
			43	0.688 6	0.629 6
16	0.404 8	0.309 8	44	0.693 6	0.635 5
17	0.425 9	0.332 1	45	0.698 5	0.641 2
18	0.445 5	0.353 0			
19	0.463 6	0.372 5	46	0.703 2	0.646 8
20	0.480 4	0.390 9	47	0.707 7	0.652 1
			48	0.712 0	0.657 3
21	0.496 1	0.408 2	49	0.716 3	0.662 3
22	0.510 7	0.424 5	50	0.720 3	0.667 2
23	0.524 4	0.439 8			
24	0.537 3	0.454 3	60	0.755 0	0.708 6
25	0.549 5	0.468 0	70	0.781 3	0.740 1
			80	0.802 1	0.765 0
26	0.560 9	0.481 0	90	0.819 0	0.7853
27	0.571 7	0.493 3	100	0.832 9	0.802 0
28	0.581 9	0.505 0			
29	0.591 6	0.516 2			
30	0.600 8	0.526 8			

3) Grubbs method for one side three or four outliers [see Reference Document D]]

(1) Purpose

This Grubbs method is used where verifying one side $k(=3$ *or* 4) pieces outliers relating to the data considered to follow the normal distribution.

(2) Method for verification

Rearrange the data and make them $x_{(1)} = x_{(2)} = \cdots x_{(n)}$. Verify k pieces $x_{(n-k-1)}, ., x_{(n)}$ on

the larger side or k piece $x_{(1)}, \cdots, x_{(k)}$ on the smaller size.

First, obtain the average value $\bar{x}$ and the sum of square S by the following formula.

$$x = \frac{1}{n}(x_{(1)} + x_{(2)} + \cdots + x_{(n)})$$

$$S = \sum_{i=1}^{n}(x_{(i)} - x)^2$$

For the verification of k pieces on the smaller side, obtain $\bar{x}_{1,k}$ and $S_{1,k}$ by the following formula.

$$x_{1,k} = \frac{1}{n-k}(x_{(k+1)} + x_{(k+2)} + \cdots + x_{(n)})$$

$$S_{1,k} = \sum_{i=k+1}^{n}(x_{(i)} - x_{1,k})^2$$

and calculate

$$g_k = \frac{S_{1,k}}{S}$$

For the verification of k pieces on the larger side, calculate

$$x_{n-k+1,n} = \frac{1}{n-k}(x_{(1)} + x_{(2)} + \cdots + x_{(n-k)})$$

$$S_{n-k+1,n} = \sum_{i=1}^{n-k}(x_{(i)} - x_{n-k+1,n})^2$$

$$g_k = \frac{S_{n-k+1,n}}{S}$$

when g_k is compared with the value $G_k(n;a)$ in Table 1.12, if. $g_k < G_k(n;a)$, all the k pieces are judged to be the outliers at the significance level of a.

Table 1.12.   Rejection limit value $G_k(n;a)$
of the Grubbs method

n	K=3		K=4	
	0.05	0.01	0.05	0.01
7	0.032	0.010		
8	0.064	0.028		
9	0.099	0.048	0.045	0.018
10	0.129	0.070	0.070	0.032
12	0.196	0.120	0.125	0.070
14	0.250	0.172	0.174	0.113
16	0.300	0.219	0.219	0.151
18	0.337	0.260	0.259	0.192
20	0.377	0.300	0.299	0.231
30	0.506	0.434	0.434	0.369
40	0.588	0.522	0.523	0.460
50	0.646	0.592	0.588	0.531

4) Grubbs method for both sides two outliers

(1) Purpose

This method is used when verifying whether both of maximum value and minimum value are outliers or not, relating to the data considered to follow the normal distribution.

(2) Method for verification

Rearrange the data and make them $x_{(1)} = x_{(2)} = \cdots x_{(n)}$, and verify the maximum value $x_{(n)}$ and the minimum value $x_{(1)}$.   First, obtain the average value $\bar{x}$ and the sum of square S by the following formula.

$$x = \frac{1}{n}(x_{(1)} + x_{(2)} + \cdots + x_{(n)})$$

$$S = \sum_{i=1}^{n}(x_{(i)} - x)^2$$

Then, obtain $x_{1,n}$ and $S_{1,n}$ from which the maximum value $x_{(n)}$ and the minimum value $x_{(1)}$ are removed and calculate the verification statistic g.

$$x_{1,n} = \frac{1}{n-2}(x_{(2)} + x_{(3)} + \cdots + x_{(n-1)})$$

$$S_{1,n} = \sum_{i=2}^{n-1}(x_{(i)} - x_{1,n})^2$$

$$g = \frac{S_{1,n}}{S}$$

When g is compared with the value $G_{1,n}(n;a)$ in Table 1.13, if $g<G_{1,n}(n;a)$, both of the maximum value and the minimum value are judged to be outliers at the significance level of a.

<div align="center">

Table 1.13   Rejection limit value $G_{1,n}(n;a)$

of the Grubbs method

</div>

n	0.05	0.01	n	0.05	0.01
4	0.000 44	0.000 01	14	0.320	0.236
5	0.011	0.002	16	0.369	0.288
6	0.044	0.014	18	0.411	0.325
7	0.078	0.033	20	0.448	0.363
8	0.120	0.06	30	0.571	0.509
9	0.159	0.093	40	0.644	0.584
10	0.195	0.122	50	0.699	0.648
12	0.266	0.181	100	0.821	0.794

Reference Documents

A) F. E. Grubbs and G. Beck: Technometrics, 14, 847(1972)

B) ISO/DIS 5725-2:A basic method for the determination of repeatability and reproducibility of a measurement method, 62(1990)

C) W. J. Dixon: Biometrics, 9, 74 (1953)

D) V. Barnett and T. Lewis: Outliers in Statistical Data, 2nd Ed.,383, John Wiley & Sons(1984)

E) V. Barnett and T. Lewis: Outliers in Statistical Data,2nd Ed.,385, John Wiley & Sons (1984)

F) H. Solomon and M.A Stephens: Journal of Quality Technology, 22, 46(1990)

1.4. Example of analysis on collaborative experiment

1.4.1. Preface

This Informative reference is to give the example that the experiment was carried out practically on the method to determine the permissible tolerance and the method to determine the critical limit from certified value specified in 1.1. and 1.2. respectively, these results were analyzed and each permissible tolerance was calculated, and is not a part of the standard.

1.4.2. Example of determination of permissible tolerance (see 1.1.)

(1) Example of analysis of the result of experiment

The collaborative experiment was carried out in order to obtain the analysis precision of Ca contents of soil. The measurement was carried out three times ([1]) at each of eight laboratories at different days and times under the within-laboratory-reproducibility condition.

Note ([1]):

The number of times of measurement specified in the measurement method standard is $n=2$, but in the collaborative experiment, the measurement was carried out three times ($m=3$).

Procedure 1: Examination of report and experiment condition survey

l) The experiment at each laboratory was carried out in accordance with the experiment instruction and there was no problem.

2) The measured values are set in order as in Table 1.14.

Table 1.14. Result of measurement   Unit: c mol(+) kg^{-1}

Laboratory	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8
Observed	6.46	6.50	6.42	6.42	6.42	6.82	6.70	6.59
Value	6.50	6.46	6.37	6.37	6.33	6.80	6.57	6.57
	6.46	6.59	6.52	6.33	6.29	6.76	6.46	6.75
Total	19.42	19.55	19.31	19.12	19.04	20.38	19.73	19.91
Average	6.47_3	6.51_7	6.43_7	6.37_3	6.34_7	6.79_3	6.57_7	6.63_7

$T_T = 156.46$

$x = 6.51_9$

Procedure 2: Preliminary examination of analysis

l) Verify the evenness of within-laboratory precision by the Cochran method.

Maximum within-laboratory variance $V_{max} = 0.1014\ 43(L_7)$

Total of each laboratory's variance $V_{total} = 0.042\ 37$

c Statistic $c = \dfrac{V_{max}}{V_{total}} = 0.340$

Rejection limit value
    (significance level 0.01) $C\,(8,3;\,0.01) = 0.615$

    (significance level 0.05) $C\,(8,3;\,0.05) = 0.516$

2) Verify the average value of each laboratory by the Grubbs method.

Measurement result being at the largest variance from total average

$$\bar{x}_6 = 6.793(L_6)$$

Total average $$\bar{x} = 6.519$$

Variance $V = 0.021\ 745$

T statistic $$T = \dfrac{\bar{x}_6 - \bar{x}}{\sqrt{V}} = 1.858$$

Rejection limit value
  (significance level 0.01) G (8, ; 0.01)=2.221

  (significance level 0.05) G (8,; 0.05)=2.032

As a result of the examination of the evenness and the average value of the within-laboratory precision, there is no laboratory judged to be abnormal even in significance level of 0.05.

Procedure 3: Analysis of variance
Carry out the analysis of variance by using the observed values of total laboratories.

1) Obtain correction term *CT*.

$$CT = \frac{T_T{}^2}{lm}$$

$$= \frac{156.462}{24} = 1019.988\ 8$$

2) Obtain total sum of square *Sr*.

$$S_T = \sum_{i=1}^{l} \sum_{j=1}^{m} (x_{ij}{}^2) - CT$$

$$= 1020.530\ 2 - 1019.988\ 8$$

$$= 0.541\ 4$$

3) Obtain sum of square inter-laboratories S_R and sum of square within-laboratory S_{Rw}.

$$S_R = \sum_{i=1}^{l} (\frac{T_i^2}{m}) - CT$$

$$= \frac{3061.336\,3}{3} - 1019.\,988\,8$$

$$= 0.456\,6$$

$$S_{Rw} = S_r - S_R$$

$$= 0.541\,4 - 0.456\,6 = 0.084\,8$$

4) Obtain total degree of freedom $\varnothing_r$, inter-laboratories degree of freedom $\varnothing_R$ and within-laboratory-reproducibility degree of freedom $\varnothing_{Rw}$.

$$\varnothing_r = lm - 1$$
$$= 24 - 1 = 23$$

$$\varnothing_R = l - 1 = 8 - 1 = 7$$

$$\varnothing_{Rw} = \varnothing_r - \varnothing_R = 23 - 7 = 16$$

5) Obtain inter-laboratories variance V_R and within-laboratory-reproducibility variance V_{Rw}.

$$V_R = \frac{S_R}{\varnothing_R} = \frac{0.0456\,6}{7} = 0.065\,2$$

$$V_{Rw} = \frac{S_{Rw}}{\varnothing_{Rw}} = \frac{0.084\,8}{16} = 0.005\,3$$

6) Put the above values in the table of analysis of variance as shown in Table 1.15.

Table 1.15.   Analysis of variance table

Factor	S	$\varnothing$	V	$E(V)$
Inter-laboratories	0.456 6	7	0.065 2	$s_{Rw}^2 + 3s_b^2$
Within-laboratory reproducibility	0.084 8	16	0.005 3	s_{Rw}^2
Total	0.541 4	23		

Procedure 4: Estimation of precision

Obtain laboratory component of variance $_{Sb}{}^2$, within-laboratory standard deviation $_{SRw}$ and inter-laboratories standard deviation $_{SR}$

$$s_{b2} = \frac{V_{R} - V_{Rw}}{m} = \frac{0.065\ 2 - 0.005\ 3}{3}$$
$$= 0.020\ 0$$

$$s_{RW} = \sqrt{V_{Rw}} = \sqrt{0.005\ 3}$$
$$= 0.073$$

As the number of times of measurement specified in the measurement method standard is *n=2,*

$$s_{R} = \sqrt{s_{b2} + \frac{s_{Rw2}}{n}} = \sqrt{0.020\ 0 + \frac{0.005\ 3}{2}}$$

$$= 0.150$$

Procedure 5: Examination of precision

1) Examination about reproducibility-within-laboratory

The required precision of reproducibility-within-laboratory is assumed to be $\boldsymbol{s}_{Rw}$=0.06 cmol(+)kg^{-1}.

The verification is carried out because S_{Rw}=0.073 cmol(+)kg^{-1} is larger than the required reproducibility-within-laboratory.

$$x_{o2} = \frac{S_{Rw}}{s_{Rw2}} = \frac{0.084\ 8}{(0.060)^2} = 23.55$$

$$x^2 \lfloor l(m-1), a \rfloor = x^2\ (16, 0.05) = 26.3$$

$$x_{o2} < x^2(16, 0.05)$$

Therefore, at the significance level of 0.05, $\boldsymbol{s}_{Rw}$ is not considered to be insufficient for the required precision of 0.06 cmol(+)kg^{-1}

Besides, the interval estimation of confidence coefficient 0.95 of reproducibility-

within-1aboratory is

$$\frac{S_{Rw}}{x^2\left[l(m-1),\dfrac{a}{2}\right]} = \frac{0.084\,8}{x^2(16,0.025)} = \boldsymbol{s}\,Rw^2 = \frac{S_{Rw}}{x^2\left[l(m-1),1-\dfrac{a}{2}\right]} = \frac{0.084\,8}{x(16,0.975)}$$

and

$$x_2(16,0.025)=28.8$$
$$x_2(16,0.975)=6.91$$

therefore

$$0.002\,94 = \boldsymbol{s}_{Rw}^{\,2} = 0.012\,3$$
$$0.054\,3 = \boldsymbol{s}_{Rw} = 0.111 \quad (c\,mol(+)kg^{-1})$$

2) Examination of inter-laboratories precision

The required precision of the inter-laboratories precision is assumed to be

$\boldsymbol{s}_R = 0.12$ cmol(+)kg^{-1} at $n=2$.

The verification is carried out because $S_R = 0.150$ (*unit*: cmol(+)kg^{-1}) is larger than the required inter-laboratories precision .

$$\varnothing_{R^*} = \frac{m_2 s_R 4}{\dfrac{V_R^2}{\varnothing_R} + \dfrac{(\dfrac{m}{n}-1)^2\,V_{Rw^2}}{\varnothing_{Rv}}} = \frac{3^2 \times 0.022\,6^2}{\dfrac{0.065\,2^2}{7} + \dfrac{(\dfrac{3}{2}-1)^2 \times 0.005\,3^2}{16}} = 7.56$$

The decimal part is discarded and $\varnothing_R^{\,*} = 7$ is used. Consequently,

$$x_o 2 = \frac{\varnothing_{R^*} s_R 2}{s_{Ro^2}} = \frac{7 \times 0.022\,6}{0.12^2} = 10.99$$

$$x^2 = (\varnothing^*_{R,a}) = x^2 = (7, 0.05) = 14.07$$

therefore , at significance level of 0.05, $\boldsymbol{s}_R$ is not considered to be insufficient for the required precision 0.12 cmol(+)kg^{-1}.

(2) Example of calculation and determination of permissible tolerance

The permissible tolerance is calculated relating to the example of (1)

Within-laboratory-reproducibility limit

$$R_w = D(2, 0.95)s_{Rw}$$
$$= 2.8 \times 0.073 = 0.204$$

Reproducibility limit

$$R = D(2, 0.95)s_R$$
$$= 2.8 \times 0.150 = 0.420$$

Remarks: If the within-laboratory precision and the inter-laboratories precision are insufficient, it is necessary to review the measurement method pursuant to 2.1. However, in the case where the inter-laboratories precision is sufficient and, to the contrary, the within-laboratory precision is insufficient , the both permissible tolerances may be calculated provided that its within-laboratory precision is not remarkably insufficient.

1.4.3. Example of determination of critical limit from certified value (refer to 1.2. in this manual)

1) Example of analysis of experiment result
The certified value of Ca content on which analysis is carried out in the example in (1) is assumed to be 6.50 cmol(+)kg^{-1}.

Procedure l: For the analysis concerning precision, see the example shown in 1.1.5 in this manual.

Procedure 2: For the examination of precision, see the example shown in 1.1.5 in this manual.

Procedure 3: Estimation of trueness. The trueness is estimated as

$$d = x - \mu = 6.519 - 6.500 = 0.019$$

The variance of assessed value of trueness is estimated as

$$s_d{}^2 = \frac{0.065^2}{3 \times 8} = 0.00272$$

The interval estimation of confidence coefficient 0.95 is

$$d - 2.0 s_{\bar{d}} = 0.019 - 2.0\sqrt{0.00272} = . \quad = d + 2.0 s_{\bar{d}} = 0.019 + 2.0\sqrt{0.00272}$$

Namely
$$-0.085 = d = 0.123 (\text{Unit}: c\ mol(+)kg^{-1})$$

Because 0 is included in this interval, it is not considered that there is the deviation in the method used for the collaborative experiment at this time.

2) Example of calculation and determination of critical limit from certified value
The permissible tolerance is calculated with respect to the example in 1).
The critical limit from certified value in the case where $n=2$ is

$$CB_{Rw} = 2.0 \times 0.150 = 0.300 (\text{Unit}: c\ mol(+)kg^{-1})$$

1.5. Collaborative experiment for obtaining precision of repeatability, reproducibility-within-laboratory and reproducibility simultaneously

1.5.1. Scope

It is impossible to obtain the repeatability limit, the within-laboratory- reproducibility limit and the reproducibility limit all together at the same time by the method specified in 1.1.1. in this manual. This section specifies the method for obtaining three permissible tolerances simultaneously. The design of experiment used for this method are

<u>Staggered type nested experiment</u>

and   <u>Completely repeated nested experiment</u>,

any of which can be used where appropriate.

1.5.2. Staggered type nested experiment

1) Design of experiment

The experimental unit of the Staggered type nested experiment in the case of obtaining the laboratory component of variance $s_b{}^2$, the within-laboratory-reproducibility component of variance $s_c{}^2$ and the repeatability variance $s_r{}^2$ is shown in Fig.1.1. Provided that x_{i1}, x_{i2} and x_{i3} are considered as the data of *No.i* laboratory and x_{i1} and x_{i2} are repeated under the repeatability condition and x_{i3} is made to be the within-laboratory-reproducibility condition for x_{i1} and x_{i2}. The example that " x_{i1} and x_{i2} are measured on the first day and x_{i3} is measured on the second day" falls on this case.

Fig.1.1.   Experimental unit of *No.i* laboratory in
Staggered type nested experiment

Laboratory

(-Reproducibility
condition)

Day of
Measurement

(Within-laboratory-
reproducibility
condition)

Measurement

(Repeatability
condition)

x_i1 x_i2 x_i3
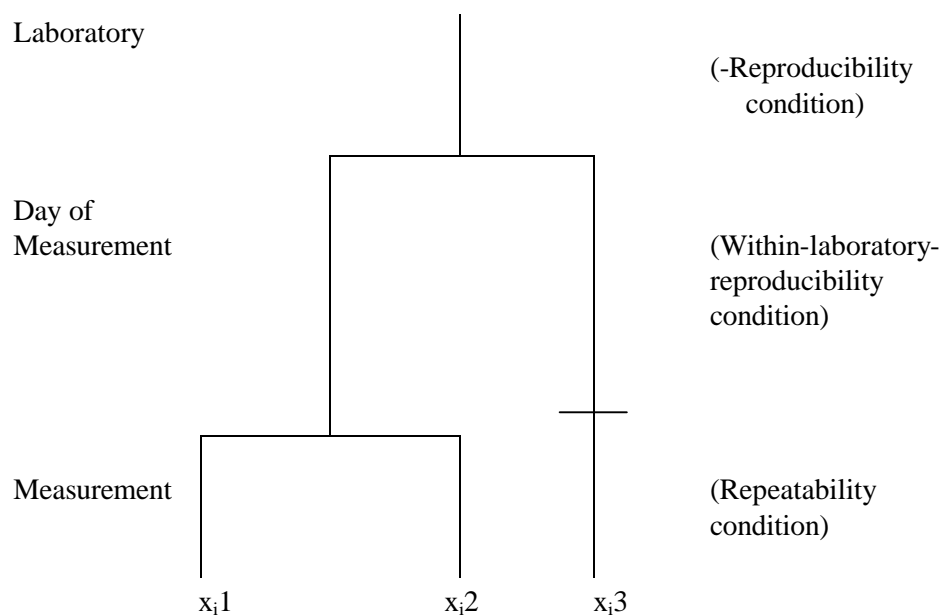
Remarks: What is change as the within-laboratory-reproducibility
condition depends on the specification of standard for
the measurement method.

2) Method for analysis

(1) Analysis of variance
Suppose *l* laboratories take part in the experiment. First, obtain $\bar{x}_{(1)i}, R_{(1)i}, x_{(2)I}$
and $R_{(2)i}$ at every laboratory by the formulas (1) to (4) .

$$x(1) = \frac{x_{i1} + x_2}{2} \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots(1)$$

$$R_{(1)i} = |\ x_{i1} - x_{i2}|\ \cdots\cdots\cdots\cdots\cdots\cdots\cdots (2)$$

$$x_{(2)\,i} = \frac{x_{i1} + x_{i2} + x_{i3}}{3} \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \quad (3)$$

$$R_{(2)i} = |\, x_{(1)i} - x_{i3}| \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \quad (4)$$

Then obtain the average $\bar{x}_r$ and the total sum of square S_r by the formulas (5) and (6).

$$x_r = \frac{1}{l} \sum x_{(2)i} \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \quad (5)$$

$$S_r = \sum_i \sum_j (x_{ij} - x_r)^2 \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \quad (6)$$

Obtain the inter-laboratories sum of square S_R, the within-laboratory reproducibility sum of square S_{Rw} and the repeatability sum of square S_r used in the analysis of variance by the formulas (7) to (9).

$$S_R = 3 \sum_j x_{(2)i}{}^2 - 3l(x_r)^2 \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \quad (7)$$

$$S_{Rw} = \frac{2}{3} \sum R_{(2)i}{}^2 \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \quad (8)$$

$$S_r = \frac{1}{2} \sum R_{(1)i}{}^2 \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \quad (9)$$

The degree of freedom of inter-laboratories sum of square S_R becomes l-1, and the degree of freedom of within-laboratory reproducibility sum of square S_{Rw} and repeatability sum of square S_r become the same l respectively. The analysis of variance table is shown in Table 1.16.

(2) Estimation of component of variance

Estimate the component of variance putting the average square equally to its expected

value.

When each average square is expressed as V_R, V_{Rw}, and V_r, the estimated values s_b^2, s_c^2 ,and s_r^2 of s_b^2, s_c^2 and s_r^2 are obtained by the formulas (10) to (12).

$$s_b{}^2 \ = \frac{1}{3}V_R - \frac{5}{12}V_{Rw} + \frac{1}{12}V_r \quad \cdots\cdots\cdots\cdots\cdots\cdots \ (10)$$

$$s_c{}^2 = \frac{3}{4}V_{Rw} - \frac{3}{4}V_r \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots \ (11)$$

$$s_r{}^2 = V_r \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \ (12)$$

Table 1.16. Analysis of variance table

Factor	S	Ø	V	E(V)
Inter-laboratories	Formula(7)	l-1	$S_R/(l-1)$	$s_r^2 + 5/3 s_c^2 + 3 s_b^2$
Within-laboratory-reproducibility	Formula(8)	l	S_{Rw}/l	$s_r^2 + 4/3 s_c^2$
Repeatability	Formula(9)	l	S_r/l	s_r^2
Total	Formula(6)	$3l$-1		

1.5.3. Completely repeated nested experiment

1) Method for experiment

The experimental unit of the completely repeated nested experiment in the case of obtaining the laboratory component of variance s_b^2, the within-laboratory-reproducibility component of variance s_c^2 and repeatability variance s_r^2 is shown in Fig.1.2. Provided that the observed value obtained as No. k in order on j th day at No. i laboratory is considered as x_{ijk}. Repeat in a day corresponds to the repeatability condition and the observed values obtained on different j at the same laboratory correspond to the within-laboratory-reproducibility condition.

Fig.1.2. Experimental unit of No.*i* laboratory in completely repeated nested experiment

Laboratory

(Reproducibility condition)

Day of measurement

(Within-laboratory-reproducibility condition)

Measurement

(Repeatability condition)

x_{i11} x_{i12} x_{i21} x_{i22}
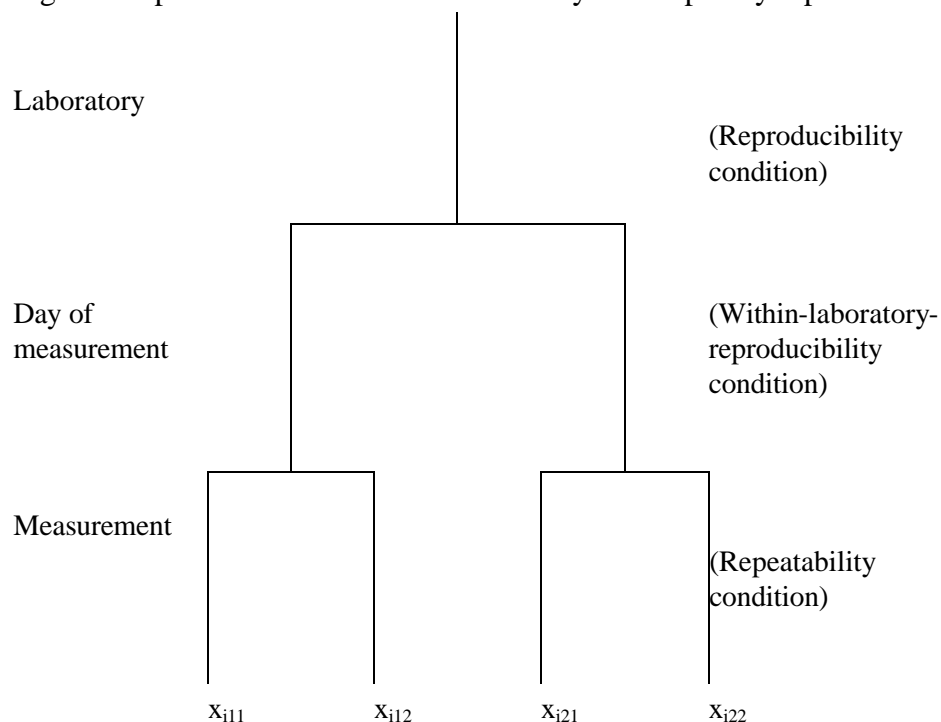
2) Method for analysis

(1) Analysis of variance Suppose that *l* laboratories take part in the measurement for *d* days measuring *m* times in a day. Obtain each sum of square by the formulas (13) to (15).

$$S_R = \sum_i \frac{1}{dm}\left(\sum_j \sum_k x_{ijk}\right)^2 - \frac{1}{ldm}\left(\sum_i \sum_j \sum_k x_{ijk}\right)^2 \quad\text{............................ (13)}$$

$$S_{Rw} = \sum_i \sum_j \frac{1}{m}\left(\sum_k x_{ijk}\right)^2 - \sum_i \frac{1}{dm}\left(\sum_j \sum_k x_{ijk}\right)^2 \quad\text{.............................. (14)}$$

$$S_r = \sum_i \sum_j \sum_k x_{ijk}^2 - \sum_i \sum_j \frac{1}{m}\left(\sum_k x_{ijk}\right)^2 \quad\text{...................................... (15)}$$

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The degrees of freedom are

$$F_R = l-1$$

$$F_{Rw} = l(d-1)$$

$$F_r = ld(m-1)$$

respectively. The analysis of variance table is shown in Table 1.17.

Table 1.17. Analysis of variance table

Factor	S	Ø	V	E(V)
Inter-laboratories	Formula(13)	$l-1$	$S_R/Ø_R$	$s_r^2 + m s_c^2 + dm s_b^2$
Within-laboratory-reproducibility	Formula(14)	$l(d-1)$	$S_{Rw}/Ø_{Rw}$	$s_r^2 + m s_c^2$
Repeatability	Formula(15)	$ld(m-1)$	$S_r/Ø_r$	s_r^2
Total		$l\,dm-1$		

(2) Estimation of component of variance

Estimate the component of variance putting the average square equally to its expected value. When each average square is expressed with V_R, V_{Rw} and V_r respectively, the expected values s_b^2, s_c^2 and s_r^2 of s_b^2, and s_c^2 and s_r^2 are obtained by the formulas (16) to (18).

$$s b2 = \frac{V_R - V_{Rw}}{d\,m} \quad\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \quad (16)$$

$$S c2 = \frac{V_{Rw} - V_r}{m} \quad\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \quad (17)$$

$$s_r^2 = V_r \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \quad (18)$$

1.6. Example of collaborative experiment for obtaining precision of repeatability, reproducibility-within-laboratory and reproducibility simultaneously

Preface

This section is to show an example of the concrete application of the collaborative experiment for obtaining at the same time the repeatability limit, within-laboratory-reproducibility limit and reproducibility limit specified in 1.5, and does not form a part of standard.

1.6.1. Example of Staggered type nested experiment (see 1.5.2)

In order to develop a manual of the method for quantitative analysis of the available phosphate of soil (P_2O_5 mgg^{-1}) by means of spectrophotometry, the collaborative experiment was carried out.

The Staggered type nested experiment was used by the participant 20 laboratories. Of the observed values x_{i1}, x_{i2}, and x_{i3} obtained at No.i laboratory, x_{i1} and x_{i2} were obtained on the first day by repeating under the repeatability condition, and x_{i3} was the result of measurement on the second day.

The original data, and $\bar{x}_{(1)i}$, $R_{(1)i}$, $x_{(2)i}$ are shown in Table1.18. Obtain the total average $\bar{x}_r$ and the total sum of square S_r by the formulas (5) and (6) in 1.5.2.

$$x_r = \frac{1}{20} \sum x_{(2)i} = 0.009\ 942$$

$$S_r = \sum_i \sum_j (x_{ij} - x_r)^2 = 0.000\ 069\ 33$$

and the inter-laboratories sum of square S_R, the within-laboratory-reproducibility sum of square S_{Rw} and the repeatability sum of square S_r become

$$S_R = 3 \sum_j x_{(2)i}^2 - 3 \times 20(x_r)^2 = 0.000\ 047\ 61$$

$$S_r = \frac{1}{2} \sum R_{(1)i}^2 = 0.000\ 002\ 76$$

$$S_{Rw} = \frac{2}{3} \sum R_{(2)i}2 = 0.000\ 018\ 96$$

The degree of freedom of repeatability sum of square S_R' becomes 19, and both degree of freedom of within-laboratory-reproducibility sum of square S_{Rw} and repeatability sum of square S_r become the same 20. The analysis of variance table is shown in Table 1.19.

Table 1.18. Original data and $\bar{x}_{(1)i}, R_{(1)i}, \bar{x}_{(2)i},$ and $R_{(2)i}$

Laboratory	First day		Second day	x(1)i	R(1) i	x(2) i	R(2) i
	x_{l1}	x_{l2}	x_{i3}				
L-01	0.009 1	0.010 2	0.009 8	0.0096 5	0.001 1	0.009 700	0.000 15
L-02	0.010 0	0.010 0	0.009 0	0.0100 0	0.000 0	0.009 667	0.001 00
L-03	0.009 5	0.009 0	0.009 4	0.0092 5	0.000 5	0.009 300	0.000 15
L-04	0.008 0	0.008 3	0.007 7	0.0081 5	0.000 3	0.008 000	0.000 45
L-05	0.010 0	0.010 0	0.010 0	0.0100 0	0.000 0	0.010 000	0.000 00
L-06	0.008 9	0.009 4	0.009 4	0.0091 5	0.000 5	0.009 233	0.000 25
L-07	0.009 8	0.009 9	0.010 1	0.0098 5	0.000 1	0.009 933	0.000 25
L-08	0.009 6	0.009 4	0.009 9	0.0095 0	0.000 2	0.009 633	0.000 40
L-09	0.010 4	0.009 4	0.010 0	0.0099 0	0.001 0	0.009 933	0.000 10
L-10	0.010 7	0.011 8	0.009 7	0.0112 5	0.001 1	0.010 733	0.001 55
L-11	0.010 0	0.010 0	0.009 0	0.0100 0	0.000 0	0.009 667	0.001 00
L-12	0.010 9	0.011 5	0.009 7	0.0112 0	0.000 6	0.010 700	0.001 50
L-13	0.010 0	0.009 5	0.009 5	0.0097 5	0.000 5	0.009 667	0.000 25
L-14	0.009 6	0.009 6	0.010 0	0.0096 0	0.000 0	0.009 733	0.000 40
L-15	0.009 9	0.009 1	0.008 2	0.0095 0	0.000 8	0.009 067	0.001 30
L-16	0.009 8	0.010 0	0.009 5	0.0099 0	0.000 2	0.009 767	0.000 40
L-17	0.010 5	0.010 2	0.011 2	0.0103 5	0.000 3	0.010 633	0.000 85
L-18	0.010 3	0.010 5	0.011 8	0.0104 0	0.000 2	0.010 867	0.001 40
L-19	0.009 8	0.009 6	0.010 4	0.0097 0	0.000 2	0.009 933	0.000 70
L-20	0.014 0	0.014 0	0.010 0	0.0140 0	0.000 0	0.012 667	0.004 00

Table 1.19. Analysis of variance table

Factor	S	Ø	V	E(V)
Inter-laboratories	0.000 047 61	19	0.000 002 505 6	$s_r^2+5/3s_c^2+3s_b^2$
Within-laboratory-reproducibility	0.000 018 96	20	0.000 000 948 0	$s_r^2+5/3s_c^2$
Repeatability	0.000 002 76	20	0.000 000 138 0	s_r^2
Total	0.000 069 33	59		

The estimate values s_b^2, s_c^2 and s_r^2 of s_b^2, s_c^2 and s_r^2 can be obtained by the formulas (10) (11) and (12) in 2.2 in 1.5.2 as shown below.

$$s_b^2 = 4.517 \times 10^{-7}$$

$$s_c^2 = 6.075 \times 10^{-7}$$

$$s_r^2 = 1.380 \times 10^{-7}$$

As in the measurement method it is specified that the number of repetition n is two and the repetition in the case where the reproducibility limit is applied is made under the within-laboratory-reproducibility condition,

$$s_R^2 = \frac{s_x^2 + s_c^2}{2} + s_b^2$$

$$s_R^2 = s_r^2 + s_c^2$$

Therefore,

$$S_R^2 = 8.244 \times 10^{-7}, \quad S_R = 9.080 \times 10^{-4}$$

$$S_{Rw}^2 = 7.455 \times 10^{-7}, \quad S_{Rw} = 8.634 \times 10^{-4}$$

$$S_r^2 = 1.380 \times 10^{-7}, \quad S_r = 3.715 \times 10^{-4}$$

Therefore, the permissible tolerances are

$$R = 2.542 \times 10^{-3}$$

$$R_w = 2.418 \times 10^{-3}$$

$$R = 1.040 \times 10^{-3}$$

2. Methods for examination for improvement of precision

2.1. Methods for examination when aiming at improvement of within-laboratory precision and reproducibility

2.1.1. Scope

This section specifies the methods for examination when aiming at the improvement of within-laboratory precision and reproducibility. The cases where the precision of measurement method is needed to be improved are as follows:

2.1.2. Examination of within-laboratory precision

The procedures to be taken up in an attempt to improve the within-laboratory precision are as follows:

Procedure 1: Divide all test operations within-laboratory in to every stage and make the flow sheet.

Procedure 2: Take up all of facilities, reagents, unit operation, etc. likely to be the factors to improve within-laboratory precision. In this case, the influence due to the combination of causes (interaction) is taken into account as well. In addition, prepare the cause and effect diagram and the like in a manner that make the relation between cause and effect understood with regard to its cause (in this case, the factors considered to be of importance from the viewpoint of those concerned should be marked).

Procedure 3: Sample is selected in accordance with 5) of 1.1.2. in this manual.

Procedure 4: For each factor taken up in Procedure 2, examine and set two to three levels as measurement condition within the limits of specified operation.

Procedure 5: Assign these factors in the orthogonal array so that the interaction the existence of which was supposed by means of multi-way layout or Procedure 2 becomes able to be estimated, practice combination of each level in random order, and carry out the chemical analysis or the physical test repeatedly (more than 2 times), if possible seven times or eight times or more.

Procedure 6: Carry out the analysis of variance or the analysis of other type taking the natural logarithm $ln\ s_{Rw}^2$ of variance s_{Rw}^2 of observed value corresponding to each combination as the characteristic.

Select, from the result of this, the combination of facility, reagent and operation so as to make $ln\ s_{Rw}^2$ minimum. In this case, cost, workability, time taken for it should be taken into consideration.

Procedure 7: When the within-laboratory precision under the best condition like this is

(1) The value satisfactory from the technical viewpoint, adopt this best condition as the draft of revision of measurement method standard and carry out, based on this, the collaborative experiment (1.1. and 1.2. in this manual).

(2) The value dissatisfied from the technical viewpoint repeats Procedures 2 to 6 furthermore.

Remark 1:

Even if the within-laboratory precision becomes satisfactory value as a result of the above, sometimes the reproducibility become large so that attention should be paid.

Remark 2:

In the case of the repeatability condition, the suffix $_{Rw}$ is replaced by the suffix r to carry out the analysis.

2.1.3. Examination of reproducibility

The procedures to be taken up in order to improve the reproducibility should be in accordance with any one of next 1) and 2).

1) When there is little information on reproducibility

In this case, it is not clear what stage in the course of the whole test operation is the factor to make the reproducibility large so that it is inevitable that the number of times of carrying out the test increases.

Consequently, it is recommended that a small number of laboratories carry out the detailed experiment first and estimate the optimum condition, than the collaborative experiment in which many laboratories take part is carried out.

The procedures for this are as follows:

Procedure 1: Select a small number, of specific laboratories. These laboratories should be of high technical level in facilities and personnel.

Remarks: In the experiment at this stage, the number of factors and levels is large, therefore, three to four laboratories should be adequate usually.

Procedure 2: Divide all measurement stages in to every stage and prepare the flow sheet.

Procedure 3: Describe at every stage all potential factors capable of bringing about the dispersion between laboratories and prepare the cause and effect diagram. At this time, the influence due to the combination of causes (interaction) should be taken into account. In addition, prepare the cause and effect diagram in a manner that make the relation between cause and effect (in this case, the factors considered to be of importance, from the viewpoint of those concerned should be marked).

Procedure 4: Sample is selected in accordance with 5) of 1.1.2. in this manual.

Procedure 5: Make the items taken up in the cause and effect diagram the factors, and examine and set two to three levels as the measurement condition within the limits of the dispersion possible of arising between laboratories (different facilities, instruments, reagents, operations, etc.).

Procedure 6: Make plan of experiment.

Assign these factors in the orthogonal array so that the interaction the existence of which was supposed in Procedure 3 becomes able to be estimated or tested by means of multi-way layout.

Procedure 7: Analyze the result of test.

The factors which become significant after carrying out the analysis of variance on the observed values and analyzing the results of the test that have the possibility of being the factors that make the reproducibility increase so that the detailed specification is needed for these factors. For the factors that do not give deviation to the observed values, the level which takes a short time and at which the operation is easy should be selected.

Procedure 8: Select, based on the result of test, the combination of levels of the condition under which the reproducibility s_R is considered to become small.

Procedure 9: When the reproducibility under the best condition like this is,

(1) The value satisfactory from the technical viewpoint, adopt this best condition as the draft of revision of measurement method standard and carry out, based on this, the collaborative experiment (1.1. and 1.2. in this manual), or carry out the experiment in accordance with next 2).

(2) The value dissatisfied from the technical viewpoint repeat Procedures 2 to 8 furthermore.

2) When the information on reproducibility has already been known

In the case where the information about the reproducibility has always been known the factors which make the reproducibility increase are relatively limited, therefore, it is possible to select direct the condition in which the reproducibility becomes the best.

The procedures in this case are as follows:

Procedure 1: Select the factor estimated to be the one which affects the reproducibility, and examine and set two to three levels as the measurement condition for the dispersion (different facilities, instruments, reagents, operation, etc.) liable to arise between laboratories.

Procedure 2: Make an experimental design.
Assign these factors in the orthogonal array or the multiway layout.

Procedure 3: Send the common samples to as many laboratories as possible to carry out measurement several times each with the above assignment.

Procedure 4: Carry out the analysis of variance or the analysis of other type taking up the natural logarithm of variance between the average values of each laboratory under every test condition as characteristic.

Procedure 5: Select the combination of facility, instrument, reagent and operation by which the variance between average values is made the smallest.

Procedure 6: When the reproducibility under the best condition like this is,

(1) Satisfactory value from the technical viewpoint, make this best condition the draft of revision of measurement method standard and carry out the collaborative experiment (1.1. and 1.2. in this manual).

(2) Dissatisfied value from the technical viewpoint repeats Procedures 2 to 5

furthermore.

2.2. Example of methods for examination when aiming at improvement of within-laboratory precision and reproducibility

2.2.1. Preface

This section shows concretely the example of application of procedures in examining how to improve the within-laboratory precision and the reproducibility specified in 2.1.

2.2.2. Example of examination of within-laboratory precision (see 2.1.2)

Examination of analysis method for ash content of peat soil.

Procedure 1: Making flow sheet

Fig. 2.1. Flow sheet of analysis
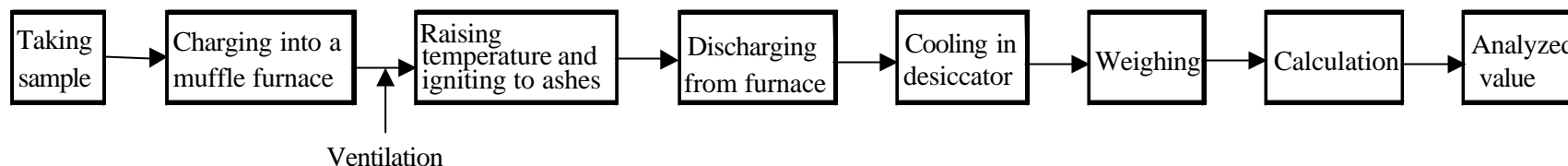
Procedure 2: Making cause and effect diagram

Fig. 2.2. Cause and effect diagram
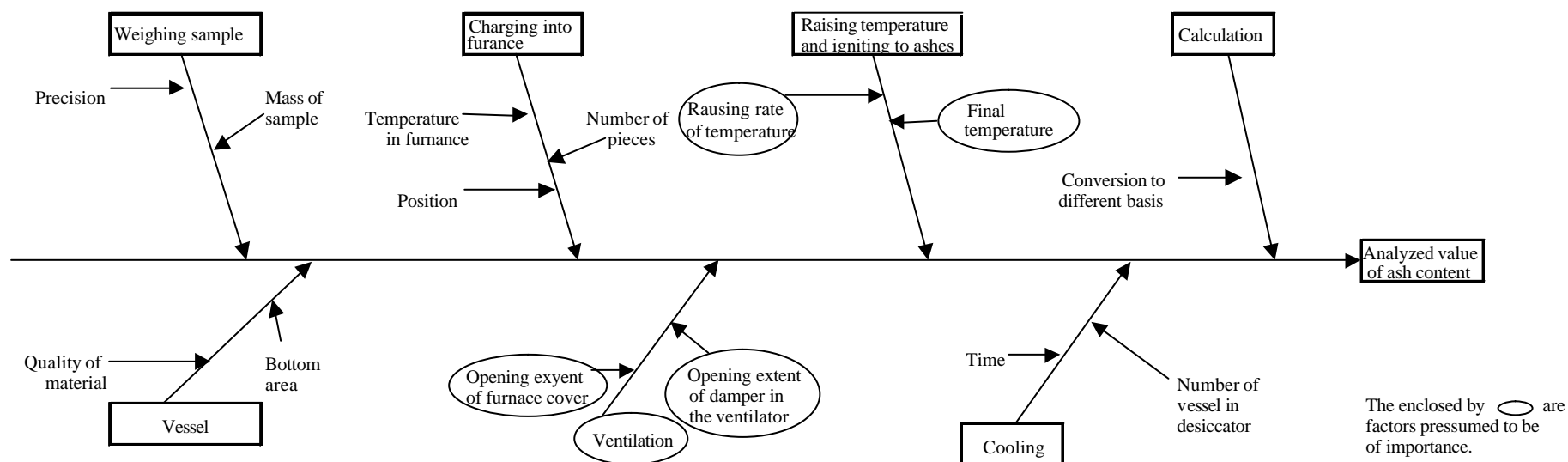
Procedure 3: Selection of factor and level

Table 2.1. Factor and level

Symbol	Factor	Level 1	Level 2
A	Ignition	Room temperature+500°C 500°C final temperature / one hour Preservation of final temperature four one hour	Room temperature? final temperature/ one hour Reservation of final temperature for one hour Re-burning for one hour
D	Extent of ventilation	Stationary air (Seal openning of furnace)	Ventilation (Open furnance cover about 2 cm)
F	Degree of final heating	$750^{+10°C}_{-20}$	$800^{+10°C}_{-20}$

Procedure 4: Obtain logarithm $ln\ s_{Rw}^2$ of variance in the test repeated two times.

Table 2.2. Result of experiment (Ash content %)

	A₁						A₂					
	D₁			D₂			D₁			D₂		
	x	s_{Rw}	$ln\ s_{Rw}^2$	x	s_{Rw}	$ln\ s_{Rw}^2$	x	s_{Rw}	$ln\ s_{Rw}^2$	x	s_{Rw}	$ln\ s_{Rw}^2$
F₁	20.44 20.20	0.170	-3.547	20.18 20.35	0.120	-4.273	20.72 20.55	0.120	-4.273	20.50 20.43	0.049 5	-6.012
F₂	20.42 20.32	0.070 7	-5.299	19.93 20.03	0.070 7	-5.298	20.58 20.42	0.113	-4.359	20.18 20.12	0.042 4	-6.320

Procedure 5: Carry out the analysis of variance supposing $ln\ s_{Rw}^2$ as the characteristic value.

If the adequate pooling is done as shown in Table 2.3., the factors D and F become significant. The optimum level becomes D_2 and F_2. This conclusion is considered to be reasonable even from technical examination. However, in this example, F value of interaction is also large. As the conclusion may be unstable according to the way of pooling, it is unable to state clearly unless the examination is carried out repeatedly a few more times.

Table 2.3. Table of analysis of variance

Factor	S	Ø	V	F_0
A	0.810	1	0.810	12.69
D	2.449	1	2.449	38.36[*]
F	1.314	1	1.314	20.58[*]
A×D	1.160	1	1.160	18.18
A×F	0.710	1	0.710	11.12
e	0.127 6	2	0.063 8	
Total	6.570	7		

2.2.3. Example of examination of reproducibility (see 2.1.3.)

1) Example that there is little information on re reproducibility

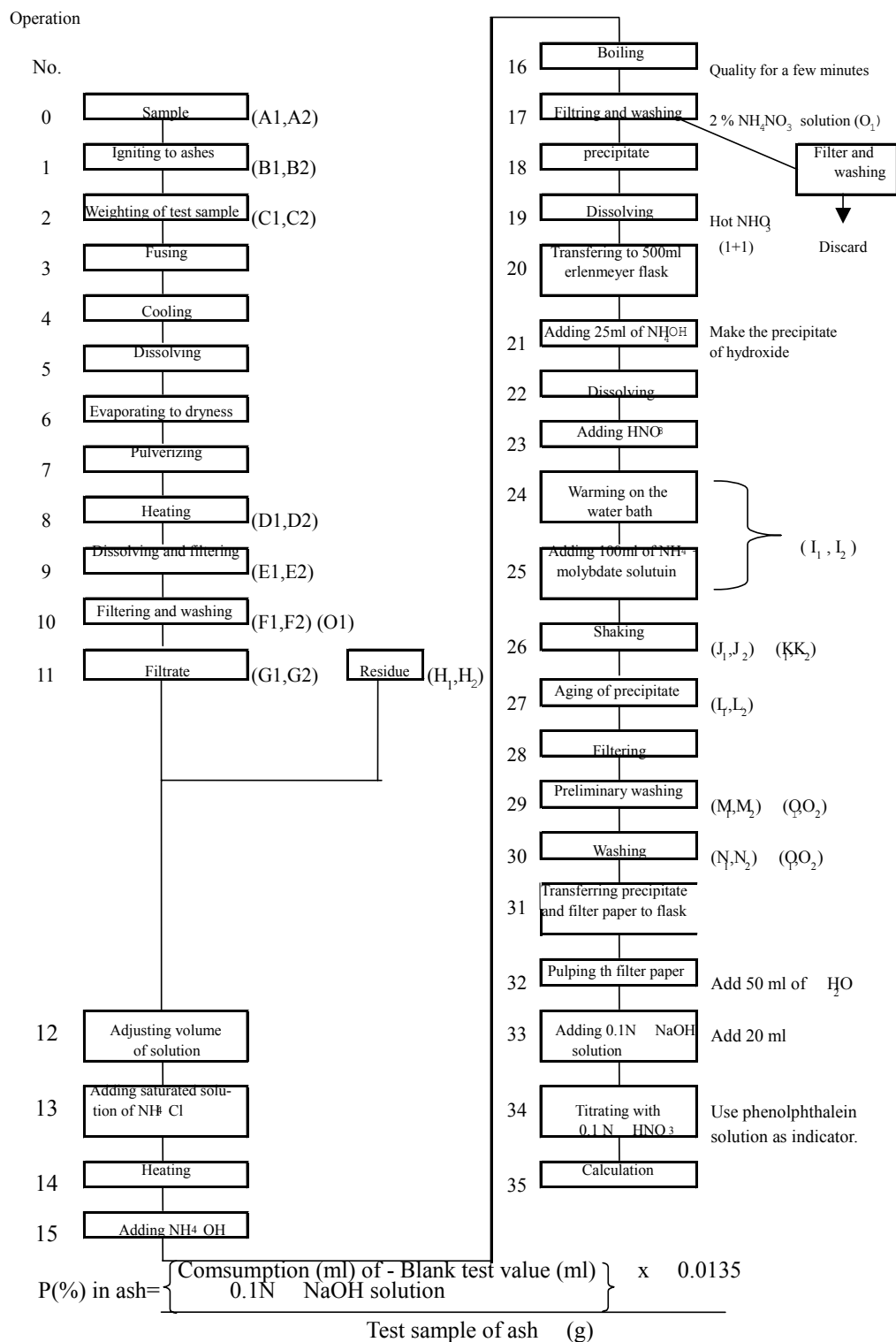Examination of analysis method for total phosphorus in soil

This experiment which is carried out in a specific institute in order to examine the error factors between analysis laboratories is an example of practice for the considerably complex analysis method.

Procedure 1: Preparation of flow sheet (It may be cause and effect diagram.)
Fig.2.3.is made by taking whole operation into pieces of every unit operation.

Fig. 2.3. Flow sheet of analysis method

Operation

No.

No.	Left column		No.	Right column	
0	Sample	(A1,A2)	16	Boiling	Quality for a few minutes
1	Igniting to ashes	(B1,B2)	17	Filtring and washing	2 % NH_4NO_3 solution (O_1)
2	Weighting of test sample	(C1,C2)	18	precipitate	
3	Fusing		19	Dissolving	Hot NHO_3
4	Cooling		20	Transfering to 500ml erlenmeyer flask	(1+1)
5	Dissolving		21	Adding 25ml of NH_4OH	Make the precipitate of hydroxide
6	Evaporating to dryness		22	Dissolving	
7	Pulverizing		23	Adding HNO_3	
8	Heating	(D1,D2)	24	Warming on the water bath	
9	Dissolving and filtering	(E1,E2)	25	Adding 100ml of NH_4 molybdate solutuin	(I_1, I_2)
10	Filtering and washing	(F1,F2) (O1)	26	Shaking	(J_1,J_2) (K_1K_2)
11	Filtrate (G1,G2) Residue	(H_1,H_2)	27	Aging of precipitate	(L_1,L_2)
			28	Filtering	
			29	Preliminary washing	(M_1,M_2) (Q_1O_2)
			30	Washing	(N_1,N_2) (Q_1O_2)
12	Adjusting volume of solution		31	Transferring precipitate and filter paper to flask	
13	Adding saturated solution of NH_4Cl		32	Pulping th filter paper	Add 50 ml of H_2O
14	Heating		33	Adding 0.1N NaOH solution	Add 20 ml
15	Adding NH_4OH		34	Titrating with 0.1 N HNO_3	Use phenolphthalein solution as indicator.
			35	Calculation	

(at operation 17) Filter and washing → Discard

$$P(\%)\ \text{in ash} = \frac{\left\{\begin{array}{c}\text{Comsumption (ml) of - Blank test value (ml)} \\ \text{0.1N NaOH solution}\end{array}\right\} \times 0.0135}{\text{Test sample of ash (g)}}$$

Procedure 2: Selection of factors and levels

As a result of discussion by many persons concerned, the factors and levels shown in Table 2.4. have been selected as those which might be the error factors between different analysis laboratories.

Procedure 3: Assignment for experiment

As shown in Table 2.4, this is an experiment for 15 factors and 2 levels. If experiment is to be carried out on all the combinations, 2^{15}=32768 experiments will be required. Therefore, it was decided to reduce the number of experiments using the orthogonal array table. The interactions that may occur from the technical point of view are presumably:

$A{\times}B$ $A{\times}C$ $C{\times}G$ $C{\times}H$ $J{\times}K$ $K{\times}L$ $M{\times}N$ $M{\times}O$ $N{\times}O$

If all other interactions are neglected, the number of experiments can be reduced to 32 (1/4096). And it was planned that each of four analysts would carry out eight analyses in order to obtain the errors between analysts and those eight analyses in two times (d_1, d_2) i.e., four analyses at a time in order to obtain the errors between days. The assignment for the experiment and experimental results are shown in Table 2.5.

Table 2.4. Factors and levels

Group of procedures		Factors	Operation No.	Level 1	Level 2
Preparation of sample	A	Type of sample	0	Coal	Coke
	B	Method of igniting to ashes	1	Muffle furnance with ventilator; Thickness of sample layer, about 3 mm at the time of igniting to adhes	Muffle furnance without ventilator; Thickness of sample layer about 6 mm at the time of igniting to adhes.
	C	Mass of ash taken	2	0.2 g (fusing mixture 2 g)	0.4 g (fusiong mixture 4 g)
Removal of SiO$_2$	D	Heating	8	60 min after dissolution (110 to 120 ^{o}C)	30 min on the send bath
	E	Treatment after dissolution to filtration	9	After 5 min of boiling, filter	Immediately after dissolution, filter
	F	Washing with HC| (2+100)	10	7 times	3 times
	G	Re-evaporating filtrate to dryness	11	Yes[1]	No
	H	Treatment of filtrate residue	12	Yes[2]	No
Reproduction of phosphorus precipitate	I	Temperature of water bath and NH$_4^-$ molybdate solution	24, 25	50 ^{o}C	60 ^{o}C
	J	Method of shaking	26	Rubber stopper, not used	Rubber stopper, used and vigorous shaking
	K	Time of shaking	26	5 min	1 min

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Table 2.4. Factors and levels (Cont'd)

Group of procedures		Factor	Operation No.	Level 1	Level 2
Production of phosphorus precipitate	L	Aging of precipitate	27	1h on 50 $^{\circ}$C water bath	1h under room temperature
	M	Preliminary washing with HNO_3 (2+100)	29	Yes	No
	N	Washing precipitate with HNO_3 (2+100)	30	10 times	Until 5 ml of washings becomes nutural
	O	Method of washing	29, 30	Method I([3])	Method II ([4])
	P	Analyst	Four		
	d	Day	Two		

Notes ([1])   Repeat operations No. 6 to 10.

Notes ([2])   Put the residue and the filter paper into a platinum crucible and burn to ashes.   After cooling misturize it with a small amount of water and H_2SO_4 (1+1) and treat with HF.

If there is any residue further,fuse by adding $K_2S_2O_7$ and then dissolve with HCl.

Notes ([3])   Method of washing I

Blow water hard along the surface of the filter paper from the washing bottle to stir up

Blow about 10 to 15ml of water at a time,and the next flow will be made after the washings   have been filtered off.

Notes ([4])   Mothod of washing II

Using the washing bottle,blow water gently onto around the upper rim of filter paper.   Next washing is continued without running out of washings.Blow about 10 to 15 ml at a time   while the washings remain on the filter paper.

Table 2.5. Assignment for experiment and observed values

Factor / Experiment No.	P (1)	P (2)	P (3)	K×L (4)	I (5)	M (6)	A (7)	A×B (8)	D (9)	C×H (10)	E (11)	C×G (12)	J (13)	N×O (14)	B (15)	A×C (16)	L (17)	M×N (18)	F (19)	N (20)	K (21)	e (22)	C (23)	J×K (24)	e (25)	O (26)	G (27)	M×O (28)	H (29)	e (30)	D (31)	Experimental result P( )
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.226
2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.215
3	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	0.243
4	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	0.215
5	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2	0.807
6	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1	2	2	2	2	1	1	1	1	0.818
7	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1	0.823
8	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1	2	2	2	2	1	1	1	1	1	1	1	1	2	2	2	2	0.818
9	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	0.707
10	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	0.773
11	1	2	2	1	1	2	2	2	2	1	1	2	2	1	1	1	1	2	2	1	1	2	2	2	2	1	1	2	2	1	1	0.705
12	1	2	2	1	1	2	2	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	1	1	2	2	1	1	2	2	0.781
13	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1	0.180
14	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1	2	2	1	1	1	1	2	2	2	2	1	1	1	1	2	2	0.191
15	1	2	2	2	2	1	1	2	2	1	1	1	1	2	2	1	1	2	2	2	2	1	1	2	2	1	1	1	1	2	2	0.154
16	1	2	2	2	2	1	1	2	2	1	1	1	1	2	2	2	2	1	1	1	1	2	2	1	1	2	2	2	2	1	1	0.177
17	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	0.776
18	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	0.837
19	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1	1	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1	0.766
20	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	1	2	1	2	1	2	1	2	0.905
21	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1	0.223
22	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1	2	1	2	1	1	2	1	2	2	1	2	1	1	2	1	2	0.233
23	2	1	2	2	1	2	1	2	1	2	1	1	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1	1	2	1	2	0.250
24	2	1	2	2	1	2	1	2	1	2	1	1	2	1	2	2	1	2	1	1	2	1	2	1	2	1	2	2	1	2	1	0.203
25	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	0.238
26	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	0.215
27	2	2	1	1	2	2	1	2	1	1	2	2	1	1	2	1	2	2	1	1	2	2	1	2	1	1	2	2	1	1	2	0.178
28	2	2	1	1	2	2	1	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	1	2	2	1	1	2	2	1	0.195
29	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2	0.726
30	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2	2	1	1	2	1	2	2	1	2	1	1	2	1	2	2	1	0.739
31	2	2	1	2	1	1	2	2	1	1	2	1	2	2	1	1	2	2	1	2	1	1	2	2	1	1	2	2	1	1	2	0.706
32	2	2	1	2	1	1	2	2	1	1	2	1	2	2	1	2	1	1	2	1	2	2	1	1	2	2	1	1	2	2	1	0.719

Procedure 4: Carry out analysis of variance as shown in Table 2.6.

Table 2.6. Analysis of variance table

Factor	$S\times10^6$	Ø	$V\times10^6$	F_0
A Type of sample	2 570 778.1	1		1450**
B Method of igniting to ashes	666.1	1		3.76
C Mass of ash taken for experiment	5 565.1	1		31.5**
D Driving	276.1	1		-
E Dissolving to filtering	364.5	1		2.06
F Washing with HCl (2+100)	3 872.0	1	Same as S	21.9**
G Re-evaporation of filtrate	40.5	1		-
H Treatment of filtration residue	990.1	1		5.60*
I Temperatuire of water bath	5 356.1	1		30.3**
J Method of shaking	21.1	1		-
K Time of shaking	630.1	1		3.56
L Aging of precipitate	50.0	1		-
M Preliminary washing with HNO$_3$ (2+100)	1 152.0	1		6.51*
N Washing precipitate with HNO$_3$ (2+10000)	2 244.5	1		12.69**
O Method of washing	171.1	1		-
P Analyst	29 839.1	3	9 946.4	56.2**
d Day of experiment	128.0	1		-
A×B	136.1	1		-
A×C	3 321.1	1		18.7**
C×G	144.5	1	Ø$_e$=9	-
C×H	1 830.1	1	V$_e$=176.86	10.35**
J×K	78.1	1		-
K×L	1 352.0	1		7.64*
M×N	136.1	1		-
M×O	924.5	1		5.23*
N×O	98.0	1		-
e	870.9	3	290.3	
Totoal	2 631 035.9	31		

Procedure 5: Prepare graphs

The graphs of the factorial effects which were significant are illustrated in Figs.2.4 and 2.5.

Procedure 6: Observation

The observations on the significant factorial effects are as follows:

(1) Mass of ash taken for experiment(*C*).

The main effect is significant, and this is due to the fact that, for a high-phosphorus sample, 0.4 g is too much, as shown in Fig.2.4, therefore it seems not necessary to take 0.5 g for a sample of containing under 0.25 % of P. It seems safer to specify a smaller mass of sample when the P content is unknown.

(2) Washing at the time of filtering SiO_2 *(F)*

The standard specifies to "wash thoroughly with HCl (2 + 100)", however as illustrated in Fig.2.4, there is a significant difference between 7-time and 3-time washing and 3-time washing is not sufficient. Therefore, in order to make the errors between laboratories smaller, it is recommended to specify the number of times of washing.

(3) Treatment of filtration residue *(H)*

The main effect is significant, however the interaction with the mass of ash taken for experiment is also significant, therefore the effect is illustrated in Fig.2.4. As is evident from the figure, when the mass of ash taken is much, a higher value is obtained if the treatment of filtration residue is carried out (though not significant), however when the mass of ash taken is little, a higher value is obtained if the treatment is not carried out. From the above, it seems that even in the case of taking 0.4 g of ash, no treatment is required.

(4) Temperature of water bath and NH_4-molybdate *(I)*

As can be seen from Fig.2.5, the measured value is higher due to the effect of impurities in the case of 60 °C , therefore the temperature of 50 °C is more preferable in this case and it is necessary that each laboratory should adhere to this temperature strictly.

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Fig.2.4.
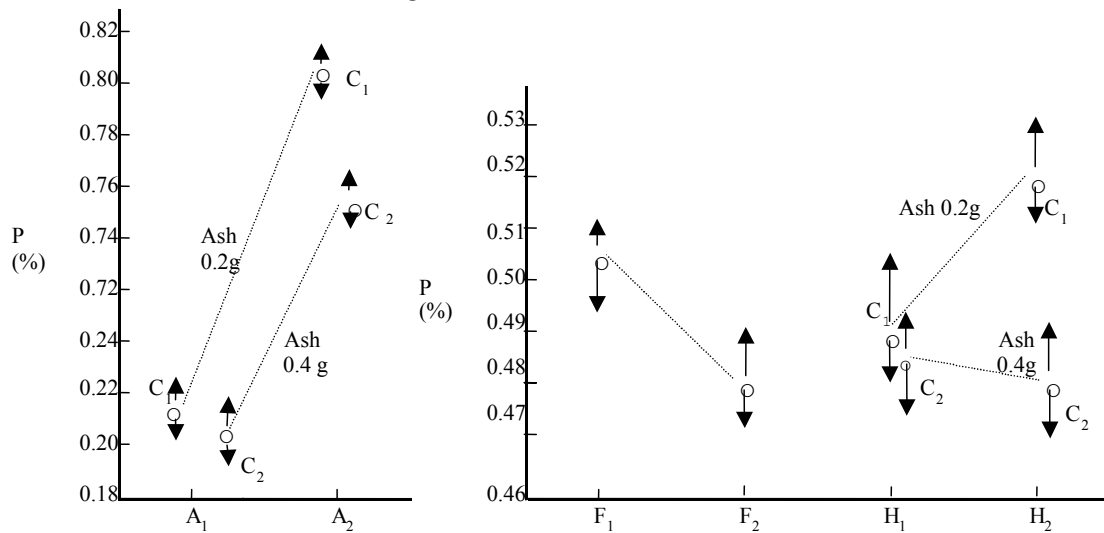
Fig.2.5.
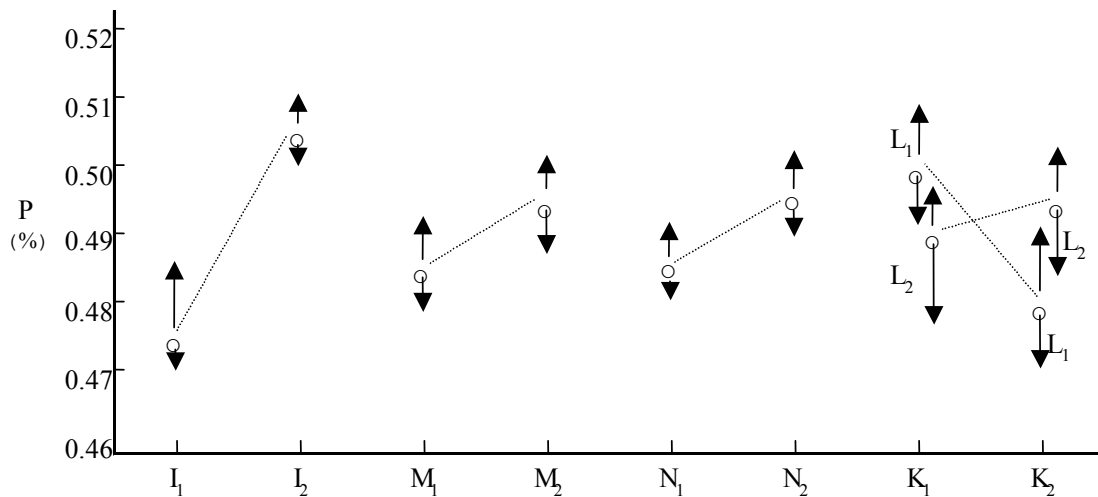
(5) Time of shaking (K)

It is rather difficult to shake for 5 min as specified in the standard, however compared to 1 min shaking, a little higher value is obtained (at 10 % significance level). The interaction with the condition of aging precipitates ($K \times L$) is also significant, but no concrete conclusion could be drawn.

(6) Preliminary washing with HNO_3 (2 + 100) (M)

As can be seen from Fig.2.5, if preliminary washing is carried out, a significantly lower value is obtained. This experiment alone can not tell whether this is due to the dissolving loss of P precipitate or insufficient washing in the case of no preliminary washing, however according to another experiment using ^{32}P tracer, it is presumed that the difference is due to the former reason.

(7) Washing with HNO_3 (2 + 10000) (N)   (interaction $A \times F$)

The effect of washing precipitates with HNO_3 (2 + 10000) is highly significant, however the result that washing ten times gives a lower value than washing until neutral washings are obtained (washing ten times or more), can not be explained from the technical point of view, therefore it seems better to consider it to be the effect of $A \times F$, another name of N. In this case, it becomes evident that for a low-phosphorus sample, 3-time washing with HCl (2 + 100) may be sufficient, however 3-time washing is not sufficient for a high-phosphorus sample (see Fig.2.6.).

Fig.2.6.
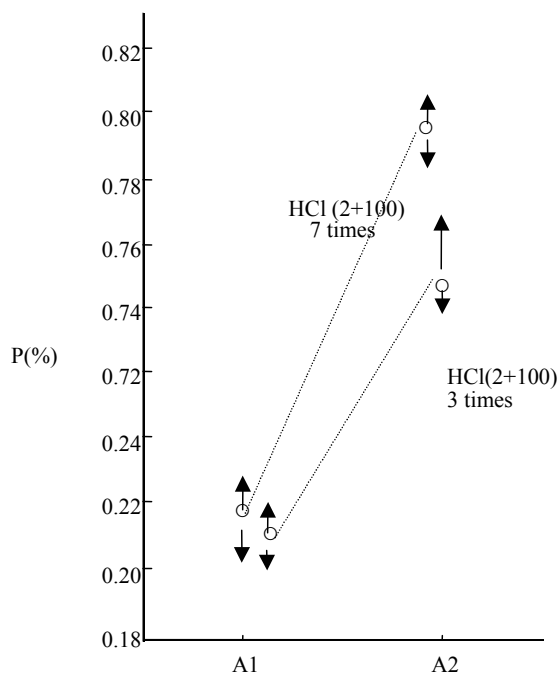
Procedure 7: Estimation of the best conditions

From the above observations, the best conditions can be constituted by

selecting:

(1) The levels showing good results from the technical point of view for the significant factors;

(2) As to the factors not to be significant, select the levels which require the simplest operational procedures and the least time. Thus, such best condition are:

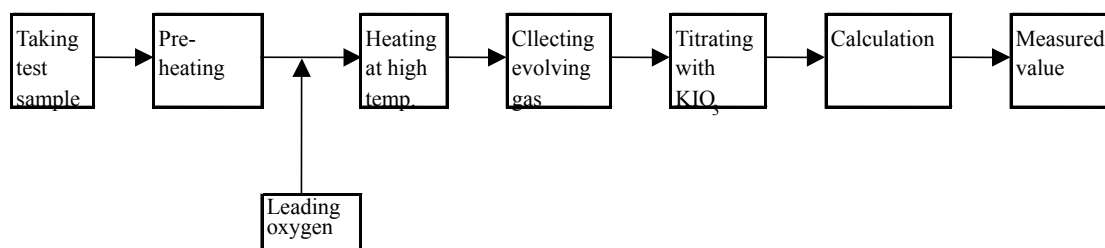$$C_{1-2}D_2E_2F_1G_2H_2I_1J_1K_1L_1M_2N_1$$

However, for a high-phosphorus sample and for a sample on which the phosphorus content is unknown, C_1 and for a low-phosphorus sample, C_2 shall be selected.

2) Example in the case where the information on reproducibility has already been obtained

Examination of analysis method for total sulfur in soil (burning method)

Procedure 1: Preparation of flow sheet

Fig.2.7.   Flow sheet up to analysis

```
┌─────────┐   ┌─────────┐        ┌─────────┐   ┌─────────┐   ┌─────────┐   ┌─────────────┐   ┌─────────┐
│Taking   │   │Pre-     │        │Heating  │   │Cllecting│   │Titrating│   │Calculation  │   │Measured │
│test     │ → │heating  │   →    │at high  │ → │evolving │ → │with     │ → │             │ → │value    │
│sample   │   │         │        │temp.    │   │gas      │   │KIO₃     │   │             │   │         │
└─────────┘   └─────────┘        └─────────┘   └─────────┘   └─────────┘   └─────────────┘   └─────────┘
                                     ↑
                                ┌─────────┐
                                │Leading  │
                                │oxygen   │
                                └─────────┘
```

Procedure 2: Preparation of cause and effect diagram

Fig.2.8.   Cause and effect diagram
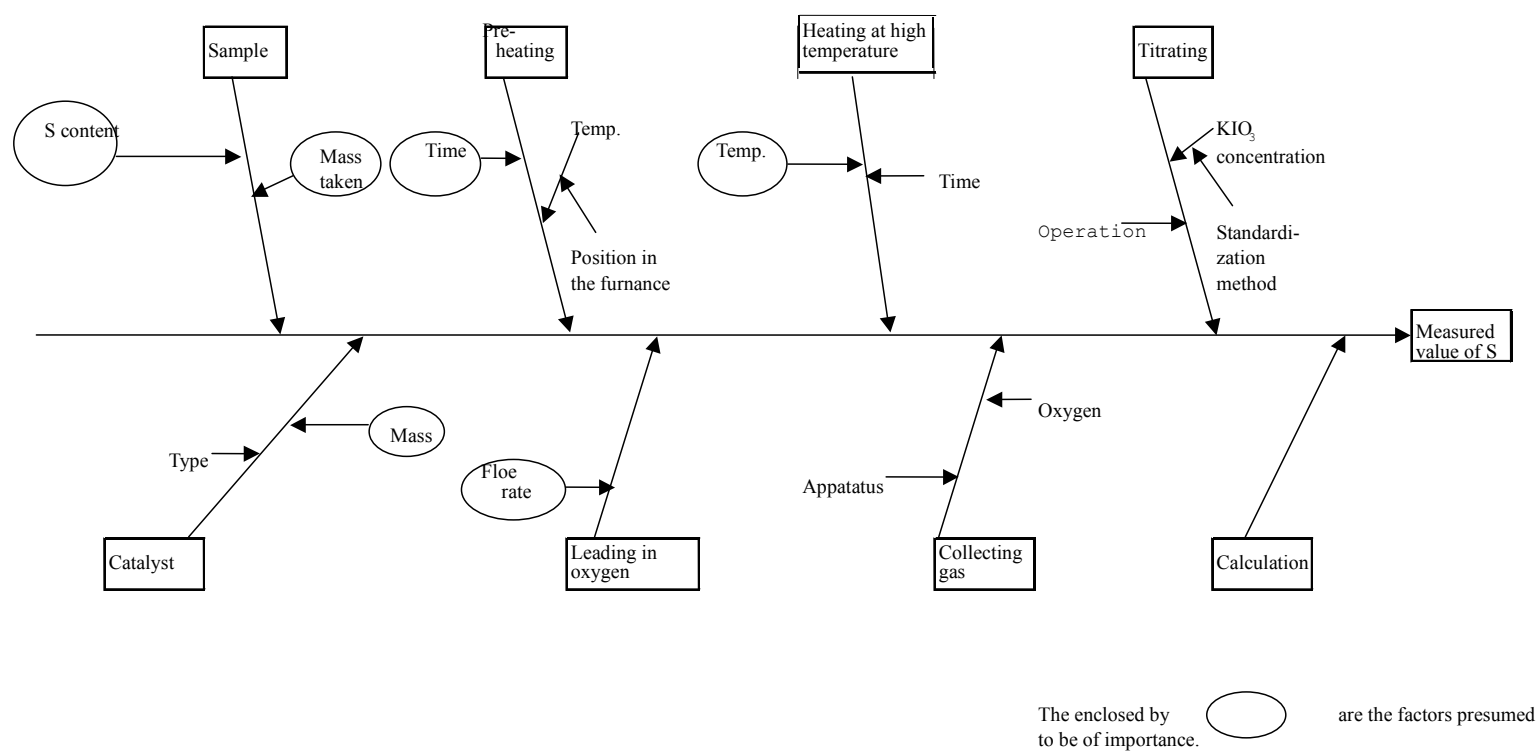
Procedure 3: Selection of factors and levels

Table 2.7.   Factors and levels

Factor	Level	
	1	2
A Type of sample	Hjgh-sulfur sample	Low-sulfur sample
B Mass of sample taken	0.5 g	1 g
C Catalyst	Cu (double the mass of sample)	No addition
D Pre-heating	3 min	No pre-heating
E Temperature in the furnance	1350 $^{\circ}$C	1400 $^{\circ}$C
F Flow rate of oxygen	150 ml/min	300 ml/min

Possible interactions;
$A \times B, A \times C, A \times D, A \times D, A \times E, B \times C, C \times D, C \times D$

Procedure 4: Assignment for experiments
The assignment for experiments and experimental results are shown in Table 2.8. and Table 2.9.

Table 2.8.   Assignment for experiments

Experiment No.	A	A×B	B	B×C	F	A×C	C	C×E	e	A×D	D	C×D	e	A×E	E
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
3	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2
4	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1
5	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2
6	1	2	2	1	1	2	2	2	2	1	1	2	2	1	1
7	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1
8	1	2	2	2	2	1	1	2	2	1	1	1	1	2	2
9	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
10	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1
11	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1
12	2	1	2	2	1	2	1	2	1	2	1	1	2	1	2
13	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1
14	2	2	1	1	2	2	1	2	1	1	2	2	1	1	2
15	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2
16	2	2	1	2	1	1	2	2	1	1	2	1	2	2	1

Table 2.9.   Measured results at each laboratory

Unit: %

Experiment No.	L1	L2	L3	L4	L5	L6	L7	Valance between average values $\times 10^5$	Logarithm of variance between average values
				Laboratories					
1	0.093	0.107	0.104	0.091	0.108	0.103	0.089	6.42	-9.65
2	0.101	0.108	0.103	0.095	0.109	0.102	0.098	2.52	-10.59
3	0.102	0.108	0.111	0.102	0.106	0.107	0.102	1.26	-11.28
4	0.090	0.106	0.112	0.097	0.104	0.104	0.103	4.89	-9.93
5	0.108	0.108	0.113	0.091	0.106	0.102	0.096	5.86	-9.74
6	0.098	0.109	0.108	0.091	0.104	0.100	0.091	5.45	-9.82
7	0.100	0.109	0.106	0.097	0.108	0.099	0.102	2.2	-10.72
8	0.103	0.110	0.109	0.100	0.109	0.102	0.102	1.73	-10.96
9	0.068	0.075	0.076	0.071	0.071	0.071	0.072	0.73	-11.82
10	0.066	0.075	0.074	0.068	0.072	0.071	0.073	1.06	-11.46
11	0.068	0.075	0.075	0.067	0.072	0.069	0.062	2.19	-10.73
12	0.066	0.075	0.078	0.069	0.069	0.069	0.051	7.41	-9.51
13	0.068	0.078	0.074	0.066	0.069	0.070	0.068	1.73	-10.97
14	0.070	0.079	0.084	0.068	0.070	0.071	0.070	3.55	-10.25
15	0.070	0.078	0.074	0.066	0.073	0.069	0.064	2.33	-10.67
16	0.070	0.071	0.078	0.065	0.070	0.070	0.061	2.79	-10.49
Average value	0.082	0.092	0.092	0.082	0.089	0.086	0.082		

Procedure 5: Analysis of variance of logarithm of variance between average value and graphing

Obtain at each treatment the variance between the average value of the observed values of seven laboratories from the observed values in Table 2.9 and, furthermore, take up the natural logarithm. The result from the analysis of variance carried out taking up this as the characteristic values are shown in Table 2.10, and the factorial effects which became significant are shown in Fig.2.9. The following matters are estimated from the Fig.2.9.

In order to improve the reproducibility,

in the case of the high-sulfur sample.....catalyst (Cu) is added and heated at the temperature of 1400 °C.

in the case of the lower sulfur sample.....Cu is not added and should be heated at the temperature of 1350 °C.

and for both samples.....the flow rate of oxygen should be 300 ml/min.

Table 2.10. Analysis of variance table of logarithm of variance between average values

Factor	S	Ø	V	F_0
A Type of sample	0.636 0	1	0.636 0	6.24
B Mass of sample taken	0.057 1	1	0.057 1	0.56
C Catalyst	0.208 1	1	0.208 1	2.04
D Pre-heating	0.000 1	1	0.000 1	0
E Temperature in the furnace	0.070 5	1	0.070 5	0.69
F Flow rate of oxygen	2.395 6	1	2.395 6	23.52[**]
A×B	0.113 7	1	0.113 7	1.12
A×C	1.073 0	1	1.073 0	10.54[*]
A×D	0.125 5	1	0.125 5	1.23
A×E	0.924 3	1	0.924 3	9.08[*]
B×C	0.000 0	1	0.000 0	0.00
C×D	0.371 9	1	0.371 9	3.65
C×D	0.420 4	1	0.420 4	4.13
e	0.557 3	2	$\emptyset'_e=6$ $V'_e=0.101\ 85$	
Total	6.953 6	15		

Fig.2.9. Relationship between standard deviation (unit:%) of average values and analysis procedure
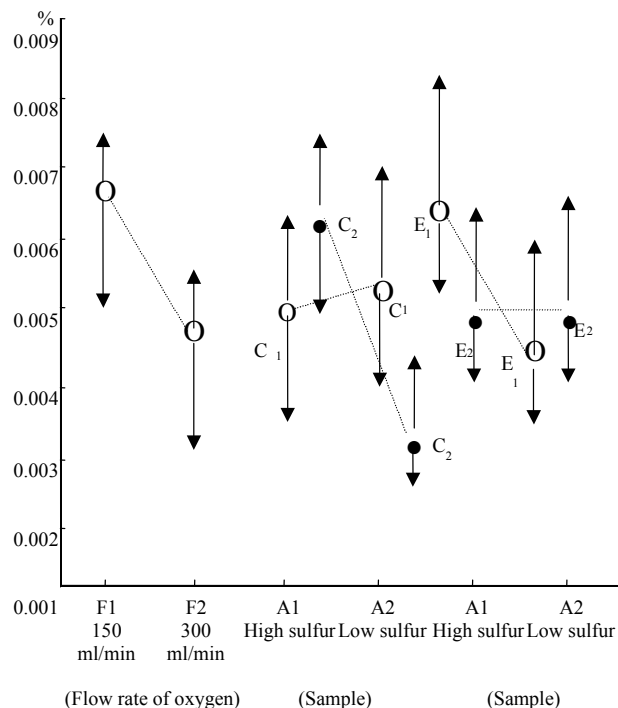
Annex III.

Periodic forest inventories

1.   Monitoring Methods for Forest Monitoring

Permanent or periodic forest inventories should be conducted to allow continuous observation of forest areas and the early recognition of development trends, to estimate standing crop, thus permitting corrective measures to be introduced in good time. One of the forest inventories suitable in monitoring activity is a working plan surveys. In this survey, the data collected were computed on a stand-by-stand basis for each species. Information on increment, detailed forest maps, data on the quality of the various sites are just as necessary as details on topography, ownership, and accessibility. Another inventory is the forest damage inventories. Subsequently, forest damage inventories record the state of health of the forest.

2.   General Description of Monitoring Forest

In order to detect the possible impact of acid deposition, a permanent monitoring site should be selected and the complete enumeration of trees and understory vegetation should be carried out regularly. Changes due to acid deposition should be described in detail.

2.1.   Site for Forest Monitoring

2.1.1. Forest Area

The forest areas should be divided into sub-units and such a classification can be based on a number of criteria:
-   existing political or administrative borders;
-    soil types;
-   land use;
-   type of vegetation;
-   site and/or climatic conditions;
-   preservation areas (nature reserves, catchment areas, etc.).

2.1.2. Selection of Plots for General Description of Monitoring Forest

When monitoring sites are selected according to the criteria described in the selection of basic survey site (which is described in 2.2.2.1.), two forest areas of more than 0.2 hectare can be selected. In each forest area, for the general description of the monitoring vegetation, a "strip" or rectangular sample area, whose length is a multiple of its width should developed for the detailed survey. In tropical forests, where the undergrowth hinders both access and visibility and large areas must be surveyed, it is usual to take rectangles or squares because such plots are the easiest to demarcate. The strips of up to 30 m wide and several hundred meters long are recommended.

The selection of the optimum size is basically determined by the spatial distribution and the variability of the forest to be surveyed. Small plots in homogeneous forests may

furnish results with higher precision, as the number of independent observations per given sampling intensity is higher. On the other hand, where heterogeneous forests are concerned, the coefficient of variation between small plots may increase so greatly that it would be better to use large ones.

The optimum plot size can be computed with

$$a = P_1 \ (t/m)^2 \quad ,$$

where a = optimum plot size, P_l = size of plot in pilot survey, t = average travel time between two neighboring plots, in = average measuring time on a plot pf size P_l (Zaide, 1980).

To compare two plot designs, their relative efficiency can be used. Here the costs and standard errors of the designs are compared as follows:

$$e = (s^2_{x1}t_1)/(s^2_{x2}t_2) \ ,$$

where s_{xi} = standard error in percent of plot designs i, t_i = time or cost for plot design i, e = relative efficiency. If e is smaller than 1, plot design I is more efficient, and if smaller than 1 less efficient than plot design 2. If e = 1, the two designs are equally efficient.

As a rule of thumb for optimizing plot designs, the plots should be large enough to contain enough trees per plot for the results to be representative, while the survey time per plot is not too great. It follows that small plots should be employed for dense stands with small trees, large ones for open stands with big trees. Very often, a distinction is made between unproductive marching times and productive survey times. This indicates that fairly large plots are preferable for surveying tropical forests. Areas between 0.4 and 0.5 ha have proved suitable.

As all data based on area are given for a horizontal plane, the sample plots in sloping terrain must be adapted accordingly. There are various means of achieving this:
-   demarcation of the plots on the incline and subsequent correction;
-   enlargement of the plot in proportion to the degree of inclination (projection of the inclined area onto the horizontal gives exactly the defined area of the sample plot);
-   demarcation of an ellipse on a slope (this becomes a circle when projected onto the horizontal);
-   measurement or computation of the horizontal distance of the trees from the plot centre (this becomes an ellipse on the slope surface).

In order to simplify the field surveys and facilitate checks, enlargement of the plot in proportion to the degree of inclination is most suitable. Particularly in mountainous regions, erroneous corrections of area lead to by no means negligible errors in extrapolating the results. For this reason, corrections for slope inclination must be extremely carefully made.

Concentric plots are employed to reduce the time used for measurement in the sample plots in areas with a high stem count which contain a large number of trees. Here, two or

more plots of differing size are demarcated around a given point. In the smallest area, all trees with diameter greater than a given minimum (e.g. 12 cm) are surveyed. In the other areas, the minimum diameter increases. This design often allows a considerable reduction of survey time without a marked decrease in efficiency. On the smaller, 200 m^2 plot, all trees are surveyed, while on the larger, 500 m^2 plot only those with a DBE of over 35 cm are included.

Sample plots on the forest edge which are the portion of the plot which actually lies within the forest changes. Yet such plots cannot be excluded, as this would mean that the border areas with their different conditions would not be included and the results would consequently not be representative. One solution is to record exactly the location of the forest edge within the sample plot and reduce the area accordingly. Another method which is very often recommended is to take the mirror reflection of the plot, as described by Schmid (1969). Here, all plots whose centers lie within the stand are surveyed, while those whose centers lie outside are neglected. In a mirror reflection of the plot from the centre point, that part of the plot lying within forest is surveyed again. Correctly applied, this method produces unbiased results, but demands that the forest margins be precisely defined.

Permanent fixed-area sample plots have the advantages that they are simple to survey, provide trend-free results and that the results are relatively easy to compute. For permanent inventories, fixed area plots are to be particularly recommended, as they allow easy determination of growth components such as in growth, mortality and cuts.

3.   Measurements and Observations and Frequency of Monitoring on Field Plots for General Description of the Monitoring Forest

3.1.   Area Information

Area information may be obtained from maps, aerial photographs, diizitat remote sensing data, or terrestrial surveys. Areas may be measured by means of area calculations, weighing, planimetry, or square or parallel grids. One of the disadvantages of measuring techniques is, that the error of the area estimation cannot be determined. The procedures are very time-consuming and not practicable for large-scale inventories. The only efficient technique is area calculation combined with GIS. Especially where large-scale inventories are concerned, areas of concern should not be delineated on the basis of the airphotos. Delineation is very time-consuming and the error of the area assessment cannot be calculated.

In forest inventories, area estimation by means of sampling is preferable to measuring techniques. There are two possibilities: dot sampling and parallel lines or "transacts". In dot sampling, points falling within the area or the class of forest concerned and the total number of points are counted. With parallel lines, it is the length of the line falling within the classification concerned which is measured. The total area is computed by adding the number of points or line sections within the area of concern and multiplication by an area expansion factor.

Sampling techniques for area estimation may be based on maps, aerial photographs, digital classification or field surveys. Where maps and airphotos are used, it must be ensured

not only that the latest data are verified but also that the precision depends on the scale. The greater the scale, the greater the accuracy. Only maps with a high dimensional stability coefficient should be employed, or even better, recent airphotos, if available.

In digital image analysis, special attention must be paid to the precision of classification. Once the algorithms for classification have been established in training areas, the precision of the classification must be checked in a completely independent area. Interpretation of confusion matrices, two-way tables based on actual class and class allocation, through image analysis is particularly important: the frequency of correct or incorrect allocation to a class may help determine the suitability of image analysis as a tool for area estimation.

Sampling for area estimation has the advantage that the sampling error can be determined. Where the sampling error of estimates related to an area is to be given, the error of area estimation has to be included in the calculation of the standard error by the rule of error propagation.

3.2. Detail Tree Information

3.2.1. Species Identification

Every field survey team should include at least one "tree finder" because of the difficulties in carrying out inventories in tropical forests. This may be an experienced forester, (though the services of such are often difficult to obtain) or one of the local inhabitants, who often possess an astonishing knowledge of the species occurring in their neighborhood. "Tree -finders" are often only familiar with the local names of tree species. Consequently it is necessary to compile a list of local and botanical species names. One species may have several local names, while one local name may be applied to a number of different species. Local names may vary with the sex or age of a tree. Consistency of the records may be enhanced by repeatedly employing the same tree finders over long periods. Apart from the advice of the tree finders, an identification key based on the main obvious characteristics of a species, such as bark, roots, leaves and fruits may be of assistance.

3.2.2. Diameter at Breast Height (DBH) and Upper Stem Diameters

Diameter at breast height should consequently be recorded for all trees on each sampling site. The diameter at 1.3 m above ground level, should be measured with the diameter tape in 0.1 cm unit. In the case of less than 4 cm, the caliper might be used. Through summation of the DBH of the individual trees, the basal area of the whole stand can be computed, which in combination with the number of trees reflects the density of the stock.

According to the standardized system of IUFRO, the following labels are used to signify the various diameter classes:

d = DBH over bark;
c = Circumference at breast height over bark;
g = basal area at breast height over bark;
d_u, c_u, g_u = corresponding values under bark;

d_b = diameter over bark at buttress height;

d_{st} = diameter over bark at stump height;

d_x = diameter over bark at x m height, e.g. d_7 = diameter in 7 m height;

$d_{o.xh}$ = diameter over bark at 0.x of tree height, e.g. $d_{0.1}$, = diameter in 1/10 of the height.

The great girth of trees in tropical forests renders diameter tapes preferable to calipers, as they provide more consistent measurements.

In tropical rain forests and mangrove swamps, where the trees display buttress or stilt roots with root collars extending far up the stem, diameter should be measured above the point at which the thickening ceases, and just like DBH is taken as a basis for the determination of volume and stem form.

3.2.3. Cross-Sectional Area Measurement

The total basal area of all trees or classes of trees per unit area (e.g., per hectare) is generally assumed that the basal area, g, is circular and can thus be directly computed either from the diameter, d, or the circumference, c, according to the following equations:

$$g = (\pi/4)d^2$$
$$g = c^2/(4\pi) ,$$

with d = diameter at 1.3 m, c = circumference at 1.3 m.

The basal area of a stand is measured in square metres or square feet. Unfortunately, the basal area of a tree is seldom really circular. Some species, e.g. *Tectonia granites*, are very irregular in form. Matern (1956) suggests that deviations from the purely circular can be described through (1) the convex deficit and (2) the isoperimetric deficit. The convex deficit is the difference between the area as determined with a measuring tape and the actual cross-sectional area of the trunk. If the cross-sectional area of the stem is not circular, the circumference is greater than that of a circle of the same area. Wherever equations for computing basal area assuming a circular form are applied, the computed area is always greater than the actual one. All areas of convex form deviating from the circular display an isoperimetric deficit.

In most cases it is sufficient to measure the smallest and largest diameters and calculate the geometric mean, $d_g = \text{í}d_l\ d_2$. Where it is not feasible to measure these two diameters, the diameter can be measured crosswise and the arithmetic mean computed.

3.2.4. Height

Tree height can be employed as a parameter in volume or increment functions and, in combination with stand age serves as a measure of site quality. The term "height" used in monitoring acid deposition can be defined as:

- Overall height. This is the distance between the ground and the tip of the tree. This distance is easier to understand than measurements at different points, but in tropical forests the tip of a tree is seldom visible from the ground.

- Bole length. This is the distance between the ground and the beginning of the crown, in other words, the point at which the first branch occurs. Stipulations for measuring lopsided crowns or crowns with dead branches must be laid down in the survey instructions.

- Stump height: this is the distance from the ground to the point at which the stem is cut. In computing volume functions, it is taken as constant.

- Crown length is the distance from the beginning of the crown to the tip of the tree.

3.2.5. Tree Form

Tree form can be described in terms of theoretical solids. The simplest solids approximating the various stem sections are the cylinder, the paraboloid, the cone, and the neiloid. Volume functions which already take "typical" stem forms into account by an upper diameter. Nevertheless, a knowledge of the theory of stem form is useful in computing volume functions.

The form factor f is the relationship between the stem volume and the volume of geometric figure - usually a cylinder - with the length and cross section g as the stem. The volume is given by

$$v = ghf.$$

Thus, the form factor for a cylinder is a reducing factor compensating for overestimation. Form factors can be calculated for stem diameters at different heights. If the DBH is taken as a basis, the resulting form function is termed the breast height or artificial form function. It is not a true expression of the stem form, as the form functions for trees with the same stem form, but differing dimensions vary. Consequently, breast height or artificial form functions are not directly comparable. The diameter at 1/10 of the tree height should be taken as the reference measurement. This allows computation of what is termed the natural form function, which in turn permits the direct comparison of stem forms independent of tree dimensions. In practice, however, the measurement of diameter at this level is very time-consuming, so that the artificial form function is commonly preferred.

Form quotient is the relationship between a given diameter to a reference diameter. Also here there are natural form quotients, which are based on clo.9 and artificial form quotients taking the DBH as a reference figure. Hohenadt (1936) divided the stem into five. sections and derived a form quotient for each section.

$$\eta_{0.9} = d_{0.9} / d_{0.9} = 1;$$
$$\eta_{0.7} = d_{0.7} / d_{0.9} ;$$
$$\eta_{0.5} = d_{0.5} / d_{0.9} ;$$

$\eta_{0.3} = d_{0.3} / d_{0.9}$;
$\eta_{0.1} = d_{0.1} / d_{0.9}$;

The form quotient according to Hohenadt (1936) can then computed from

$$\lambda_{0.9} = 0.2 \, (1 + \eta^2_{0.7} + \eta^2_{0.5} + \eta^2_{0.3} + \eta^2_{0.1})$$

and used for the immediate calculation of volume,

$$V = \Pi / 4 \, d^2_{0.9} \, h \, \lambda_{0.9}.$$

Analogue series can be calculated fort the DBH as a reference diameter.

3.2.6. Volume

Volume is the most important parameter determined in forest inventory and almost always the one for which sampling designs are optimized. Procedures for volume determination are manifold and cannot be discussed in detail here. For those interested, textbooks such as Prodan (1965) or Hush et al. (1982) provide more thorough information. Methods and problems of volume determination are outlined in Cunia (1979) and Kublin (1987). As the computation of volume functions requires a solid basis in regression analysis, the book by Draper and Smith (1981) may be mentioned here as recommended reading.

The selection of the method to be used for volume determination is one of the most important points in the planning of an inventory, as biased volume estimates may place the entire results of the inventory in question (Cunia 1981; Gertner and Kohl 1992).   It is of the utmost importance that the volume to be quantified in the inventory be precisely defined. To this end, it is necessary to stipulate from the beginning:

- The minimum and maximum diameter of the trees to be surveyed;

- To what minimum diameter of the upper stem the volume is to be calculated (this gives together with the maximum and minimum diameter the defined spectrum of tree forms);

- Whether the volume is to be determined inside or outside bark;

- Whether branches, roots, and stump are to be included;

- Whether damaged stem sections are to be excluded or not;

- Whether the cross or only the merchantable volume is to be determined;

- Whether the term "usable volume" refers to the volume without logging loss or loss through processing at the sawmill.

The FAO has proposed certain definitions of volume to be applied in forest inventories (FAO 1973, p. 105). Volume may be expressed in several different units. However, it is strongly advisable to use the metric system (m^3).

3.2.7. Weight and Biomass

The biomass of a tree, divided into components such as roots, branches, stem, bark, top sections and even needle or leaf mass, related to a unit area (Young et al. 1964), may be taken as a measure of the productivity of a site. Biomass is expressed in kilograms of dry weight. Where data on biomass are required, a rough estimate can be made on the basis of previous stem volume measurements.

3.2.8. Density

Density may be expressed in terms of stem count, volume, or biomass. One important variable for ecological estimation is crown density, which gives the proportion of ground covered by crowns (Whitmore 1989). Particularly in exploited areas, this parameter should not be left out of consideration. Aerial photography constitutes the most convenient technique. In tropical forests, stem count rather than crown density per unit area should be determined due to closed canopy.

Annex IV.

Data and Information on Ecological Impact Monitoring from Malaysian Site for EANET: Pasoh Forest Reserve

Introduction

Pasoh Forest Reserve is an inland forest reserve situated at 2° 59'N and 102° 19'E, about 37 km from Kuala Pilah in the south and about 140 km southeast of Kuala Lumpur. The nearest coastline is about 70 km to the southwest. Pasoh Forest Reserve lies approximately at the junction of Dale's (1959) West and Southwest regions, and in Jelebu district which is the driest climatological station in Peninsular Malaysia on record. The daily air temperature in the forest ranges from 17.5^oC to 29^oC with a monthly mean of 23.0^oC. At Kuala Pilah the monthly mean varies from 26.0^oC to 27.5^oC. The greatest fluctuations in the forest are above the canopy, and these diminish towards the forest floor. The average daily number of hours of sunshine varies between 3 and 5, the longer duration being during the months of April and May, and these decrease towards the end of the year.

Soil and Geological Aspect

Pasoh Forest Reserve is underlain by two types of rock formation (Gobbett, 1972). A north-south ridge of igneous rocks and an acid granite nature on the east of the reserve is of Carboniferous to Cenozoic age. The central and western parts of the reserve are composed of sedimentary rocks of Middle or upper Triassic age.

The soil profile in Pasoh can be categorized into three group (Allbrook, 1973). The Batu Anam, Malacca and Durian series are of shale parent material and are characteristically inter bedded with sandstone and quartzite. the Bangor, Serdang and Munching series are of shale while the Rengam and Tampin series are of granite parent material. Lastly, the Akob and Telemong series are derived from fluviatile granite alluvium and have permanent but variable water table. Pasoh Forest is drain by two tributaries of the westward flowing Manong Kaman River. Except for the northeastern corner of the area, which consists of a gentles slope rising to an altitude of 570 m, the terrain is for the most part relatively flat and at an altitude of approximately 90 m. It is well-drained but swamp area occur along some of the tributaries.

Historical Background

Pasoh Forest Reserve, constituted nearly 1917, was identified as the site of collaborative research (1970-74) between the Malaysian national Committee for the International Biological Program (IBP), the University of Malaya, the Forestry Department of Malaysia, The Royal Society of London and Japanese Society for the promotion of Science. The Government of Negeri Sembilan State, in which the reserve is situated, allocated about 1950 ha of the reserve in 1968 and another 450 ha or so in 19771 to the IBP for the integrated study on dynamic and productivity. The remaining 1975 ha of the reserve in the northwest through west to the south, was logged and converted to oil palm (*Elaeis guineensis* Jacq.) plantations in 1971 (Manokaran and Swaine, 1994).

Pasoh Forest Reserve now consists of a core research area of about 650 ha of primary lowland forest surrounded by a buffer zone of about 650 ha of logged and regenerating forest (lowland dipterocarp forest in which the natural regeneration, mainly seedlings but also including advance growth, had been released and assisted by silvicultural treatment after exploitation) and about 100 ha of primary hill forest. the regenerating buffer zone, as well as much of the area under oil palm now, was selectively logged between 1955 and 1960 and the silvicultural treatment of GCL carried out in the logged area, also between 1955 and 1960.

Between 1973 and 1979, Pasoh Forest reserve was the site of the University of Malaya-University of Aberdeen project on reproductive biology of rain forest trees. In 1977, the reserve became a substation of the FRI. It is now known as the Pasoh Forestry Research Center of FRIM. It is a center for rain forest research by scientist from the Institute and from local and foreign institutions and universities. beginning in 1985, a demographical study is in progress of all woody plants of 1 cm diameter and larger in a 50 ha plot in the primary part of the forest (Manokaran *et al*., 1990; Manokaran *et al*., 1992).

Vegetation

Pasoh Forest Reserve belongs to the lowland dipterocarp forest which includes all the well-drained primary forests of the plains, undulating land and foothills up to about 300 m altitude, with exception of the narrow strip of beach forest (Symington 1943, Wyatt-Smith 1961, 1964). This forest are part of the lowland evergreen rain forest

formation of Burtt-Davy (1983). This forest formation in Peninsular Malaysia is characterized by family dominance of the Dipterocarpaceae, and may be regarded as composed of three tree layers; the emergent layer of trees, usually with spreading crowns nestling above but in contact with those of the main canopy, the main-storey trees forming the continuous canopy at 20-30m height, and the understorey trees below the main canopy.

Pasoh Forest Reserve was described as "Mixed Red Meranti" forest merging to "Red Meranti" forest by Salleh (1968) but was considered by Wyatt-Smith (1987) as "Red Meranti-Keruing" forest of the Central and Southern type. Species of Dipterocarpaceae are abundant, and include *Shorea macroptera, S. pauciflora, S. acuminata, S. parvifolia, S. leprosula, S. lepidota, S. maxwelliana, Dipterocarpus cornutus, D. crinitus, D. costulatus, D. sublamellatus, and Neobalanocarpus.* Emergent from other families, as revealed by the survey undertaken by Ashton (1971a, 1971b), include *Koompassia malaccensis, Intsia palembanica* (both Leguminosae), *Triomma malaccensis* (Burseraceae), *Dyera costulata* (Apocynceae) and *Durio oxyleyanus* (Bombacaceae).

Pasoh Plots

Studies of tree species distribution with soil variations were carried out by Wong and Whitmore (1970), and later with environmental variations by Ashton (1976). Wong and Whitmore (1970) selected randomly ten 1-acre (0.4 ha) plots of 1x10 chains (20.1 x 201.2 m) within the core area in 1959. The long axis of each plot was made to run north-south and the plot number 1 to 10. All plots except 1 and 2 were considered to be on flat land, and all except 4,5 and 8 considered to be well-drained. Ashton in 1971 enlarged fove of those plots to a size of 2 ha (100x 200 m) by extending the shorter sides of the old plot. Five plots (1, 3, 4, 6 and 7) were selected due to their site diversity and accessibility and each plot were divided into 50 quadrats (20 x 20m). The forest was chosen as the location of study as it contained a core primary forest are which was uniform in relief but having different geology and soil series.

Species Groups

Emergent (E)
Mature phase light demanding long lived species growing above the main canopy of primary forest to more than 30m, usually with spreading crowns.

Main canopy (MC)

Mature phase light-demanding relatively long lived species that form the main canopy of primary forest, growing to heights between 20 to 30 m.

Understorey-Mature phase shade-tolerant tree species forming the lower strata of the primary forest, with maximum heights below 20m.

Pioneer (P)

Short-lived tree species requiring a gap for germination and establishment, showing rapid growth; extremely light demanding and intolerant of shade.

Late-seral (LS)

Light-demanding tree species but relatively tolerant of shade, prominent during the late stages of succession. They can persist to form part of the mature forest.

This classification is based partly on knowledge of the tree species from field experience, and is augmented by reference to the tree Flora of Malaya (Whitmore 1972, 1973, Ng, 1978, 1989) to the Foresters' Manual of Dipterocarps (Symington, 1943) and to the Pocket Check List of Timber Trees (Kochummen, 1979).

Species composition

Pasoh Forest have 45-48 number of families, 125-141 number of genera and 235-276 species. Of the ten commonest families, in terms of tree density, in each of three sites, four, Dipterocarpaceae, Euphorbiaceae, Myristaceae and Burseraceae, were common to all three sites. Dipterocarpaceae is a second common species in Pasoh Forest Reserve. Basal value of the commonest families were 43.6% of the total at Pasoh Reserve Forest.

Table 1. Main characteristic features of Pasoh Forest Reserve

Parameter	Pasoh
Forest type	Lowland dipterocarp forest
Forest sub type	Red-Meranti Keruing
Altitude	90 m a.s.l.

Plot Size	4x 2 ha
Mean annual temperature (nearest station)	27.1°C
Mean annual rainfall (nearest station)	1850 mm
Soil	Sedimentary -derived soil mainly
Tree density (ha^{-1})	545.5
Basal area (m^2 ha^{-1})	29.13

Note: Based on Wyatt-Smith (1987)

Source: Modified from Manokaran and Swaine (1994)

Table 2. Density and Basal Arae of the ten commonest families in 1971 at Pasoh Forest Reserve.

Family	Pasoh	
	Density (ha^{-1})	Basal Area (m^2 ha^{-1})
Dipterocarpaceae	52	9.04
Flacourtiaceae	11.4	0.25
Euphorbiaceae	66.1	1.82
Rhizophoraceae	6.1	0.22
Tiliaceae	4.8	0.19
Sterculaceae	12.9	0.67
Lauraceae	10.6	0.38
Anarcadaceae	19.4	1.03
Myristicaeae	21.9	0.69
Burseraceae	40.3	1.85
Annonaceae	30.3	0.71
Rubiaceae	15	0.33
Meliaceae	11.4	0.24
Guttiferae	16.3	0.53
Sapindaceae	23.1	0.7
Leguminosae	36.9	3.3
Myrtaceae	28.9	1.07
Polygalaceae	17.5	0.48
total for 10 commonest families	336.4	20.69
Total for remaining families	209.1	8.44
Grand total	545.5	29.13

In the 50-ha plot in Pasoh, the Dipterocarpaceae was the second most frequent family at the size of 1 cm diameter and larger, forming just over 9% of the total stems, and dominated basal area from this lower size class level, accounting from 24 % of the total (Manokaran *et al*., 1991). Euphorbiaceae, the commonest family at Pasoh, formed only 6.2% of the total basal area; members of the family are mostly sub canopy and understorey species in lowland and hill forests in Peninsular Malaysia (Whitmore, 1973). Collectively, the ten commonest families contributed 71.0% to the total basal area   at Pasoh.

Table 3. Density and basal Area of the ten Commonest Species in 1971 at The Pasoh Forest reserve.

E=Emergent species, MC=main canopy species, U= understorey species.

Species	Pasoh	
	Density (ha^{-1})	Basal Area $(m^2\ ha^{-1})$
Scaphium macropodum (MC Sterculaceae)	6.9	0.41
Anisophyllea corneri (U Rhizophoracea)	0.1	0.03
Gonocaryum gracile (U Icacinaceae)	0.3	0
Gluta elegans (MC Anarcadaceae)	0.1	0
Sanitary laevigara (MC Burseraceae)	3.3	0.19
Ixonaanthes icosandra (MC Linaceae)	14.4	0.99
Shorea macroptera (E Dipteracarpaceae)	4.1	0.32
Ochanostachys amentaceae (MC Olacaceae)	6.3	0.21
Porterandia anisophylla (U Rubiaceae)	2.6	0.05
Shorea parvifolia E Dipterocarpaceae)	3.4	0.54
Shorea parvifolia (E Dipterocarpaceae)	3.6	0.52
Sanitary tomentosa (MC Burseraceae)	3.8	0.26
Trigoniastrum hypoleucum (MC Trigoniaceae)	1.3	0.03

Xerospermum noronhianum (U Spaindaceae)	15.6	0.41
Cleistanthus sumatranus (U Euphorbiaceae)	12	0.18
Dacryodes rugosa (U Burseraceae)	10.6	0.24
Xantophyllum eurhynchum (U Polygalaceae)	7.4	0.11
Saraca declinata (U Leguminosae)	7.1	0.42
Koompasia malaccensis (E Leguminosae)	6.5	1.06
Penstapadon motleyi (MC Anacardiaceae)	6.5	0.52
All species of shorea	29.3	5.12
All species of Dipterocarpus	10.1	2.13
Total for 10 commonest species	93.3	4.55
Total for remaining species	452.2	24.58
grand total	545.5	29.13

Species groups

The proportion of emergent species as a group were very high in Pasoh. Emergent comprised of dipterocarpaceae in Pasoh Forest

Table 4. Proportion of the Species Group and Their Densities and Basal Area in 1971 at Pasoh Forest Reserve.

Species Group	Pasoh
Emergent	
Species (% of total spp.)	12.6
Density (ha^{-1})	82.4
Basal area $(m^2\ ha^{-1})$	12.13
Main canopy species	
Species (% of total spp.)	46.2
density (ha^{-1})	213.3
Basal area $(m^2\ ha^{-1})$	10.92
Understorey species	
Species (% of total spp.)	39.6
Density (ha^{-1})	246.4

Basal area (m^2 ha^{-1})		5.99
(Pioneer + Late serial species)		
Species (% of total spp.)	1.4	(0.5+0.9)
Density (ha^{-1})	3.4	(0.4+3.0)
Basal area (m^2 ha^{-1})	0.09	(0.01+0.08)
Total Density (ha^{-1})		545.5
Basal area (m^2 ha^{-1})		29.13

Source: Manokaran and Kochummen, 1990.

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Appendix 1. Sensitivity of soil types to acid deposition classified according to their inherent soil factor in Japan

Low sensitive soils
Fluvisols (Gley soils, Gray lowland soils, Brown lowland soils)
Rendzic Leptosols (Rendzina-like soils)
Chromic Luvisols (Terra Rossa-like soils, Chocolate Brown soils)
Chromic/Eutric Cambisols (Terra Fuska-like soils)
Vertic Cambisols and Chromic Vertisols(Grumsol-like soils)
Dystric/Humic Gleysols and Dystric Planosols (Stagnogley soils)
Umblic Andosols (Kuroboku soils)
Moderately sensitive soils
Cambisols (Brown forest soils, Yellow-brown soils)
Podzols (Podzolic soils)
Haplic Andosols (Light colored Andosols)
Greysols (Illuvial paddy soils, Gray paddy soils)
High sensitive soils
Vitric Andosols (Pyroclastic regosol)
Umblic Andosols (Regosolic Andosol)
Fiblic Histosols (Peat soils)
Haplic/Ferric Alisols and Haplic/Ferric Acrisols (Red and yellow soils)
Dystric Gleysols/Planosols (Pseudo-gleys)
Lithic Leptosols (Coagulated lithosol)
Dystric/Eutric Regosols (Non-coagulated lithsol)

Note: Names of soil types are followed by FAO/UNESCO(1990). However, names in parentheses are by "The Group of Japanese Pedologists"

Appendix 2. Decline scale

Items	Decline scale and observations	
Vitality of tree	0. No damage	1. Slightly damaged
	2. Evidently damaged	3. Severely damaged
	4. Dead	
Form of tree	0. Normal	1. Some branches lacking
	2. Evidently wanting	3. Severely wanting
	4. Dead or almost dead	
Branch growth	0. Normal growth	1. Somewhat reduced
	2. Short and slender	3. Extremely short
Dieback of stem	0. Not found	1. Slightly dieback
	2. Evidently dieback	3. Severe dieback if found
	4. Completely dead	
Density of foliage	0. Healthy foliage	1. Unbalanced foliage
	2. Sparse foliage	3. Very sparse with dead leaves
Deformation of leaves	0. Normal	1. Slightly deformed
	2. Evidently deformed	3. Completely deformed
Size of leaves	0. Normal	1. Somewhat small
	2. Evidently small	3. Very small
Color of leaves	0. Normal	1. Somewhat abnormal
	2. Abnormal	3. Very abnormal
Injury of leaves	0. No symptoms	1. Slightly injured
	2. Evidently injured	3. Severely injured