Technical Manual
for
Soil and Vegetation Monitoring
in East Asia

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of

Acid Deposition Monitoring Network in East Asia

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1. Introduction

1.1. Background

Acid deposition due to acid precipitation and acidic gases may cause soil acidification, nutrient imbalance, and/or direct damage to plant body, and may become a cause of forest decline. Since the forest/tree declining process is relatively slow and complicated, it is important to monitor soil and vegetation for a long-term evaluation for description of the acidification and declining processes.

Since the preparatory-phase activities of Acid Deposition Monitoring Network in East Asia (EANET) started in 1998, experiences in soil and vegetation monitoring have been gradually accumulated in the participating countries of EANET. During the preparatory phase, the Monitoring Guidelines and Technical Manuals for Acid Deposition Monitoring Network in East Asia (Environment Agency, Government of Japan, 1997), which had been adopted by Expert Meetings on Acid Deposition Monitoring Network in East Asia, have been used for the monitoring. However, the conditions of the acid deposition monitoring in the participating countries are quite diverse, especially in the soil and vegetation monitoring. The methodologies described in the manual are not suitable for certain areas. In addition, experiences in the participating countries are limited in this field. For improving this situation, The Workshop on Ecological Impact Monitoring of Acid Deposition in East Asia (the 2nd Training Workshop on EANET) was held in Beijing on 31 August - 3 September, 1999. Technical issues were discussed in detail among experts from the participating countries and an international organization. The workshop clarified objectives of soil and vegetation monitoring and provided guidances on the technical issues. The proposed revision of this technical manual is mainly based on the outcomes of the workshop.

1.2. Objectives of soil and vegetation monitoring

The ultimate objective of the soil and vegetation monitoring will be to assess the impacts of acid deposition on terrestrial ecosystems in a comprehensive and systematic manner through establishment and maintenance of good quality database. To achieve this ultimate objective, step-wise approach should be adopted. The initial objectives of the soil and vegetation monitoring could be an establishment of baseline data, and also early detection of possible impacts of acid deposition, particularly on plants and forest

ecosystems.

1.3. Outline of the manual

In this manual, forest area is mainly focused as monitoring site, and vegetation means trees and understory vegetation in forest area. Soil and vegetation monitoring should be carried out according to step-wise approach on the monitoring objectives. Concept of approach on soil and vegetation monitoring is described in Table 1.1.

For the initial objectives, basic survey of soil and forest is proposed in the chapter 2. Physical and chemical properties of soil should be analyzed as the soil monitoring items, and description of trees, understory vegetation monitoring and survey of tree decline should be carried out as the forest monitoring items. These monitoring items should be surveyed in basic survey sites. The baseline data on soil and forest may be established through these activities, then changes in soil and forest properties can be detected. The possible impact of acid deposition on terrestrial ecosystems may be estimated based on the data. When some changes in properties of soil and forest would be detected, intensive survey would be carried out for the detailed survey. For the purpose, grasp of accurate deposition of acidic substances in the forest area and application of new techniques should be discussed.

For further discussions toward the ultimate objectives, terrestrial ecosystem analysis is proposed in the chapter 3. As one of comprehensive and systematic approaches, catchment analysis can be proposed. Elemental dynamics and nutrient status may be described through the analysis, which may be useful for evaluation of acid deposition impact on terrestrial ecosystem. Such sites should be classified to ecosystem analysis sites.

This technical manual describes representative methodologies for all the participating countries in East Asia. However, soil and vegetation in these countries are quite diverse. Therefore, possible alternate methods are also proposed in some cases. The methodologies for grassland are not described here although the acidification may also be an important issue for such area.

This manual should be reviewed and revised with experiences of the participating countries in EANET.

2. Basic survey of soil and forest

2.1.   Overall process of basic survey of soil and forest

Overall process of basic survey of soil and forest is described in Fig. 2.1. If some changes are detected in the basic survey site, intensive survey would be undertaken to assess the implication with acid deposition. Terrestrial ecosystem analysis should be carried out independently from these surveys. As described below (in 2.2.2.1.), a soil monitoring site will be established in a forest monitoring site. Thus, throughout the survey from preliminary surveys to establishment of permanent monitoring site, the common procedures should be carried out for the basic survey.

2.2.   Selection of basic survey site

Soil and forest properties may be characterized by area specific factors such as climate, geological and geographical features. For the evaluation of data in soil and forest monitoring, these area specific factors should be reported.

In order to select sites for permanent monitoring, preliminary surveys should be carried out. Conditions of monitoring areas should be clarified, and reproducibility conditions of data should be recorded precisely.

2.2.1.   Preliminary surveys

Preliminary surveys should be conducted over extensive areas in order to select sites for continuous monitoring to detect possible impacts of acid deposition on forest ecosystem. Preliminary surveys should be carried out as follows, and every necessary item should be described. Such survey sites should preferably be located within a radius of approximately 50km of (wet and dry) deposition monitoring sites.

2.2.1.1.   Characterization of basic survey site

The characterization of a basic survey site is described in the monitoring guidelines. Soil and forest monitoring should be carried out in a basic survey site. When some symptoms would be detected in the basic survey site, intensive survey for clarification of the implication with acid deposition would be carried out.

2.2.1.2.   Collection of information on soils, vegetation, and other characteristics

In preliminary surveys, the following information on soils, vegetation, geography, and meteorology should be collected. Fieldwork should be carried out, if necessary. Information on monitoring sites should be recorded correctly, and the characteristics of the sites in the country should be clarified. It is desirable to collect comparable maps with standardized international taxonomy of soil and vegetation. Soil and vegetation classification should be unified according to the FAO/UNESCO Soil Map of the World (FAO/UNESCO, 1977).

a) Soil information

Most East Asian countries already possess their own soil maps. However, these maps are sometimes described using specific soil units in individual countries. For comparison purpose, it is desirable to collect comparable maps with standardized international taxonomy, such as the FAO/UNESCO Soil Map of the World. If they are not available, it is preferable that the maps in each country will be accompanied by columnar sections of representative soil profiles and analytical data, which clarify the nature of the soils. Mineralogical composition and land use history are useful.

In order to perform preliminary survey, soil maps using a scale of 1:50,000, preferably 1:25,000, should be collected. If they are not available, maps as large-scale as possible should be collected.

Note:
- If any soil maps are not available for the areas of preliminary surveys, fieldwork should be carried out to collect geological, geographical and/or land-use information.
- Surface geological maps, geographical maps and land use maps may also be useful for evaluation of characteristics of monitoring sites. Thus, for the preliminary survey, any kind of relevant available information should be collected.

b) Vegetation information

Most East Asian countries already possess their own vegetation (plant-sociological) maps, physiognomic vegetation maps and/or land-use maps. As mentioned above, for comparison purpose, it is desirable to collect maps that correspond to the international

taxonomy, such as FAO/UNESCO Soil Map of the World.

Note:
- If any vegetation maps are not available for the area of preliminary surveys, it is also effective to use aerial photographs and/or satellite images, which show vegetation.

c) Climate and meteorological information

Each country should use meteorological observation stations to collect meteorological data, including temperature, precipitation, evapotranspiration, wind direction, wind speed and insolation (e.g. photosynthetically active radiation, PAR). Especially annual mean temperature and annual precipitation should be required for more than 10 years in the past. These meteorological data will be collected from the observation stations in the area within a radius of approximately 50km of deposition monitoring sites.

All the items of climate and meteorological information for the preliminary surveys are not mandatory. The items, which can be obtained in accordance with the procedures of the meteorological monitoring system of each country, could be used.

2.2.1.3. Soil preliminary survey

According to the above information concerning soil, soils should be classified into high, low, and moderately sensitive types with respect to acid deposition, and the distribution of each soil type should be described. If necessary, fieldwork should be done in order to obtain more detailed distributions of soils for selection of permanent monitoring sites. These results should be described on maps, then the area of distribution of each soil should be described.

The standard of sensitivity of soil types is shown in Appendix 1 in this manual. Capacity of soil to neutralize acid deposition varies depending on the type of bedrock, the type of soil, the content of exchangeable bases, topography and other factors. In particular, sensitivity to acid deposition tends to be high in areas where acidic bedrock is distributed, the soil pH is low, the soil is oligotrophic with a low base saturation percentage and the content of exchangeable aluminum is high. In such areas, continuous acid deposition tends to suppress the growth of plants.

2.2.1.4. Forest preliminary survey

According to the above information concerning vegetation and land-use, distribution of forest area and each forest type should be clarified. This distribution map should be evaluated with respect to soil type, and some forest areas whose soil types are different may be identified.

2.2.2. Establishment of the permanent monitoring sites

2.2.2.1. Site selection criteria

The following criteria should be addressed at the selection of the monitoring site. Image of permanent monitoring sites is shown in Fig.2.2.

(a) Two forest sites, whose soils have different sensitivities to acid deposition, are recommended to be selected.
(b) Each site should be established in a continuous forest area of more than one hectare. If the area is surrounded with a suitable shelter belt, 0.2 hectare is sufficient.
(c) Sites must be accessible for surveying over a long period (decades). Therefore, sites on which land use patterns do not change over this period of observation should be selected.
(d) Preferably, a common tree species or the dominant vegetation type between the sites will be selected.

Note:
- When two forest sites are selected according to criterion (a), distance between each site and emission sources should also be considered. If one site is closer to emission sources than the other site, effect of emission sources and soil type at both sites should be evaluated.
- When the site would be established in afforestation area, management record is necessary.
- In the case that there are some difficulties in complying with these criteria, flexibility should be given and the differences with the siting criteria should be clearly recorded.

2.3.   Monitoring methods for soil monitoring

2.3.1.   Selection of plots for soil monitoring

As shown in Fig. 2.2., two forest sites, whose soils have different sensitivities to acid deposition, should be selected. Several plots, at least two plots, occupying areas from 5 m x 5 m to 10 m x 10 m, should be selected randomly at each monitoring soil type.

2.3.1.1.   Soil profile description

For the establishment of plots, the soil profile description should be prepared. The soil profile should be described near the center of the candidate plots before soil sampling. The methods, including practical procedures, survey parameters, and soil classification, should be unified according to FAO-guidelines for soil description (FAO, 1990). A description made according to domestic standard soil survey methods should also be shown to ensure the compatibility with the information obtained by other soil survey programs in each country. A large-scale geographical map (e.g. 1:1000) and /or sketch of the area around the plot and a photo of the soil profile with the scale should also be attached to the soil profile description. When the results of soil profile description are similar to the expectation, five subplots should be selected in the plot as described below.

Note:
- If the result of the soil profile description is extremely different, the soil profile description should be prepared for additional several plots in the same monitoring site.

2.3.1.2.   Selection of subplots for soil sampling

In the plot, five subplots, each occupying 1m x 1m, are selected in principle at the center and on the diagonal lines of the plot as shown in Fig. 2.3. However, the location may be modified to obtain soil samples which have not received the direct effect of stemflow.

Note:
- The above sampling procedure, which is called the multi-stage sampling, is

described in Fig. 2.4. As mentioned later, this sampling procedure is also useful for Quality Control/Quality Assurance.

2.3.1.3. Conservation of plot for soil monitoring

The environment of the plots should be maintained without any anthropogenic disturbance throughout the soil monitoring period.

Permanent signs which are made by stainless steel or plastics to identify the sampling plots, occupying area from 5m x 5m to 10m x 10 m, should be maintained in order that all samples to be taken at the same location every sampling time. The areas disturbed by soil sampling are marked to distinguish them from virgin areas at the time of the next sampling.

2.3.1.4. Record of plot for soil monitoring

Following general description of the monitoring site should be described in Form (Soil & Vegetation B). When the site has particular characteristics that differ from the selection criteria, these characteristics should be recorded.
  1) Locality name of the site in detail.
  2) Latitude and longitude of the center of monitoring site.
  3) Altitude of the site in 10 m unit.
  4) Direction of the slope should be measured with a clinometer and shown as the angle from the north or south.
  5) Slope degree shown as an average level of the plots.
  6) Type of forest (natural, secondary, man-made).
  7) Management records as to years of regeneration, year and times of slashing, thinning, fertilization and the special treatment.
  8) The stand age means the year since the regeneration. The true age of tree should be written in the parentheses if the age of planted tree is higher.
The soil profile should also be described in Form (Soil & Vegetation C).

2.3.2. Monitoring parameters and frequency of analysis

Monitoring parameters, frequency of analysis and suggested analytical methods are summarized in the following Tables.

Table 2.1. Monitoring parameters for soil

Parameters	Unit	Criterion	Frequency of analysis
Chemical Properties of Soil			
a) Moisture Content	wt%	M	
b) pH (H_2O) and pH (KCl)		M	
c) Exchangeable Base Cations (Ca, Mg, K and Na)	cmol(+) kg^{-1}	M	
d) Exchangeable Acidity	cmol(+) kg^{-1}	M	
e) Exchangeable Al , H	cmol(+) kg^{-1}	O	Every 3 – 5 years
f) Effective Cation Exchangeable Capacity (ECEC)	cmol(+) kg^{-1}	M	
g) Carbonate Content (for calcareous soil)	%$CaCO_3$	M*	
h) Total Carbon Content	g kg^{-1}	O	
i) Total Nitrogen Content	g kg^{-1}	O	
j) Available Phosphate	P mg kg^{-1}	V	
k) Sulfate	S mg kg^{-1}	V	
Physical Properties of Soil			
a) Fine Earth Bulk Density	Mg m^{-3}	O	
b) Penetration Resistance (in the fieldwork)	kg cm^{-2}	O	

M: Mandatory items; O: Optional items; V: Voluntary items. Carbonate content is mandatory item only for calcareous soil.

Table 2.2. Analytical equipment and methods for soil monitoring

Parameters	Equipment/methods
Chemical Properties of Soil	
a) Moisture Content	Drying oven, Balance
b) pH (H_2O) and pH (KCl)	Glass electrode
c) Exchangeable Base Cations (Ca, Mg, K and Na)	AAS, ICP-AES or ICP-MS (CH_3COONH_4-Extraction)
d) Exchangeable Acidity	Titration (KCl-Extraction)
e) Exchangeable Al , H	ibid.
f) Effective Cation Exchangeable Capacity (ECEC)	Calculation (as sum of exchangeable cations)
g) Carbonate Content (for calcareous soil)	Volumetric calcimeter
h) Total Carbon Content	Titration (Walkley-Black method) or CN-analyzer
i) Total Nitrogen Content	Titration (Kjeldahl method) or CN-analyzer
j) Available Phosphate	Spectrophotometry (Bray-1 test)
k) Sulfate	Turbidimetry, IC, ICP-AES or ICP-MS
Physical Properties of Soil	
a) Fine Earth Bulk Density	Metal sampling cylinder, Drying oven, Balance
b) Penetration Resistance (in the fieldwork)	Pocket penetrometer

2.3.3. Soil sampling

In each subplot, after removing the litter layer (O horizon), 1-2 kg soil samples are collected by fixed depth. Two layers, uppermost (0-10cm) and underlying (10-20cm) layers should be collected with shovel or metal sampling cylinder. The sample is collected in equal proportions over the whole layer.

In the center subplot soil samples should be collected beside the hole for soil profile description. For the corner subplots, appropriate size of holes for sampling should be dug, then similar sampling procedures should be employed. From the next sampling, the same procedures should be adopted for the center subplot.

Note:
- Soil whose layers have limited depth may be sensitive to acid deposition. When the soil layers are significantly thin (e.g. less than 5 cm), each horizons should be collected for chemical analysis.
- Preferably, the uppermost layer (0-10cm) will be divided into two sub-layers (0-5cm and 5-10cm) in order to detect small change of chemical properties of the uppermost layer.

2.3.4. Pretreatment of soil samples

The soil samples collected are air dried and passed through a 2 mm sieve. Stony materials, macroscopic roots and plant residues larger than 2 mm must be manually removed. They are ground immediately before the analysis when ground material is necessary for analyses.

2.3.5. Chemical properties of soil

For each method of chemical and physical analysis, serial number in the manual is given. The number starts from SA-001 for chemical analysis and from SA-101 for physical analysis.

a) Moisture Content (Mandatory)

Method SA-001
Reference Methods: ISRIC 1993
Method suitable for Mineral Horizon

I. Principle

Calculation of the soil analysis is done on basis of oven-dry soil. The moisture content of the sample should be determined shortly before soil analysis.

II. Apparatus

1. Moisture tins or flasks with fitting lid
2. Drying oven

III. Procedure

1. Transfer approx. 5 g of air-dried fine earth to a tared moisture tin and weigh with 0.001 g accuracy (A gram).
2. Dry overnight at 105ºC (lid removed).
3. Remove tin from oven, close with lid, cool in desiccator and weigh (B gram).

IV. Calculation

The moisture content in wt% (w / w) is obtained by:

$$\text{Moist(wt\%)} = [(A-B) / (B-\text{tare tin})] \times 100$$

The corresponding moisture correction factor (mcf) for analytical results or the multiplication factor for the amount of sample to be weighed in for analysis is:

$$\text{Moisture correction factor} = (100 + \%\text{moist}) / 100$$

V. Report

Report moisture content in % with 1 decimal place. All the analytical data have to be corrected with moisture correction factor, and expressed on basis of oven-dry soil.

b) pH (H$_2$O), pH (KCl) (Mandatory)

Method SA-002
Reference Methods: ISRIC 1993
Method suitable for Mineral Horizon

I. Principle

The pH of the soil is potentiometrically measured in the supernatant suspension of a 1:2.5 soil:liquid mixture . The liquid is either water (pH(H$_2$O)) or a 1 M KCl solution (pH(KCl)).

II. Apparatus

1. pH meter with glass-calomel combination electrode.
2. Reciprocating shaking machine.

III. Reagents

1. 1 M (mol/L) Potassium chloride (KCl) solution, 1M: Dissolve 74.5 g KCl in deionized water and make to 1 L.
2. Buffer solutions, pH 4.00, 7.00 and 9.00(or 10.00): Dilute standard analytical concentrate ampoules according to instruction.

IV. Procedure

1. Weigh 20 g fine earth into a 100 ml polyethylene wide-mouth type bottle.
2. Add 50 ml liquid (water or 1 M KCl solution) and cap the bottle.
3. Shake for 2 hours.
4. Before opening the bottle for measurement, shake by hand once or twice.
5. Immerse electrode in upper part of suspension.
6. Read pH when reading has stabilized (accuracy 0.1 unit).

Note: The reading is considered stable when it dose not change more than 0.1 unit per 30 seconds. (or 0.02 units per 5 secs.).In calcareous soils stabilization may be difficult to achieve because of non-equilibrium conditions.

V. Remarks

1. Prior to reading, calibrate the pH meter with buffer solutions for the range in which is measured. Because of differences in slope of the calibration line measurements outside a calibration range may be in error.
2. Buffer solutions should not be stores for too long. Especially the pH 9 and 10

solutions are sensitive to CO_2 and may soon become unreliable.

VI. Report

pH values are reported with accuracy of 0.1 unit.

c) Exchangeable Base Cations (Ex-Ca, Mg, K and Na) (Mandatory)

Method SA-003
Reference Methods: ISRIC 1993
Method suitable for Mineral Horizon

I. Principle

The sample is percolated with ammonium acetate and bases are measured in the percolate. Two techniques are described, they differ only in technique, not in principle:

> **1. Percolation tube procedure**
> **2. Automatic extractor procedure**

Alternative extraction procedure

1. **Buchner funnel procedure:** Filtration with light suction using a 55 mm Buchner funnel or Pyrex Buchner funnel (Corning size No. 40) is also applicable. This method is described on p. 160, Method of Soil Analysis, Part 2 (American Society of Agronomy 1982)
2. **Centrifuge procedure:** Also the centrifugation procedure described on p. 161, Method of Soil Analysis, Part 2 (American Society of Agronomy 1982) is also applicable.

II. Apparatus

1. Either: Percolation tubes, 2-2.5 cm diameter, approx. 30 cm length (or a 60 ml syringe), with adjustable outlet (rubber or plastic tube with screw-clamp or stopcock);

> Or.

Automatic extractor* (Holmgren et al, 1977).

2. pH-meter.
3. AAS or ICP.

III. Reagents

Either: For percolation tubes: ignited and washed sea sand; cotton wool.

> Or:

For automatic extractor: filter pulp. Standard grade.

1. Ammonium acetate solution, 1 M: Dissolve 385 g NH_4Ac in water in a 5 L beaker and make to 5 L. Adjust the pH to 7.0 with ammonia or acetic acid 1 M. For calcareous soils pH of the acetate solutions is adjusted to pH 8.2.

IV. Procedure

1. Percolation

Two alternative techniques are described successively.

1) The percolation tube procedure

2) The automatic extractor procedure

1) Percolation tube procedure

1)-1 Preparation

1. Install percolation tube in vertical position in a stand or rack.
2. Close the bottom of the tube with some cotton wool, compress with a plunger. Add two tea-spoons of sea-sand (approx. 10 g, giving a layer of about 1 cm thick).
3. Weigh 5 g of sample (accuracy 0.01 g) into a porcelain dish, add approx. 25 g sea-sand and mix well with a spatula.
4. Transfer quantitatively to the percolation tube and level the mixture with a long spatula or rod.
5. Add two tea-spoons of sea-sand to make an approx. 1 cm cover on the sample (to avoid splashing and compaction of the sample). Include two blanks (approx. 25 g sea-sand, no soil) and a reference sample.

1)-2 Exchangeable bases

The presence of soluble salts may give an unacceptable overestimate of the exchangeable bases. Therefore, in the procedure, a distinction can be made on basis of the presence or absence of soluble salts. Usually the conductivity of the pH-H_2O suspensions is used to test this based on the method mentioned below:

a. EC25 > 0.5 ms: soluble salts need to be washed out first
b. EC25 < 0.5 ms: soluble salts negligible, no pre-washing needed.

Warning: Washing out soluble salts will change the so-called Reduced Ratio (Sodium Adsorption Ratio, SAR). Consequently, the composition of the exchange complex will be changed (upon dilution the proportion of monovalent cations will be reduced) which leads to erroneous results. In cases where these errors are suspected to be unacceptably high, the determination of exchangeable bases should be regarded as impossible.

a. If EC25 > 0.5 mS (pre-washing)

1. Open outlet of percolation tube and place 150 mL beaker under it.
2. From a 100 mL volumetric flask, filled approx. to the mark with ethanol 80%, add about 25 mL to the tube.
3. When the first drops come from the outlet, close this. (The outlet was open to facilitate air expulsion from the sample.)
4. Check tube on entrapped air-bubbles and, if necessary, try to remove these by light tapping against the tube. Should this fail, then carefully disturb the soil / sand mixture with an extended mounting needle. Allow to stand for 20 minutes.
5. Place volumetric flask, with the remainder of the ethanol, upside down in the percolation tube.
   Note: When using a relatively short percolation tube such as a syringe, the flask has to be supported above this tube by a stand or rack. Adjust distance between sampleand flask-mouth to approx. 5 cm.
6. Open and adjust outlet to percolate the 100 mL in 2 hours (approx. 20 drops / min.).
7. Discard percolate and place a 100 mL volumetric flask under outlet.
8. From a 100 mL volumetric flask filled with NH_4Ac 1M almost to the mark add about 25 mL to the tube and then place the flask upside down in the tube.
9. Adjust outlet so that the 100 mL percolates in 4 hours (approx. 10 drops / min.).
10. Make collecting volumetric flasks to volume with NH_4Ac 1 M, homogenize.
11. Measure Ca, Mg in this extract by AAS or ICP, and K and Na by AAS, FES or ICP.

b. If EC25 < 0.5 mS (no pre-washing)

1. Open outlet of percolation tube and place 100 mL volumetric flask under it.
2. From a 100 volumetric flask filled almost to the mark with NH_4Ac 1 M, add about 25 mL to the tube.
3. When the first drops come from the outlet, close this. (The outlet was open to facilitate air expulsion from the sample.)
4. Check tube on entrapped air bubbles and, if necessary, try to remove these by light tapping against the tube. Should this fail, then carefully disturb the soil / sand mixture with an extended mounting needle. Allow to stand for 20 minutes.
5. Place volumetric flask with the remainder of the NH_4Ac solution upside down on the percolation tube and allow to stand for 20 minutes.
   Note: When using a relatively short percolation tube such as a syringe, the flask has to supported above this tube by a stand or rack. Adjust distance between sample and flask- mouth to approx. 5 cm.

6. Open and adjust outlet to percolate the 100 mL in 4 hours (approx.10 drops / min)

7. Make collecting volumetric flask to volume with NH_4Ac 1 M, homogenize.

8. Measure Ca, Mg in this extract by AAS or ICP, and K and Na by AAS, FES or ICP.

2) The automatic extractor procedure

2)-1 Preparation

1. Close the bottom of the sample tube with approx. 1 g of filter pulp. Compress with a plunger.

2. Add 2.5 g fine earth (accuracy 0.01 g) and place sample tube in upper disc of extractor. If necessary, level sample to even thickness with a spatula. Include a reference sample and two blanks.

3. Connect sample tube with collecting syringe the plunger of which is inserted in slot of stationary disc of extractor.

2)-2 Exchangeable bases

The presence of soluble salts may give an unacceptable overestimate of the exchangeable bases. Therefore, in the procedure, a distinction can be made on basis of the presence or absence of soluble salts. Usually the conductivity of the pH-H_2O suspensions (chapter 4) is used to test this:

a. EC25 > 0.5 ms: soluble salts need to be washed out first

b. EC25 < 0.5 ms: soluble salts negligible, no pre-washing needed.

Warning: Washing out soluble salts will change the so-called Reduced Ratio (Sodium Adsorption Ratio, SAR).Consequently, the composition of the exchange complex will be changed (upon dilution the proportion of monovalent cations will be reduced) which leads to erroneous results. In cases where these errors are suspected to be unacceptably high, the determination of exchangeable bases should be regarded as impossible.

a. If EC25 > 0.5 mS (pre-washing)

1. Rinse wall of sample tube with some ethanol 80% from wash bottle.

2. Carefully fill sample tube to the 25 ml mark with ethanol for 20 minutes.

3. If necessary, fill to 25 ml mark again and place reservoir tube on top of sample tube. Add about 40 ml ethanol 80% to reservoir tube, start extractor and complete percolation in 2 hours.

4. Remove both reservoir tube and collecting syringe. Discard percolate and replace collecting syringe by a clean one. Proceed with step b. 2 (next section).

b. If EC25 < 0.5 mS (no pre-washing)

Rinse wall of sample tube with some NH_4Ac 1 M from wash bottle.

1. Carefully fill sample tube to the 25 ml mark with NH_4Ac to stand for 20 minutes.
   Note: If pre-washed, omit standing for 20 minutes.
2. If necessary, fill to 25 mL mark again and place reservoir tube on top of sample tube. Add about 40 ml NH_4Ac 1 M to reservoir tube, start extractor and complete percolation in 8 hours.
3. Disconnect collecting syringe, transfer contents quantitatively to 100 ml volumetric flask and make to volume with NH_4Ac 1 M solution.
4. Measure Ca, Mg in this extract by AAS or ICP, and K and Na by AAS, FES or ICP.

V. Calculations

Exch. Ca (cmol (+) / kg soil) = [(a-b)*10*100*mcf]/[10*20.04*s]

Exch. Mg (cmol (+) / kg soil) = [(a-b)*10*100*mcf]/[10*12.15*s]

Exch. K (cmol (+) / kg soil) = [(a-b)*2*100*mcf]/[10*39.10*s]

Exch. Na (cmol (+) / kg soil) = [(a-b)*2*100*mcf]/[10*23.00*s]

Base saturation = [Exch.(Ca + Mg + K + Na)/ECEC]*100

where

a = mg/L Ca, Mg, K or Na in the diluted sample percolate A(dilution 10* and 2* respectively)

b = ditto in the diluted blank percolate A

s = air-dry sample weight in gram

mcf = moisture correction factor

VI. Report

Results in cmol (+) / kg soil for respective cations and in % for base saturation with 2 singnificant digits.

VII. Remark

Application of the described method to calcareous (and gypsiferous) soils leads to erroneous results (as dose application of many other method). Dissolution of carbonates

interferes particularly with the determination of exchangeable Ca (over-estimation). Results can be improved to some extent by raising the pH of acetate buffer solution to 8.2 where the solubility of Ca (and Mg) carbonate is reduced. This can also be achieved by using acetate buffer (pH 7)/ethanol mixture (e.g. 1:1), Since in neither case the solubility is reduced to zero the results remain unreliable.

A better alternative would seem to be silver thiourea method described in p. 10-1, Procedure for Soil Analysis, fourth edition (ISRIC 1993)

The result of Exchangeable base cations should be used for calculation of Effective Cation Exchangeable Capacity (ECEC).

Measurement of electrical conductivity (ISRIC 1993)

I. Apparatus

1. Conductivity meter with dip cell and pipette cell

II. Reagents

1. Standard potassium chloride solution, 0.01 M: Dilute standard analytical concentrate ampoule of 0.100 M KCl according to instruction. Pipette 10 mL of the standard 0.100 M KCl solution into a 100 mL volumetric flask and make to volume with water. Alternatively, dissolve 0.7456 g of oven-dried (105C) KCl in water in a 1L volumetric flask and make to volume with water.

III. Calibration of conductivity meter and measuring cell

1. Add about 30 mL standard 0.01 M KCl solution to a 50 mL beaker and measure the temperature.
2. Rinse and fill pipette cell with the standard KCl solution or insert dip cell in this solution.
3. Set temperature compensation dial at measured temperature and adjust reading of the meter to 1.412 mS/cm with cell-constant dial.

IV. Measurement

1. Measure the temperature of the extract and set temperature compensation dial at this temperature. (The reading is then automatically corrected to 25 $^{\circ}$C.)
2. Fill pipette cell with extract is available for rinsing the cell between measurements (usually the case with the saturation extract) then rinse the cell with water and acetone and dry with air-jet.

d) Exchangeable Acidity (Mandatory)

Method SA-004
Reference Methods: ISRIC 1993
Method suitable for Mineral Horizon

I. Principle

The sample is percolated with a 1 M KCl solution. The acidity brought into solution from various sources in the soils is measured by titration.

II. Apparatus

1. Burette

III. Reagents

1. Potassium chloride (KCl) solution, 1 M: Dissolve 373 g KCl in deionized water and make to 5 L.

2. Hydrochloric acid (HCl), 0.02 M (standard solution): Dilute standard analytical concentrate ampoule (1 g / 1 L) according to instruction.

3. Sodium hydroxide (NaOH) solution, approx. 0.025 M (standardized): Dissolve 1 g of NaOH in deionized water in a 1 L volumetric flask. Cool and make to volume. Standardize this solution by titration against the 0.02 M standard HCl solution.
   Note: This is an alternative for making the solution with a standard solution concentrate ampoule (which may be used also). Sodium hydroxide standard solution has a limited life and need to be re-standardized after storage: the effect of a CO_2 tap is limited by (frequent) open of the bottle.

4. Phenolphthalein indicator solution, 0.1 %: Dissolve 100 mg phenolphthalein in 100 mL ethanol 96 %.

IV. Procedure

1. Percolation

A. Transfer 10 g of fine earth (accuracy 0.05 g) to a dry filter paper in a funnel placed in a 100 mL volumetric flask. Include two blanks.

B. Add ten portion of 10 mL 1 M KCl solution with 15-minute intervals so that percolation takes about 2 and 1/2 hours.

C. After the last portion has percolated, remove the funnel and fill the volumetric flask to the mark with 1M KCL solution and homogenize.

2. Determination of exchangeable acidity

A. Pipette a 25 mL aliquot of percolate into a 250 mL erlenmeyer flask and add 3-5 drops of phenolphthalein solution.

B. Titrate with 0.025 M NaOH until the color turns just permanently pink (in practice: wait for 1 min).

Note 1: Weakening of the pink color can be caused by hydroxy-Al precipitate. This can be remedied by adding another drop of phenolphthalein.

Note 2: When using automatic titrator, set end-point pH at 7.60.

V. Calculation

$$\text{Exchangeable acidity (cmol (+) / kg soil)} = [(a-b) * M * 4 * 100 * mcf] / s$$

where

a = mL NaOH neede for percolate

b = mL NaOH needed for blank

M = molarity of NaOH solution

s = air-dry sample weight in gram

4 = aliquot factor

mcf = moisture correction factor

VI. Report

Results in cmol (+)/ kg soil with 2 significant digit.

e) Exchangeable Al and H (sum is equal to exchangeable acidity) (Optional)

Method SA-005
Reference Methods: Method of Soil Analysis (1965), p. 994.
Method suitable for Mineral Horizon

I. Principle

Unbuffered 1 M KCl solution replaces exchangeable H and Al from soil. Exchangeable Al and H in KCl extract are measured separately by titration.

II. Apparatus

1. Burette

III. Reagents

1. Hydrochloric acid (HCl), 0.1 M (standard solution): Dilute standard analytical concentrate ampoule (1 g / 1 L) according to instruction.
2. Sodium hydroxide (NaOH) solution, approx. 0.1 M (standardized): Dissolve 4 g of NaOH in deionized water in a 1 L volumetric flask. Cool and make to volume. Standardize this solution by titration against the 0.1 M standard HCl solution.
3. Sodium fluoride (NaF) solution: Dissolve 40 g of NaF in 1 litter of water.
4. Phenolphthalein indicator solution, 0.1 %: Dissolve 100 mg phenolphthalein in 100 mL ethanol 96 %.

IV. Procedure

1. Pipette into a 250-mL beaker a known volume (the major portion) of the KCl percolate from section d) containing the exchangeable Al (and H), and add 5 drops of phenolphthalein indicator.
2. Titrate the solution with 0.1 N NaOH to a permanent pink end point with alternate stirring and standing. If needed, add a few more drops of indicator to replace that adsorbed by the precipitate of $Al(OH)_3$. The amount of base used is equivalent to the total amount of acidity in the aliquot taken.
3. Add 1 drop of 0.1 N HCl to bring the solution back to the colorless condition, and add 10 ml of NaF solution.
4. Titrate the solution with 0.1 N HCl until the color just disappears while stirring the solution constantly. Add 1 or 2 drops of indicator. If the color appears, continue addition of acid until the color just disappears and dose not return within 2 minutes.

V. Calculation

Exchangeable Al (cmol (+) / kg soil) = [b * M_{HCl} * c * 100 * mcf] / s

     and

Exchangeable H (cmol (+) / kg soil) = [(a * M_{NaOH} - b * M_{HCl}) * c * 100 * mcf] / s

where
a = mL NaOH needed for percolate
b = mL HCl needed for percolate
M_{HCl} = molarity of HCl solution
M_{NaOH} = molarity of NaOH solution
s = air-dry sample weight in gram
c = aliquot factor (factor = 2, in case of 50 mL percolate is used)
mcf = moisture correction factor

VI. Remark: If colorimetric determination of Al or by AAS or ICP these can be done according to ones of the procedures described by Bertsch and Bloom in Method of Soil Analysis; Part 3-Chemical Methods (1996) p. 538, Soil Science Society of America, Inc., Madison, USA.

VII. Report
Results in cmol (+) / kg soil with 2 significant digit.

f) Effective Cation Exchangeable Capacity (ECEC) (Mandatory)

Method SA-006
Reference Methods: ISRIC 1993
Method suitable for Mineral Horizon

I. Principle

The concept of effective cation exchange capacity (ECEC) was first formalized be Coleman et al. (1959) as the sum of the exchangeable Ca, Mg and Al displaced from the soil using 1 M KCl, but has evolved to include Na and K. There is considerable evidence to show that the quantities of exchangeable cations extracted from nonsaline noncalcareous soils by any of the common extracting solutions are very similar (Grove et al., 1982). Thus it is possible to use this technique to estimate cation exchange capacity in soils which do not contain salts and carbonates.

II. Apparatus

As listed under Preparations for Exchangeable base cations, and Apparatus for Exchangeable acidity or "Exchangeable Al and H"

III. Reagents

As listed under Preparations for Exchangeable base cations, and Apparatus for Exchangeable acidity or "Exchangeable Al and H"

IV. Procedure

Determine Ca, Mg, K, Na and Al by ABS, FES, ICP or titration.

V. Calculations

ECEC = Ca + Mg + K + Na + Exchangeable acidity

     Or.

ECEC = Ca + Mg + K + Na + Al + H

where

Ca = exchangeable Ca in centimoles of cation charge per kilogram

Mg = exchangeable Mg in centimoles of cation charge per kilogram

K = exchangeable K in centimoles of cation charge per kilogram

Na = exchangeable Na in centimoles of cation charge per kilogram

Al = exchangeable Al in centimoles of cation charge per kilogram

H = exchangeable H in centimoles of cation charge per kilogram

For the calculation, the results of Exchangeable base cations described in pp.14-19 and Exchangeable acidity described in pp.20-21 (or Exchangeable Al and H described in pp.22-23) should be used.

VI. Report

Results in cmol (+) / kg soil with 2 significant digit.

VII. Remark

Over a wide variety of soils, values for the sum of basic cations extracted by a variety of extractants such as 1 M NH_4OAc, 0.2 M NH_4Cl, 0.2 M $BaCl_2$, 0.2 M $CaCl_2$, 1 M $BaCl_2$-TEA and 0.01 M $SrCl_2$ plus Al extracted with 1 M KCl or 0.2 M NH_4Cl were essentially the same, indicating that almost any extractant is suitable for estimation of ECEC.

g) Carbonate Content (Mandatory item if pH(H_2O)>7)

Method SA-007
Reference Methods: SSSA 1996
Method suitable for Mineral Horizon

I. Principle

The soil sample is treated with a strong acid. The loss of CO_2 gas is measured volumetrically.

II. Apparatus

1. Volumetric calcimeter (Dreimanis 1962). The volumetric calcimeter is shown Fig. 2.5. The principle components are (A) 250-mL Florence flask with two-hole rubber stopper, (B) 25-mL addition tube with stopcock, (C) three-way stopcock, (D) manometer, and (E) leveling bulb. Tubing connections can be made by Tygon tubing.

2. Magnetic stirrer.

III. Reagents

1. Hydrochloric acid (HCl), 6M, with 5 % (by weight) ferrous chloride ($FeCl_2 \cdot 4H_2O$): Add 500 mL of concentrated HCl to 400 mL of deionized water, then add 50 g of $FeCl_2 \cdot 4H_2O$, and dilute to a total volume of 1 L.

2. n-amyl alcohol.

3. Reagent-grade calcium carbonate ($CaCO_3$).

IV. Procedure

1. Calibration of calcimeter Sample preparation

A. Weight dry fine-grind (small enough particle size to pass through a 100 mesh in.-1 sieve; 150–μm nominal pore size) reagent-grade $CaCO_3$ to the nearest 0.1 mg into separate decomposition flasks. Samples of approximately 10, 20, 30, 50, 75, 150, 200, 300, 400, 600 and 800 mg are recommended.

B. Place a stirring bar in the flask, and add two drops of amyl alcohol.

C. Install the sample flask in the system, and fill the graduated funnel (B) to the 25-mL mark with HCL-$FeCl_2$ solution.

D. Open the three-way stopcock (C) to the atmosphere, and adjust the liquid level of the measuring burette (D) to exactly 0 mL by adjusting the height of the leveling bulb (E).

E. Close the system to the atmosphere with tree-way stopcock (C) (180 degree rotation), and lower the leveling bulb about 2 cm.

F. Simultaneously begin to add HCl-FeCl$_2$ solution from the graduated funnel (B) to the sample and begin lowering the level bulb. The leveling bulb liquid level should be kept 1 to 2 cm below the liquid level in the measuring burette (D).

G. After the sample is moistened, turn the magnetic stirrer (slow stirring rate).

H. Close the stopcock of the graduated funnel (B) after 20 mL of acid has been dispensed.

When the level in the gas burette ceased to drop (usually less than 3 min), equalize liquid levels in the leveling bulb (E) and the measuring burette (D), and read and record the volume of CO$_2$ that has been evolved. Also record the temperature (T) and barometric pressure (P).

2. Determination of total carbonate in soils

A. Add 0.5 to 5.0 g plus-minus 1 mg of soil which has been ground to pass a 100 mesh in sieve (150-μm minimal pore size) to the decomposition flask (A). The sample should contain no more than 600 mg CaCO$_3$ equivalent.

B. Perform steps (b) through (i) as described above. Longer reaction times will be required if the soil contains dolomite.

3. Calculation

1) Calibration of calcimeter:

A. Correct the CO$_2$ volume for the standards by subtracting the average CO$_2$ volumes for the reagent blanks as follows:

$$V_{CO_2(corr)} = V_{CO_2(std)} - V_{CO_2(blank)}$$

B. Reduce all corrected CO$_2$ volumes to those at standard temperature and pressure (STP) using the following equation:

$$V_{CO_2(STP)} = V_{CO_2(corr)} (273 \text{ K} / T \text{ K}) (P \text{ mmHg} / 760 \text{ mmHg})$$

C. Determine the calculated CaCO$_3$, W_{CaCO_3}(cal), from the $V_{CO_2(STP)}$ values using the following equation:

$$W_{CaCO_3}(cal) = [V_{CO_2(corr)}] (100 \text{ g CaCO}_3 \text{ mol}^{-1} / 22.414 \text{ L mol}^{-1}) (1 \text{ L} / 1000 \text{ mL})$$

D. Plot actual $CaCO_3$, Wc_aco_3, on the y-axis vs. $Wc_aco_3(cal)$ on the x-axis. The plot should be close to a straight line. The slope C is the correction factor between the actual and calculated $CaCO_3$. The value of C should be less than 1 and is related to the actual quantity of CO_2 remaining dissolved in the HCl digestion agent under the conditions of analysis.

$$Wc_aco_3 = (C) [Wc_aco_3(cal)]$$

2) Determination of carbonate in soils

A. The $V_{CO_2(STP)}$ is calculated as in (a) and (b) above.

B. The weight of $CaCO_3$ is calculated as follows

$$Wc_aco_3 = (c) [V_{CO_2(corr)}](100 \text{ g } CaCO_3 \text{ mol}^{-1} / 22.414 \text{ L mol}^{-1}) (1 \text{ L} / 1000 \text{ mL})$$

C. Calcium carbonate equivalent is calculated as follows

$$CaCO_3 \text{ equivalent, \%} = (Wc_aco_3 \text{ g} / W_{soil} \text{ g})(100)$$

V. Report

Report % $CaCO_3$ with 1 decimal place.

h) Total Carbon Content (Organic Carbon Content) (Optional)

Method SA-008
Reference Methods: ISRIC 1993
Method suitable for Mineral Horizon

The method for the determination of soil organic carbon operate on principles of wet oxidation and dry oxidation. Dry oxidation method is performed semi-automatically by commercially available C and N analyser.

Out of wet oxidation methods, Walkley-Black method is appropriate for routine analysis because of its simplicity.

I. Principle

The walkley-Black procedure is followed. This involves a wet combustion of the organic matter with a mixture of potassium dichromate and sulfuric acid at about 125ºC. The residual dichromate is titrated against ferrous sulfate.

II. Apparatus

1. Burette.

2. Safety pipette 10 ml.

3. Illuminated magnetic stirrer.

4. Measuring cylinder 25 ml.

III. Reagents

1. Potassium dichromate standard solution, 0.1667 M. Dissolve 49.04 g $K_2Cr_2O_7$ A.R.(dried at 105ºC) in deionized water in a 1 L volumetric flask and make to volume with water.

2. Concentrated sulfuric acid (96%).

3. Concentrated phosphoric acid (85%).

4. Barium diphenylamine sulfonate, 0.16%(indicator): Dissolve 1.6 g barium diphenylamine sulfonate in 1 L of deionized water.

5. Ferrous sulfate solution, 1 M (approx.): Dissolve 278 g $FeSO_4.7H_2O$ in ca. 750 ml water and add 15 ml conc.H_2SO_4. Transfer to a 1 L volumetric flask and make to volume with water.

IV. Procedure

1. Grind approx. 5 g fine earth to pass a 0.5 mm sieve.

2. Weigh 1 g of this material (accuracy 0.01 g) into a 500 ml wide-mouth erlenmeyer flask. Include a reference sample.

Note: In case of soils containing more than 2.5% C proportionally less sample should be weighed in.

3. Add 10.00 ml dichromate solution. Include two blanks (erlenmeyer flasks without soil) to determine the molarity of the ferrous sulfate solution.

4. Carefully add 20 ml sulfuric acid with a measuring cylinder, swirl the flask and allow to stand on a pad for 30 minutes (in fume cupboard!).

5. Add about 250 ml water and 10 ml phosphoric acid with a measuring cylinder and allow to cool.

6. Add 1 ml indicator solution and titrate with ferrous sulfate solution while the mixture is being stirred. Near the end-point the brown color becomes purple or violet-blue and the titration must be slowed down. At the end-point the color changes sharply to green. If more than 8 of the 10 ml dichromate added has been reduced then repeat the determination with less soil (see also step 2).

Note: The end-point is easily overshot, in that case add 0.50 ml of the dichromate solution and titrate again dropwise (change calculation accordingly).

V. Calculation

The carbon content of the soil is obtained by

$$\% \ C = M * [(V1 - V2) / S] * 0.39 * mcf$$

where
M = molarity of ferrous sulfate solution (from blank titration)
V1 = ml ferrous sulfate solution reqired for blank
V2 = ml ferrous sulfate solution reqired for sample
S = weight of air-dry sample in gram
$0.39 = 3 * 10^{-3} * 100\% * 1.3$ 　　　　　　　(3 = equivalent weight of carbon)
mcf = moisture correction factor

Note: The factor 1.3 is a compensation factor for the incomplete combustion of organic matter in this procedure. This ineffectiveness varies with the type of organic matter and the factor 1.3 is a compromise.

VI. Report

Report organic carbon content (%) with 1 decimal place.

i) Total Nitrogen Content (Optional)

Method SA-009
Reference Methods
Method suitable for Mineral Horizon

The method for the determination of soil total nitrogen operate on principles of dry combustion and distillation. Dry oxidation (Dumas) method is performed semi-automatically by a commercially available C and N analyser.

Kjeldahl distillation method is most commonly used.

I. Principle

The Kjeldahl procedure is followed. The sample is digested in sulfuric acid and hydrogen peroxide with selenium as catalyst and whereby organic nitrogen is converted to ammonium sulfate. The solution is then made alkaline and ammonia is distilled. The evolved ammonia is trapped in boric acid and titrated with standard acid. The procedure determines all soil nitrogen (including adsorbed NH_4^+) except that in nitrates.

II. Apparatus

1. Digestor (Kjeldahl digestion tubes in heating block).
2. Steam-distillation unit (fitted to accept digestion tubes).
3. Burette 25 ml.

III. Reagents

1. Sulfuric acid selenium digestion mixture: Dissolve 3.5 g of selenium powder in 1 L of concentrated (96%) sulfuric acid by mixing and heating at approx. 350ºC. The dark color of the suspension turns into clear light-yellow. When this is reached, continue heating for 2 hours.
2. Hydrogen peroxide 30%.
3. Sodium Hydroxide solution, 38%: Dissolve 1.90 kg NaOH pellets in 2L water in a heavy-walled 5 L flask. Cool the solution with the flask stopperd to prevent absorption of atmospheric CO_2. Make up the volume to 5L with freshly boiled and cooled deionized water. Mix well.
4. Mixed indicator solution: Dissolve 0.13 g methyl red and 0.2 g bromocresol green in 200 mL ethanol.
5. Boric acid-indicator solution, 1%: Dissolve 10 g of H_3BO_3 in 900 ml hot water, cool and add 20 ml mixed indicator solution. Make to 1 L with deionized water and mix thoroughly.
6. Hydrochloric acid, 0.010 M standard. Dilute standard analytical concentrate ampoule

according to instruction.

IV. Procedure

1. Digestion

A. Grind approx. 5 g fine earth to pass a 0.5 mm sieve.

B. Weigh 1 g of this material (accuracy 0.01 g) into a digestion tube. Of soils, rich in organic matter (>10%), 0.5 g is weighed in (see Remark 1). In each batch, include two blanks and a reference sample.

C. Add 2.5 ml digestion mixture.

D. Add successively 3 aliquots of 1 ml hydrogen peroxide. The next aliquot can be added when frothing has subsided. If frothing is excessive, cool the tube in water.
   Note: In step 3 and 4 use a measuring pipette with balloon or a dispensing pipette.

E. Place the tubes on the heater and heat for about 1 hour at moderate temperature (200 ^{o}C).

F. Turn up the temperature to approx. 330 ^{o}C (just below boiling temp.) and continue heating until mixture is transparent (this should take about two hours).

G. Remove tubes from heater, allow to cool and add approx. 10 ml water with a wash bottle while swirling.

2. Distillation

A. Add 20 ml boric acid-indicator to a 250 ml beaker and place beaker on stand beneath the condenser tip.

B. Add 20 ml NaOH 38% to digestion tube and distil for about 7 minutes during which approx. 75 ml distillate is produced.
   Note: the distillation time and amount of distillate may need to be increased for complete distillation (see Remark 2).

C. Remove beaker from distiller, rinse condenser tip, and titrate distillate with 0.01 M HCl unit color changes from green to pink.
   Note: When using automatic titrator: set end-point pH at 4.60.

V. Remarks

1. The described procedure is suitable for soil samples with a nitrogen content of up to 10 mg N. This corresponds with a carbon content of roughly 10 % C. Of soils with higher contents, less sample material is weighed in. Sample size of less than 250 mg should not be used because of sample bias.

2. The capacity of the procedure with respect to the amount of N that can be determined

depends to a large extent on the efficiency of the distillation.

VI. Calculation

$$\% \, N = [(a-b) / s] * M * 1.4 * mcf$$

where

a = mL HCl required for titration sample

b = mL HCl require for titration blank

s = air-dry sample weight in gram

M = molarity HCl

$1.4 = 14 * 10^{-3} * 100 \%$

mcf = moisture correction factor

VII. Report

Report total nitrogen content (%) with 1 decimal place.

j) Available Phosphate (Voluntary)

Method SA-010
Reference Methods: SSSA (Bray-2 test)
Method suitable for Mineral Horizon

I. Principle

For acid soil, F- promotes P desorption by decreasing Al activity through the formation of Al and F complexes. Fluoride also is effective in suppressing the readsorption of sulbilized P by soil colloids. However, the Bray-1 test (Bray & Kurtz, 1945) performs unsatisfactorily in highly calcareous soils due to the neutralization of the acid by calcium carbonate ($CaCO_3$) and formation of CaF, that react with dissolved P to secondary precipitates.

II. Reagents

1. Ammonium fluoride, 1 M: Dissolve 37 g of NH_4F in deionized water and dilute to 1 L. Store the solution in a polypropylene bottle.
2. Hydrochloric acid, 0.5 M: Dilute 20.8 mL of concentrated HCl (12 M) to 500 mL with deionized water.
3. Extracting solution for acid soluble and absorbable phosphate: Add 15 mL of 1.0 M NH_4F and 100 mL of 0.5 M HCl to 385 mL of deionized water to obtain a solution containing 0.03 M NH_4F and 0.1 M HCl. It will keep in glass more than 1 year.

III. Procedure

1. Weigh 1.0 g of air-dried soil (<2 mm) into a flat-bottomed glass vial bottle, and add 7 mL of the extracting solution.
2. Shake the suspension vigorously for 40 sec and filter through a membrane filter (0.45 µm) or Whatman NO. 42 filter paper (if Whatman NO. 42 filter paper is used and the filtrate is not clear, pour the filtrate back though the same filter).
3. Transfer an aliquot containing 1 to 20 µg of P to a 25-mL volumetric flask and determine the P concentration by the Ascorbic Acid Method or by the Modified Ascorbic Acid Method mentioned below.

IV. Calculation

P_{ex} (mg kg^{-1}) = [P concentration (µg/mL)]* [25/v1] * [v2/g soil used]

where

v1 = volume of extract used for P determination,

v2 = volume of extract

V. Report

Report phosphorus content in mg/kg with 2 significant digit.

Phosphorus determination

Apparatus

1. Spectrophotometer.

Ascorbic Acid Method

Reagents

1. Sulfuric acid, 2.5 M: Dilute 70mL of concentrated H_2SO_4 (18M) to 500mL with deionized water.

2. Ammonium molybdate: Dissolve 20 g of $[(NH_4)6Mo_7O_{24} \cdot 4H_2O]$ in 500mL of deionaized water. Store the solution in a glass-stoppered bottle.

3. Antimony potassium tartrate $[K(SbO) \cdot C_4H_4O_6 \cdot 1/2H_2O](1mg\ Sb\ mL^{-1})$: Dissolve 0.2728 g of $K(SbO) \cdot C_4H_4O_6 \cdot 1/2H_2O$ in 100 mL of deionized water.

4. Ascorbic acid, 0.1 M: Dissolve 1.76 g of $C_6H_8O_6$ in 100 mL of deionaized water. Prepare the solution on the day it is required.

5. Mixed reagent: Mix throughly 50 mL of 2.5 M H_2SO_4, 15 mL of ammonium molybdate solution, 30 mL of ascorbic acid solution and 5 mL of antimony potassium tartarate solution. Prepare a fresh mixed reagent daily.

6. Phosphate stock solution, 50 mg P L^{-1}: Dissolve 0.2197 g of oven-dried (40 ^{o}C) KH_2PO_4 in deionized water/ Add 25 mL of 3.5 M H_2SO_4, and dilute to 1 L with deionized water.

7. Working phosphate standard solution: 5 mg P L^{-1}: Dilute 10 mL of the 50 mg P L^{-1} stock solution to 100 mL with deionized water.

Procedure

Transfer a aliquot of sample or P standard solution that contain 2 to 40 μg P to a 50-mL

volumetric flask. Dilute with deionized water to about 25 mL, and add 8 mL of mixed reagent. Dilute the solution to volume and mix well. Measure the absorbance at 880 nm after 10 min. Prepare a blank that contain all reagents except the P solution.

Modified Ascorbic Acid Method

Reagents

1. Reagent A-ascorbic acid (0.1 M)-trichloroacetic acid ($C_2HCl_3O_2$) (0.5 M): Dissolve 8.8 g of ascorbic acid and 40.9 g of $C_2HCl_3O_2$ in about 400 mL of deionized water and dilute to 500 mL with deionized water.

2. Reagent B-ammonium molybdate (0.01 M): Dissolve 6.2 g of ammonium molybdate in about 400 mL of deionized water and dilute to 500 mL with deionized water.

3. Reagent C-sodium citrate ($Na_3C_6H_5O_7$) (0.1 M)-sodium arsenite ($NaAsO_2$) (0.2 M)-acetic acid (5 %): dissolve 29.4 g of $Na_3C_6H_5O_7 \cdot 2H_2O$ and 26.0 g of $NaAsO_2$ in about 800 mL of deionized water, add 50 mL of glacial acetic acid (99.9 %) and dilute to 1 L with deionized water.

4. Phosphate stock solution, 50 mg P L^{-1}: Dissolve 0.2197 g of oven-dried (40 ^{o}C) KH_2PO_4 in deionized water/ Add 25 mL of 3.5 M H_2SO_4, and dilute to 1 L with deionized water.

5. Working phosphate standard solution: 5 mg P L^{-1}: Dilute 10 mL fo the 50 mg P L^{-1} stock solution to 100 mL with deionized water.

Procedure

Add 10 mL of reagent A to a 25-mL volumetric flask and transfer an aliquot of sample or P standard solution containing 2 to 25 μg of P to the flask. Add 2 mL of Reagent B and 5 mL of Reagent C to the flask immediately and mix the content well. Dilute to volume with deionized water and mix well. After 10 min, measure the absorbance at 700 nm. Prepare a blank containing all reagents but P solution.

k) Sulfate (Voluntary)

Method SA-011
Reference Methods: SSSA
Method suitable for Mineral Horizon

I. Principle

The alkaline extraction of soils $NaHCO_3$ is effective in solubilizing and replacing anions as well as some organic fractions. Values obtained with this method generally account for more S than is present as acetate-soluble sulfate in most soils and probably include both organic and inorganic S to some extent.

II. Apparatus

1. Reciprocating shaking machine.

III. Reagents

1. Sodium bicarbonate solution, 0.5M, pH 8.5: Dissolve 42 g of $NaHCO_3$ in deionized water and make to 1 L. Adjust the pH to 8.5 by adding NaOH 1M (4 g /100 mL). In case of overshooting pH 8.5 add some $NaHCO_3$ 0.5 M.
2. Sulfate stock solution, 1 g S L^{-1}: Dissolve 5.434 g of potassium sulfate (K_2SO_4) in about 800 mL of deionized water, and dilute to 1 L with deionized water.

IV. Procedure

1. Weigh 10 g of soil (<2 mm) in a 125 mL Erlenmeyer flask or other appropriate vessels, and add 40 ml. of 0.5 M $NaHCO_3$ (adjusted to pH 8.5 with NaOH).
2. Shake the suspension for 60 minutes.
3. Filter the suspension through dry Whatman No. 42 filter paper or equivalent.
4. Determine the S concentration by turbidimetry , IC or ICP.

Note: An alternative acid extraction, i.e. CH_3COONa-CH_3COOH buffer solution (pH 4.5, containing 100g of CH_3COONa and 30mL of CH_3COOHin 1 L of deionized water), is also available if organic matter extracted in the alkaline $NaHCO_3$ solution interferes with the determination of sulfur. Sulfur concentration determined by acid extraction is sometimes lower than that determined by alkaline extraction.

V. Report

Report sulfur content in mg/kg with 2 significant digit.

2.3.6. Physical properties of soil

a) Fine Earth Bulk Density (Optional)

Method SA-101
Reference Methods: Method of Soil Analysis, Part 1, p.364 (1986)
Method suitable for Mineral Horizon

I. Principle

Soil sample taken from the soil profile by metal sampling cylinder of a known volume is sieved, oven-dried and weighed.

II. Apparatus

1. Metal sampling cylinder; 100 ml ($20cm^2$ * 5cm) or equivalents.
2. Drying oven.

III. Procedure

1. After smoothing soil profile surface, drive or press sampling cylinders into either vertical or horizontal soil surface far enough to fill the sampler, but not so far as to compress the soil in the confined space to the sampler. More than three samples have to be taken from each soil horizon.
2. Carefully remove the sampling cylinder and its contents so as to preserve the natural structure and packing of the soils as nearly as possible. A shovel, alongside and under the cylinder may be needed in some soils to remove the sample without disturbance.
3. Trim the soils extending beyond each end of the cylinder, and flush with each end with straight-edged knife or sharp spatula. The soil sample is thus established to the same as the volume of the sampling cylinder.
4. Pass the soil sample through a 2mm sieve. Transfer the sample to a container, place it in as oven at 105 ^{o}C until constant weight is reached, and weigh it.

IV. Caluculation

Fine Earth Bulk density (Mg m^{-3}) = g / V

Where   g = oven-dry mass of the sieved soil (g), V = sample volume (mL)

V. Report

The bulk density is recorded in Mg m^3 for respective measurements, and their average value is reported with two significant digit.

b) Penetration resistance (in the fieldwork) (Optional)

Method SA-102
Reference Methods
Method suitable for Mineral Horizon

I. Principle

When conducting the soil profile description, the penetration resistance of the uppermost and the underlying soil horizons is measured in situ by a direst-reading pocket penetrometer.

II. Apparatus

1. Pocket penetrometer (Yamanaka's penetrometer)

III. Procedure

1. After smoothen the soil profile surface by shaving off in the soil pit, the penetrometer is pushed slowly into the desired soil horizon until penetration reaches the designated depth.
2. The measurement must be repeated more than 5 times at different points of each soil horizon. In case the cone of a penetrometer hits stony materials or plant roots, the measurement has to be done once again.

IV. Report

The compressive strength indicated on the penetrometer scale is recorded in kg cm^{-2} for respective measurements, and their average value is reported. If the direct reading is given in mm, it should be converted into kg cm^{-2}.

2.4. Monitoring methods for forest monitoring

For the initial objectives of forest monitoring, the following two items have been proposed: General description of forest, and Survey of tree decline. However, some of these methodologies are not suitable for tropical region. In 2.4.3., methodologies for tropical region are also proposed.

2.4.1. General description of forest

Flora may change due to various environmental factors. For detection of the possible impact of acid deposition, these changes should be described in detail. For the description of the floral change in the forest area, Description of trees and Understory vegetation survey should be carried out regularly.

Note:

- Site for forest monitoring should be increased accordingly, and data of wide area should be accumulated. Cooperation with other forest research may also be useful.

2.4.1.1. Selection of plots for general description of the forest

When monitoring sites are selected according to the criteria which is described in 2.2.2.1., two forest areas of more than 0.2 hectare are selected. In each forest area, for the general description of the forest, a measuring plot should be subdivided to three coaxial circles of 1000, 400 and 200 square meter for the detailed survey (Table 2.3. Fig.2.6.).

Table 2.3. Measuring plot for the detailed survey of forest

Area	Trees to be measured
1 ($200m^2$: radius 7.98m)	Tree height above 1.3m
2 ($400m^2$: radius 11.28m)	DBH more than 4 cm
3 ($1000m^2$: radius 17.85m)	DBH more than 18 cm

The center and borders should be marked by the stakes and color painting of the bark. It is suggested to mark the serial number and the point of DBH measurements on tree stems for the succeeding investigations. For marking the serial number, painting or nailing small aluminum plates on the bark may be useful.

Note:

- It is preferable to select plots as many as possible in the monitoring site.

2.4.1.2. Monitoring items and frequency of monitoring for general description of the forest

The following items should be described.

Table 2.4. Monitoring items for general description of the forest

Items	Classification	Frequency of monitoring
Description of trees		Every 3-5 years
Name of species	M	
Diameter at Breast Height	M	
Height of tree	M	
Understory vegetation survey	M	

M: Mandatory items

a) Description of trees (Mandatory)

For each monitoring tree in the plot, Name of species, Diameter at breast height (DBH) and Height of tree should be described. The results should be reported in Form (Soil and Vegetation F).

- Name of species: Latin name should be described. At least, genus name should be clarified.

- DBH: The diameter at 1.3m high in the upper side of the trunk should be measured with the diameter tape in 0.1cm unit. In the case of less than 4cm, the caliper may be used.

- Height of tree: Height of tree should be measured with a suitable instrument as the height meter, or measuring pole, describe in 0.1m unit. Selected trees, more than twenty stems including the highest and the smallest ones, may be measured, if it is difficult to measure all trees. The heights of the rest are estimated according to the diameter-height curve.

b) Understory vegetation survey (<u>Mandatory</u>)

Understory vegetation may be more sensitive to environmental changes than trees. Floral changes in understory vegetation should be recorded preferably in every 3-5 years. The species names of all trees smaller than 1.3m height, herbs and ferns found in the small circle ($200m^2$), should be described with their dominance scale. The results should be reported in Form (Soil and Vegetation G).

Table 2.5. Expression of dominance

Dominance scale	Percent coverage of the species
5	over 75 %
4	51 - 75 %
3	26 - 50 %
2	6 - 25 %
1	1 - 5 %, or large number with low cover
+	less than 1 %

<u>Note:</u>

- In a forest, which consists of numerous tree species, such as tropical rainforest, the methodologies above may not be suitable. For these regions, the methodologies for forest monitoring should be elaborated. Some candidate methodologies are proposed in 2.4.3. for tropical region.

The results of Description of trees and Understory vegetation survey should be summarized in Form (Soil & Vegetation H).

2.4.2. Survey of tree decline

The tree decline can be caused by factors of air pollution and acid deposition as well as by external growth factors such as climatological (strong wind, cold wind, thunder, heavy rain, drought), biotic (animal, insect, disease, wood rot,) and human impacts (silvicultural practice, harvesting). For estimation of these causes, long term monitoring may be necessary. In the survey of tree decline, characteristics of tree decline should be observed and recorded regularly, then the cause of decline should be estimated considering environmental factors.

2.4.2.1.   Selection of monitoring trees

The sample trees are selected systematically. Mark four points of north, south, east and west, 12m apart from the center of permanent site. Five dominant trees should be selected randomly around the each point, then total of twenty trees is selected as the monitoring trees. The serial number of each monitoring tree should be noted as numbering had been done at the description of trees.

Note:
- Even if it is difficult to select five trees at each point, at least two trees should be selected, and total of 10 sample trees should be selected.

2.4.2.2. Monitoring items and frequency of monitoring for survey of tree decline

Table 2.6. Monitoring items for survey of tree decline.

Items	Classification	Frequency of Monitoring
Observation of tree decline	M	Every 3 – 5 years
Photographic Record of tree decline	O	
Estimation of decline causes	O	

M: Mandatory; O: Optional

These monitoring items should be surveyed in the season when density of foliage reaches peak.

a) Observation of tree decline

Points of the observation are necrosis, chlorosis, discoloration or abnormal defoliation of the leaves, die back at shoot. When any abnormal symptoms are found, the detailed condition should be described considering the following aspects:

- Position of the injured leaves: direction in a tree crown in relation to dominant wind direction, upper and bottom on crown, immature, matured or old leaves, sunny or shady.
- Pattern and position of the injury in leaf: such as a spot between veins, small spots scatter entirely, discoloration from the pointed end of leafs, defoliation without discolor, broken leaves, etc. For recording leaf color, Mansel color chart is preferably used.
- Observation of dust on the leaves: scanning electron microscope

observation may be useful.

● Existence of injured or dead trees or reduction of growth rate.

● Date when the symptom of damages is found.

To evaluate the total decline class, the following items should be observed according to the Decline Scale (Appendix 2): vitality of tree, form of tree, growth of branch, die back of top stem, density of foliage, deformation of leaves, size of leaves, coloration of leaves, injury of leaves. The results should be reported in Form (Soil and Vegetation E).

b) Photographic record of tree decline (Fig. 2.7.a) and b))

Photograph of tree crowns from the four fixed points mentioned above should be taken.

Set wide (28mm focal distance) lens upward using the tripod and the level, top of the camera toward north direction. The exposure level should be adjusted to the brightness of gap of tree crown. Shape and width of the gap will be recorded with high shutter speed.

Time and date, kind of film, exposure level, focal distance of the lens, film height from the ground level should be recorded. Four photographs should be attached to Form (Soil and Vegetation E) above. Each photograph should be glued on A4-sized sheet. Information concerning the record should also be reported on the sheet.

Note:
● Data analysis such as digitizing the photographs by computer image and comparison with habitus changes of digitized data are preferable for the statistical evaluation.
● It is desirable, if possible, to take an overview photograph from the higher position where whole tree crowns can be observed.

c) Estimation of decline causes

In case that the cause of a decline can be estimated, write the presumed cause

with the reasons. Scientific and specialized detail surveys such as pest and disease analysis are necessary for this identification. On this matter, standardization and training of tree decline assessment for surveyors is essential for this aspect domestically and internationally.

2.4.3.   Methodologies for tropical region

Tropical ecosystems are significantly complicated, and the soil and vegetation are quite diverse. Some methodologies for forest monitoring in the technical manual are not suitable for tropical ecosystems, and new approaches should be developed. In the workshop summary of the 2nd EANET Training Workshop, needs for intensive research activities on tropical forests was emphasized and integration of methodologies suitable for vegetation monitoring in tropical region was recommended.

Basically, concerning the mandatory items for the forest monitoring, the standard method described in the previous subchapters should be applied for every ecological survey site. In this subchapter, some methodologies possibly applicable for tropical forests are described, based on experiences. It is expected that these methodologies could be used in the tropical region and elaborated with further accumulated experiences.

2.4.3.1.   Periodic forest inventories

Many international research works have been promoted in tropical forests in the East Asian region, through which experiences of forest monitoring have been accumulated. These experiences may be useful for EANET activities, especially for accumulation of baseline data in tropical forests.

In Annex III, periodic forest inventories survey is proposed for forest monitoring in the tropical region. This survey is developed by Malaysian Experts, based on their experiences. It is more detailed than the general description of forest described in 2.4.1. Area information and detailed tree information should be surveyed for description of the periodic forest inventories. As detailed tree information, species identification, DBH and upper stem diameter, cross-sectional area measurement, height, tree form, volume, biomass (weight), and density should be measured. The case study in Malaysia is shown in Annex IV.

For implementation of this survey, specific information and skills concerning forest science are required, and therefore, the survey should be supported by specialists in this field.

2.4.3.2.   Canopy analysis using hemispherical photograph

For the evaluation of tree decline, photographic record is proposed in the subchapter 2.4.2. According to this method, five trees of dominant species should be selected at four points and total of twenty trees should be selected. The method may be applicable for the afforestation area in the temperate region. For the forests such as tropical rain forest, which consists of many tree species, it may not be applicable. As one of the possible methods for photographic record in the tropical region, canopy analysis using hemispherical photograph may be proposed. Basic procedures of the canopy analysis are as follows.

Using digital or film camera with fish-eye lens, hemispherical photograph will be taken in forest area (Fig.2.8.). It will preferably be carried out regularly. The photographs will be converted to digital image, which can be processed in computer. Canopy gap (open space) of the forest can be imaged and the gap size (area) can be estimated (Fig. 2.9.). Seasonal change of canopy structure, which was estimated from the computer image, is shown in Fig. 2.10. Diffuse light penetration decreases with increase of Plant Area Index. These changes may correlate with the growth pattern of the forest. For the evaluation of growth pattern, long term monitoring is necessary.

The procedures of hemispherical photographic record are relatively easy and the image can be analyzed by using a computer. Although some tools such as fish-eye lens and relevant softwares are necessary for the implementation of the method, the proposed method may be one of the promising methods suitable for the tropical region.

Note:
- Detailed information of the software for canopy analysis is in the following URL.
  SCANOPY home page: www.regent.qc.ca

2.5. Intensive survey

2.5.1. Objectives of intensive survey

Through the basic survey of soil and forest, baseline data will be accumulated, and trends in properties of soil and forest may be grasped. If forest decline or other changes in ecosystems are detected at these sites, more intensive surveys on soil and vegetation should be undertaken to assess the implication with acid deposition.

2.5.2. Possible methodologies for intensive survey

For the assessment of the implication between the changes and acid deposition in the intensive survey, various environmental factors should be discussed and application of new methodologies should be tried. As the initial step of the intensive survey, Grasp of accurate deposition on forest area can be proposed.

2.5.2.1. Grasp of accurate deposition on forest area

In basic survey site, data of soil and forest should be evaluated using data of the nearest wet and dry deposition monitoring site. Since most deposition monitoring sites may be outside of forest area and apart from the soil and forest monitoring sites, intensive survey in the soil and forest monitoring site is necessary in order to grasp accurate acid deposition on forest area.

To estimate the dry and wet deposition accumulated to the forest area, rain water including throughfall and stemflow will be collected in each permanent plots.

a) Rain (Precipitation)

1) Setting the rain sampler
    Simple rain sampler, plastic funnel (30 cm in diameter) connected to 20 to 30 litters plastic tank, can be used. The mouth of funnel should be set more than 1.3m high. Polyethylene or polypropylene may be useful as the material for the sampler.

    The sampler should be set in open place near the monitoring site. It should be apart more than 500 m from the heavy traffic road, and 50 m from tall trees and houses.

Note:
- The membrane filter (pore size 0.8μm) should be set between the funnel and the bottle of the sampler if possible.
- If the electricity can be supplied to the forest site, wet only sampler is preferable for rain sampling.

2) Frequency of sampling

Daily sampling (when it rains) at a fixed time (for example 9:00 am) is preferable if wet only sampler is available. In remote monitoring site, weekly collection is preferable to biweekly collection. Record the daily meteorological condition.

3) Sampling and chemical analysis

On the site, amount of rain water should be measured using measuring cylinder. Two liters of sample should be brought back to laboratory and filtrated by means of filter paper or membrane filter in order to remove particulate. Filtered water should be stored in a 500ml plastic bottle and kept in refrigerator until chemical analysis.

Parameters for chemical analysis:
- Acidity (pH)
- Electric conductivity (EC or Λ)
- Cations: Na^+, K^+, Ca^{2+}, Mg^{2+}, and NH_4^+
- Anions: NO_2^-, NO_3^-, SO_4^{2-}, Cl^-, and PO_4^{3-},

b) Throughfall

1) Setting the sampler

Same type of the sampler as the rain sampler should be used for through fall. At least three sets of the samplers are recommended to be set under the canopy in the forest. It should not be placed near trunks.

2) Frequency of sampling

Daily sampling (when it rains) at a fixed time (for example 9:00 am) is preferable if wet only sampler is available. In remote monitoring site, weekly collection is preferable to biweekly collection. Record the daily meteorological condition.

3) Sampling and chemical analysis

Sampling and chemical analysis procedures are same as the precipitation sample.

c) Stemflow

1) Sampling equipment

Stemflow should be dammed by collar of elastic plastic band (3 cm thick and 30 cm width, polyethylene form is useful) at the height of 1.3m of the trunk, and get flow water using a polyvinyl tube (Fig. 2.11.). When the plastic collar would be set on trunks, it is necessary to be careful not to injure the cambium, and any adhesives are not allowed except silicone for sealing gap between the bark and the plastic collar. Three sets of the sampler should be set for a species. More than one hundred-liter capacity may be needed for weekly collection.

2) Frequency of sampling

Weekly collection is preferable. Biweekly collection is acceptable.

3) Sampling and chemical analysis

Sampling and chemical analysis procedures are same as the precipitation sample.

3. Terrestrial ecosystem analysis

As described in the chapter 1, the ultimate objective of soil and vegetation monitoring on EANET will be to assess the impacts of acid deposition on terrestrial ecosystems in a comprehensive and systematic manner through establishment and maintenance of good quality database. To achieve the ultimate objectives, catchment analysis can be one of the useful approaches, especially for description of nutrient dynamics and nutrient status.

The area, where terrestrial ecosystem analysis will be carried out, should be classified as the ecosystem analysis site.

3.1 Catchment analysis

Terrestrial ecosystems consist of plants, animals, fish, insects and microbes and relevant interacting factors. Environmental impacts such as air pollution and acid deposition are estimated as the long term effects on not only floral or faunal change but also nutrient cycling and nutrient status.

For description of nutrient (elemental) dynamics, at least, inputs and outputs of the elements in question should be evaluated. Especially, for investigation of outputs, main part of outputs, such as stream and river, should be estimated. In this point of view, certain extent of catchment area can be a candidate area.

Though the methodologies for catchment analysis have not been established, the experiences for description of nutrient dynamics have been accumulated. In annex I, some information on candidate parameters is described.

4. Quality assurance and quality control in monitoring soil

The objective of QA/QC activity is to obtain reliable data that can be comparable among the members' countries of the EANET to evaluate effects of acid deposition on soil in the region. QA/QC should be incorporated in all aspects of designing, sampling, measurement and operations in a field and a laboratory, and data management and processing. During and after sampling, it is important to practice the condition control and the statistical procedures.

4.1. Condition control

Permanent signs which are made by, e.g., stainless steel to identify the sampling plots, occupying area from 5m x 5m to 10m x 10m in the case of soil should be maintained in order that all samples to be taken at the same location every sampling time.

a) Notes should be recorded of relevant facts related to the processes of sampling, transportation, sample storage, physical test, preparation and chemical analysis of samples, etc.

b) If samples are analyzes in different laboratories, the accuracy of the analysis should be crosschecked between the laboratories according to the statistical procedures shown in 4.4.

c) If necessary data is lacking after assessing data accuracy by the crosschecks or other ways, it is desired that the data are obtained through repeated sampling and/or analysis.

d) The results of QA/QC should be stored. This information will improve the usefulness of the reported data, in particular its future application.

e) Training of specialists is an important part of QA/QC. Training programs should include training in monitoring methods at the monitoring sites and laboratory analysis, evaluation of results, and QA/QC methods.

4.2. Data management and processing

a) All data should be recorded according to the manual and filled the forms, which are attached in the reporting procedures and formats.

b) Description concerning the state of the effects of acid deposition on soil should be included in the data report along with maps, photographs, figures and/or tables.

c) Duplicated samples should be taken in each increment (subplot in the case of a soil) at least. If samples are analyzed in different laboratories, check all the steps of analysis comparing with the analytical manual and the precision of the analysis should be clarified by crosschecks between the laboratories.

d) Integrated evaluation of the monitoring results should also be included. Statistical analysis including ANOVA and their interpretation must be often useful for the purpose.

e) In order to make a comprehensive assessment of acid deposition issue in the region, as well as to facilitate data exchange among participating countries should be promoted. Standardization of reporting methods and formats which are shown in the manual should be promoted and improved according to common experiences and agreement on it. Moreover, results can be compiled by an appropriate organization in the region, and comprehensive assessments performed using the compiled data.

4.3. Statistical procedures

Soils and vegetation are characterized by several types of variation. Quality of data from monitoring depends mainly on such variability, besides statistical error on each step of a process from an increment sampling to chemical or physical analysis. A hierarchical system of sampling units is employed for soil monitoring of EANET as shown in Table 6.1. The statistical design of experiment used for evaluation of the variation and/or precision are 1) staggered type nested experiment, and 2) completely repeated nested experiment, any of which can be used where appropriate.

Table 4.1. Hierarchical concepts of the sampling units in soil monitoring

Unit	Number	Size	Reference	Examples in Japan.
Horizontal				
-Area	1	<50km in radius	2.2.1.1	15 prefectures
-Soil type	2	0.1-1 ha	2.3.1	35 in an area
-Plot	Several	5m×5m/10m×10m	2.3.1	2-4 in a site
-Subplot	5	1m×1m	2.3.1.2	5 in a plot
Vertical				
-Horizon	2 layers	-	2.3.3	2(uppermost & subsequent)
Temporal				
-Period	1 time	Every 3-5 years*	2.3.2	every 3 years

*Depending on the pollution regime

4.3.1. Definitions

Some important definitions for the purpose of the main words used in this manual, the following definitions apply.

(1) Trueness: The degree of bias.

(2) Precision: The degree of dispersion.

(3) Accuracy: The degree of error. The general expression of trueness and precision.

(4) Permissible tolerance:

  (a) The permissible tolerance expressed with performance, accuracy, trueness, precision, etc. related to the error of observed value or measurement result for the identical material under the specific measurement condition.

  (b) The critical range or critical difference in which n pieces of the ranges of observed value or measurement result obtained under the specific measurement condition are included in a given probability with respect to the identical material.

  (c) The critical difference in which the difference between the certified value and the observed value or the measurement result obtained under the specific measurement condition is included in a given probability with respect to the reference material or the certified reference material.

(5) Measurement condition: The condition concerning the repetition when obtaining

the plural number of observed values or measurement results from the identical material. The measurement condition includes repeatability condition, within-laboratory-reproducibility condition and reproducibility condition each of which shall be defined concretely in each measurement methods.

(6) Repeatability condition: The measurement condition under which man, time and facility are all identical in measurement of identical material.

(7) Within-laboratory-reproducibility condition: The measurement condition under which man, time and facility are different partially or in measurement of identical material in the same laboratory.

(8) Reproducibility condition: The measurement condition under which laboratory, man, time and facility are all different in measurement of identical material.

(9) Repeatability: The precision of observed value under the repeatability condition. It is called the repeatability standard deviation when expressed with standard deviation and the repeatability variance when expressed with variance.

(10) Reproducibility-within-laboratory: The precision of observed value or measurement result under the within-laboratory-reproducibility condition. It is called within-laboratory-reproducibility standard deviation when expressed with standard deviation and within-laboratory-reproducibility variance when expressed with variance.

Informative reference: Repeatability and reproducibility-within-laboratory are called precision-within-laboratory in generic use.

(11) Reproducibility: The precision of measurement results under the reproducibility condition. Sometimes it is called briefly inter-laboratory precision. Moreover, it is called reproducibility standard deviation when expressed with standard deviation and reproducibility variance when expressed with variance.

(12) Repeatability limit: The permissible tolerance under the repeatability condition.

(13) Within-laboratory-reproducibility limit: The permissible tolerance under the within-laboratory-reproducibility condition.

Informative reference: Repeatability limit and within-laboratory-reproducibility limit are called permissible tolerance within-laboratory in generic use.

(14) Reproducibility limit: The permissible tolerance under the reproducibility

condition.

4.3.2. Model of monitored data

The following model is assumed for the monitored value *x* according to the hierarchical sampling design:

$$x = \boldsymbol{m} + h + a + s + p + i + b + c + e$$

where,   μ: mean value

e: error under the repeatability condition

c: of the error under the within-laboratory-reproducibility condition, the part unable to be explained by *e*.

b: of the error under the reproducibility condition , the part unable to be explained by $c + e$.

$i:$ random effect of inclement (subplot)

p: random effect of plot

s: random effect of soil type

a: random effect of area

h: fixed effect of horizon

Expected value and degree of freedom in variance of each stage of sampling can be calculated by ANOVA in a branching experimental plan using statistical variation model.

4.3.3. Estimating mean value and variability of each stage of the hierarchical sampling

Table 4.2 shows an example of a result in the multi-stage sampling and ANOVA table.

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Table 4.2. Calculated average values at each stage with calculated standard errors (Virtual)

Country	Area	Soil type	Plot	Subplot
5.1±0.2 Japan	5.1±0.2 Tokyo (Urban)	4.9±0.2 Sensitive soil	4.7±0.1 No.1	4.8±0.1
				4.7±0.1
				4.9±0.1
				5.0±0.2
				4.6±0.1
			5.2±0.1 No.2	5.3
				5.2
				5.1
				5.1
				5.3
		5.3±0.1 Non-sensitive soil		5.3±0.1
				5.4±0.2
				5.2±0.1
				5.2±0.1
				5.4±0.1
	5.1±0.1 Tsukuba (Rural)	5.0±0.2 Sensitive soil	4.7±0.3 No.1	4.6±0.1
				4.8±0.2
				5.0±0.3
				4.5±0.3
				4.7±0.2
			5.3±0.1 No.2	5.0±0.1
				5.1±0.2
				5.1±0.1
				5.4±0.2
				5.4±0.1
		5.2±0.1 Non-sensitive soil	5.0±0.2 No.1	4.8±0.1
				4.9±0.2
				5.0±0.1
				5.2±0.2
				5.1±0.2
			5.4±0.1 No.2	5.5±0.1
				5.5±0.1
				5.3±0.2
				5.3±0.1
				5.4±0.1

ANOVA table

Source of variance	Variance
Country	-
Area	0.174
Soil type	0.263
Plot	0.061
Subplot	0.052
Error	0.011
Total	**0.561**

Data of Tokyo-sensitive Soil –No.2 have no standard error, because they were analyzed without repetition.

4.3.4. Estimating precision of monitored data: collaborative experiment

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 in the 1st section of the Annex II.

4.3.5. Methods for examination for improvement of precision

The cases where the precision of measurement method is necessary to be improved are shown in the 2nd section of the Annex II. The methods for examination in these cases are also described in the Annex II.

References

Bartels, J. M. (ed.) 1996; Method of Soil Analysis, Part 3, Chemical Method, Soil Science Society of American (SSSA), Inc, Publisher, Madison, Wisconsin USA

Black, C. A. (ed.) 1965; Method of Soil Analysis, Part 2, Chemical and Microbiological Properties, American Society of Agronomy, Inc, Publisher, Madison, Wisconsin USA

Environment Agency, Government of Japan 1997. Guidelines and Technical Manuals for Acid Deposition Monitoring Network in East Asia.

FAO 1990. Guidelines for Soil Profile Description (third edition). Soil Resources Develop. Conser. Service, Land and Water Develop. Div., FAO, Rome, 68pp.

FAO-UNESCO 1977. Soil Map of the World.

Japanese Standards Association 1991. General rules for permissible tolerance of chemical analyses and physical tests (JIS Z 8402-1991). Japanese Industrial Standard.

Ishizuka, M., L. Puangchit and P. Phasuk 2000. Simulation of leaf and canopy photosynthesis in a tropical deciduous forest in western Thailand. In: Proceedings of the International Workshop on the Response of Tropical Forest Ecosystem to Long Term Cyclic Climate Changes (preparing).

Klute, A. (ed.) 1986; Method of Soil Analysis, Part 1, Physical and Mineralogical Methods, Second edition, American Society of Agronomy, Inc and Soil Science Society of America, Inc., Publisher, Madison, Wisconsin USA

Page, A. L. (ed.) 1982; Method of Soil Analysis, Part 2, Chemical and Microbiological Properties, Second edition, American Society of Agronomy, Inc and Soil Science Society of America, Inc., Publisher, Madison, Wisconsin USA

Snedecor, G.W. and W.G.Cochran 1991. Statistical Methods. Eigth edition. Iowa State Univ. Press, Ames. 503pp.

Van Reeuwijk, L.P. (ed.) 1993; Procedure for Soil Analysis, Fourth edition, International

Soil Reference and Information Center (ISRIC), Wageningen, Netherlands

Table 1.1. Concept of approach on soil and vegetation monitoring (Ecological survey site)

Step	Objectives	Expected results	Methodologies	Site classification
Initial objectives ↓ Ultimate objectives	Establishment of baseline data Early detection of possible impact	Accumulation of baseline data on soil and forest. Trends in properties of soil and forest.	Basic survey - Soil monitoring (Chemical and Physical analysis) - Forest monitoring (Description of trees, Understory vegetation survey, and forest decline survey)	Basic survey site (possible same site as deposition monitoring site)
		Clarification of the implication with acid deposition (when some changes are detected)	Intensive survey (grasp of deposition, application of new methodologies, etc.)	
	To assess impact of acid deposition on terrestrial ecosystem with good quality database and comprehensive and systematic manner	Description of elemental dynamics in ecosystem. Estimation of environmental capacity Development of acidification model	Terrestrial ecosystem analysis (Catchment analysis, etc.)	Ecosystem analysis site
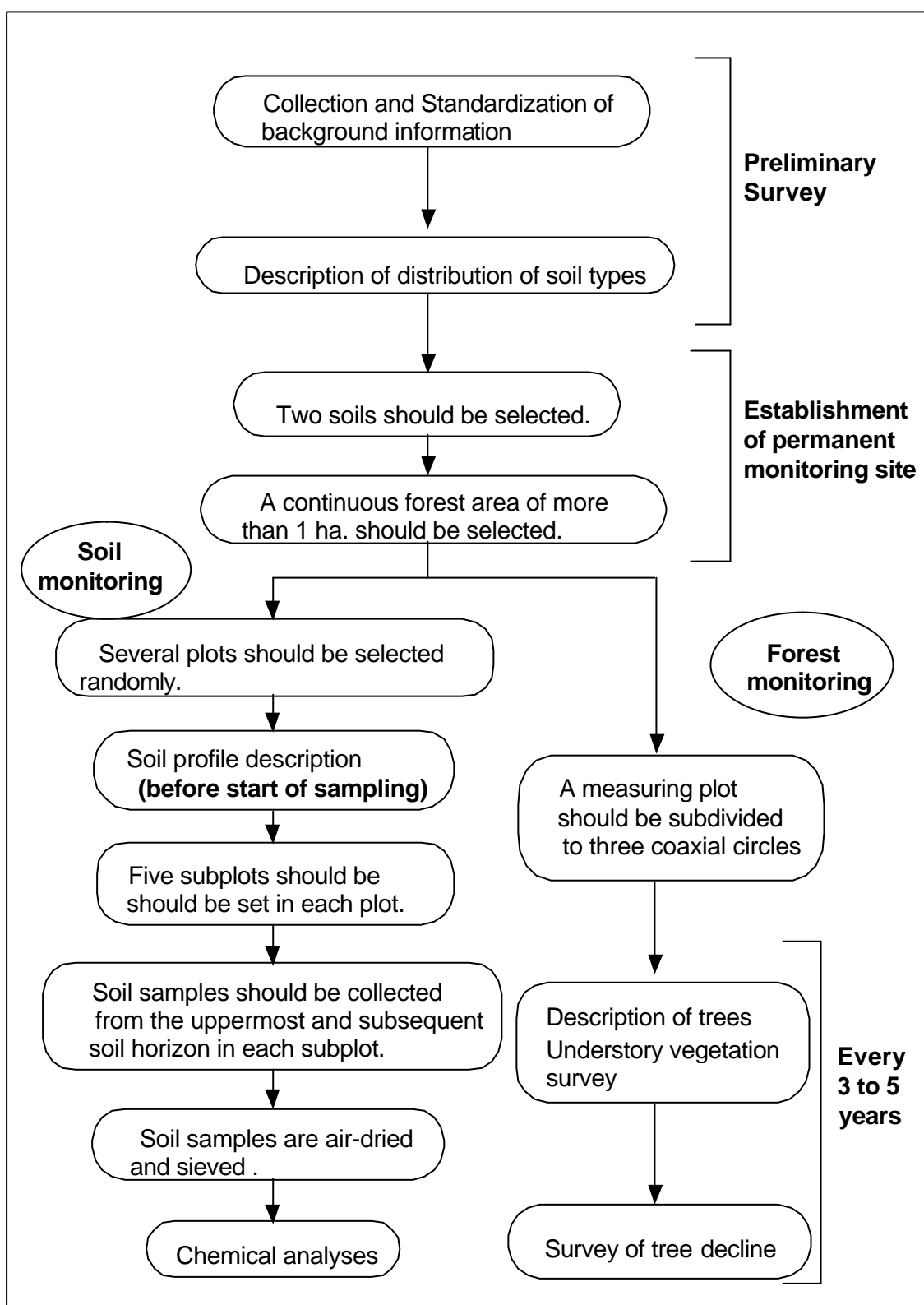
Figure 2.1. Overall process of basic survey of soil and forest

If some changes are detected through the above survey, more intensive survey should be undertaken to assess the implication with acid deposition. The terrestrial ecosystem analysis will be carried out independently of these surveys.
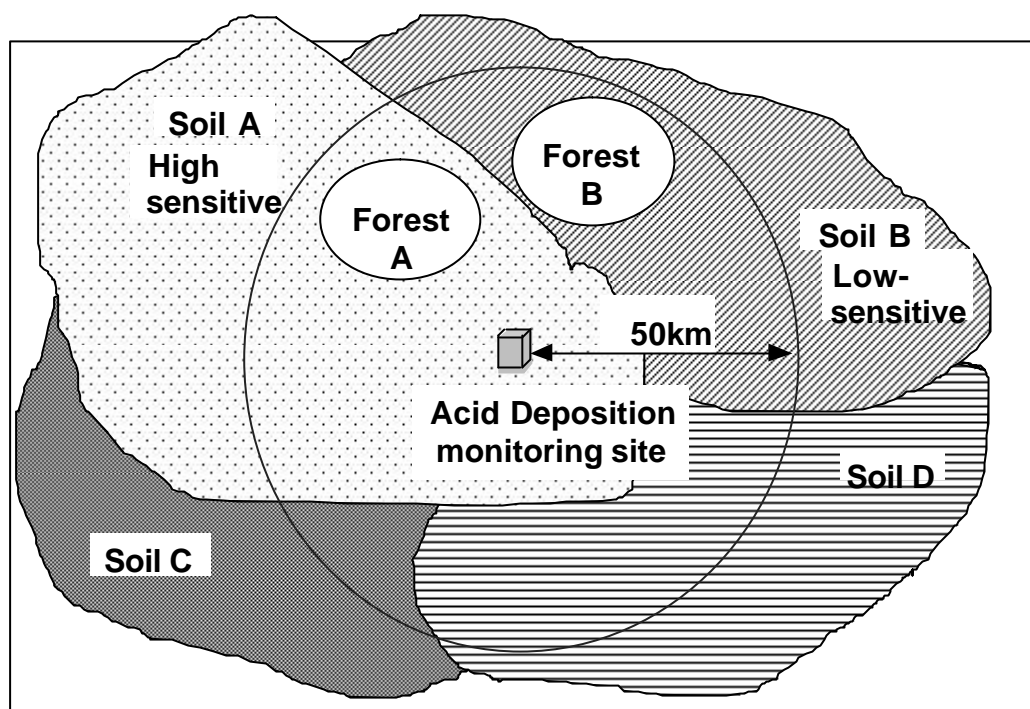
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Figure 2.2. Image of the permanent monitoring sites

Within a radius of approximately 50km of acid deposition monitoring sites, two forest sites, whose soils have different sensitivities to acid deposition, are recommended to be selected. In each forest, plots for soil and vegetation monitoring should be established.
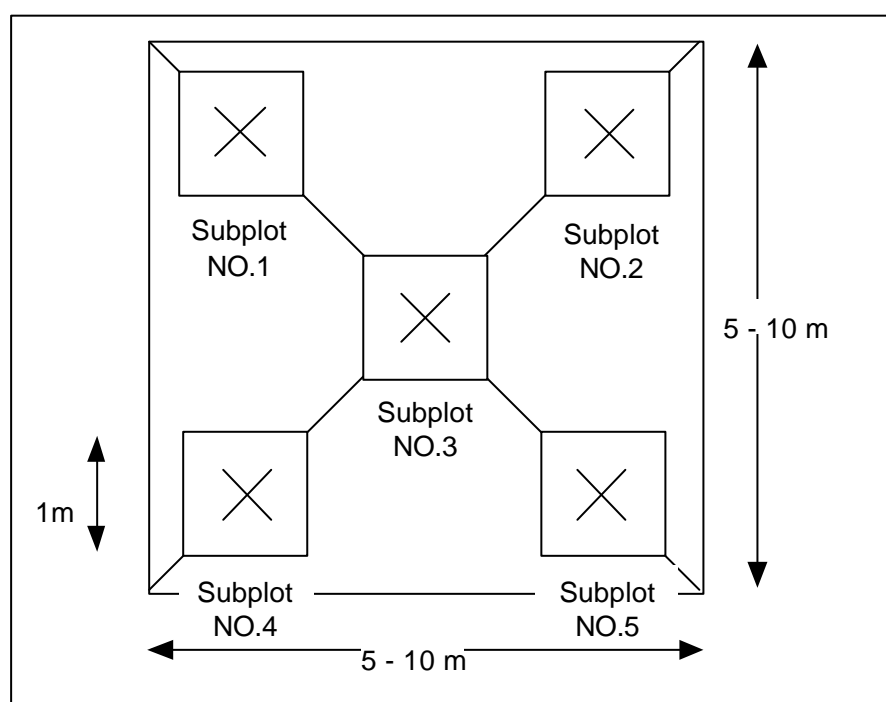
Figure 2.3. Selection of plots and subplots for soil monitoring

Several plots, at least two plots, occupying areas from 5 m x 5 m to 10 m x 10 m, should be selected randomly at each soil type. Five subplots for soil sampling, each occupying 1 m x 1 m, are selected at the center and on the diagonal lines of the plot.
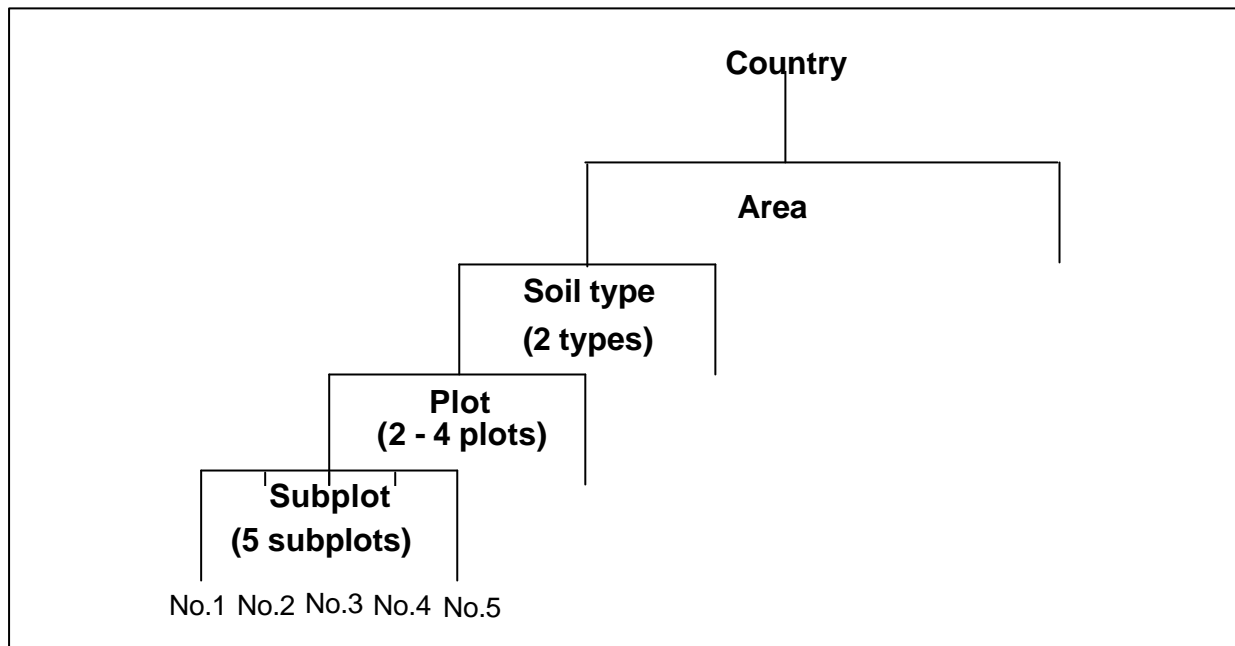
Figure 2.4. Image of the multi-stage sampling for soil monitoring
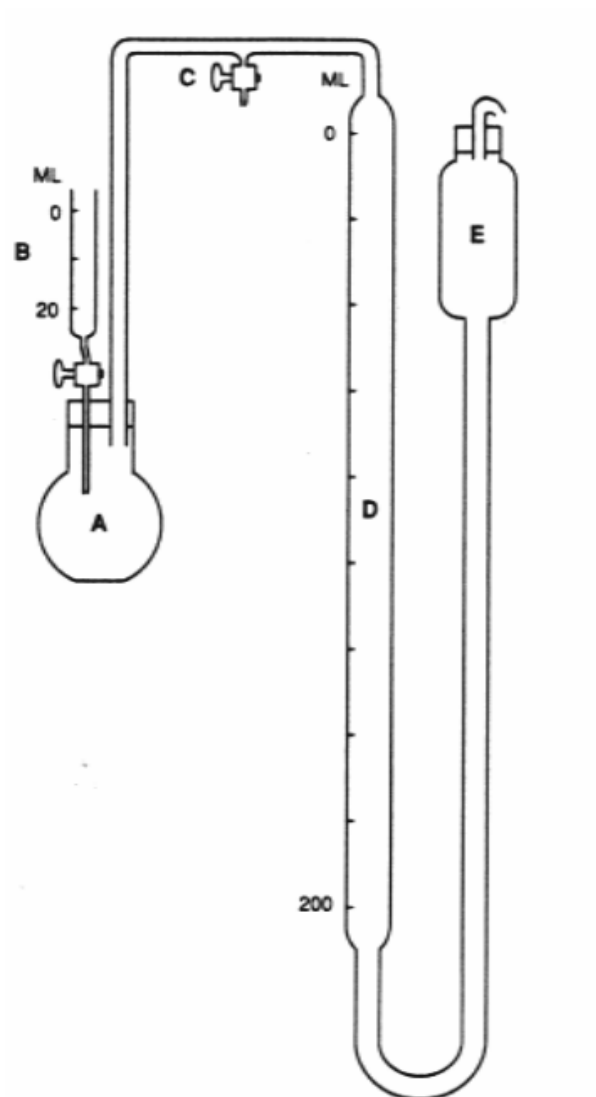
Figure 2.5. Volumetric calcimeter for the determination of carbonate
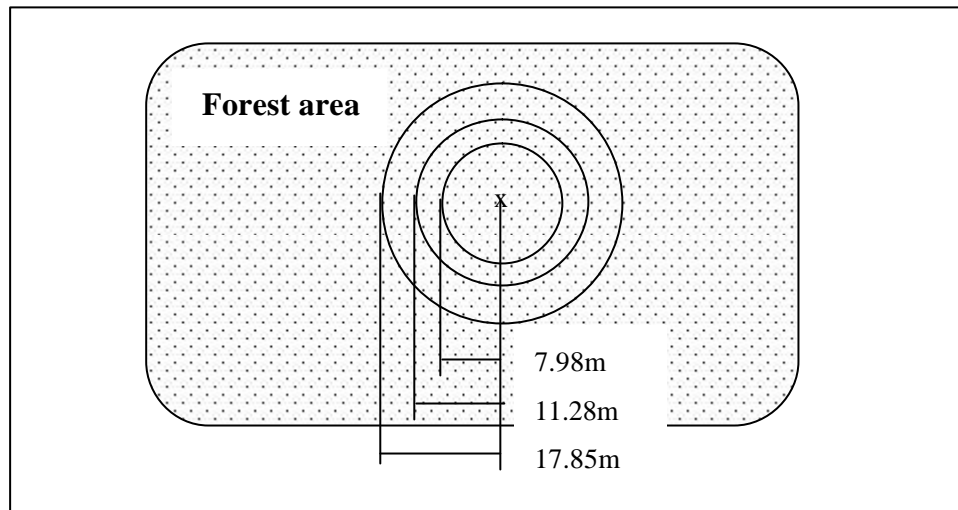
Figure 2.6. Selection of plots for description of trees

For description of trees, a measuring plot should be subdivided to three coaxial circles of 1000, 400 and 200 square meter for the detailed survey when average tree height is around 20m.
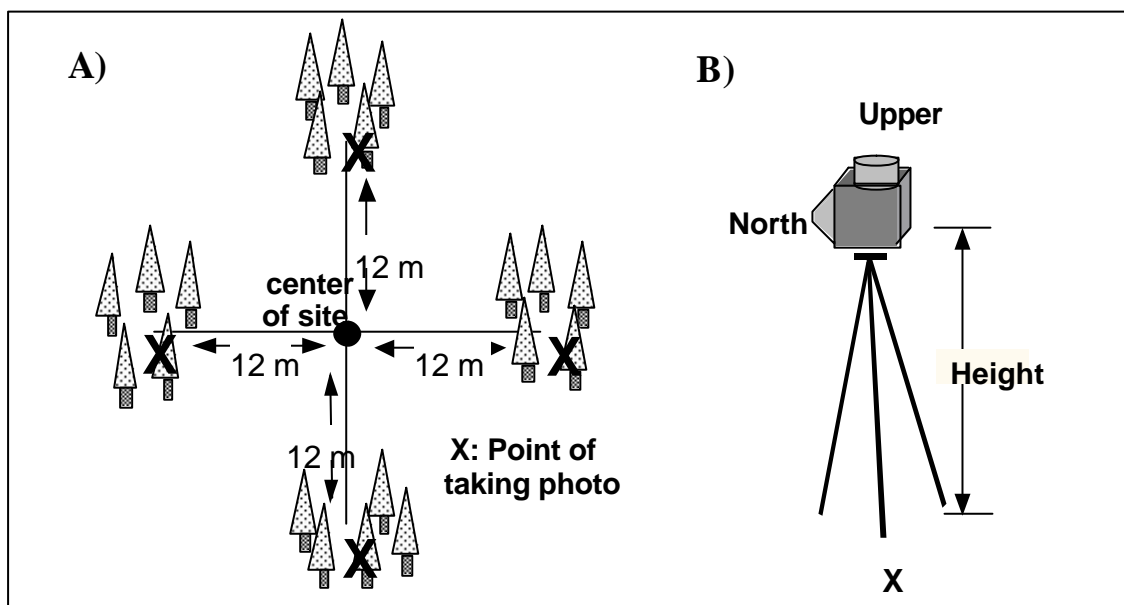

Figure 2.7. Survey of tree decline

For the twenty trees, observation and evaluation of decline, record of foliage by photograph, and estimation of a decline cause should be carried out.

A) Position of recording: Around four points of north, south, east and west, 12 m apart from the center of permanent site, five dominant trees should be selected.

B) Standing position of camera: The lens of camera should be set upward and top of the camera should be set toward north direction.
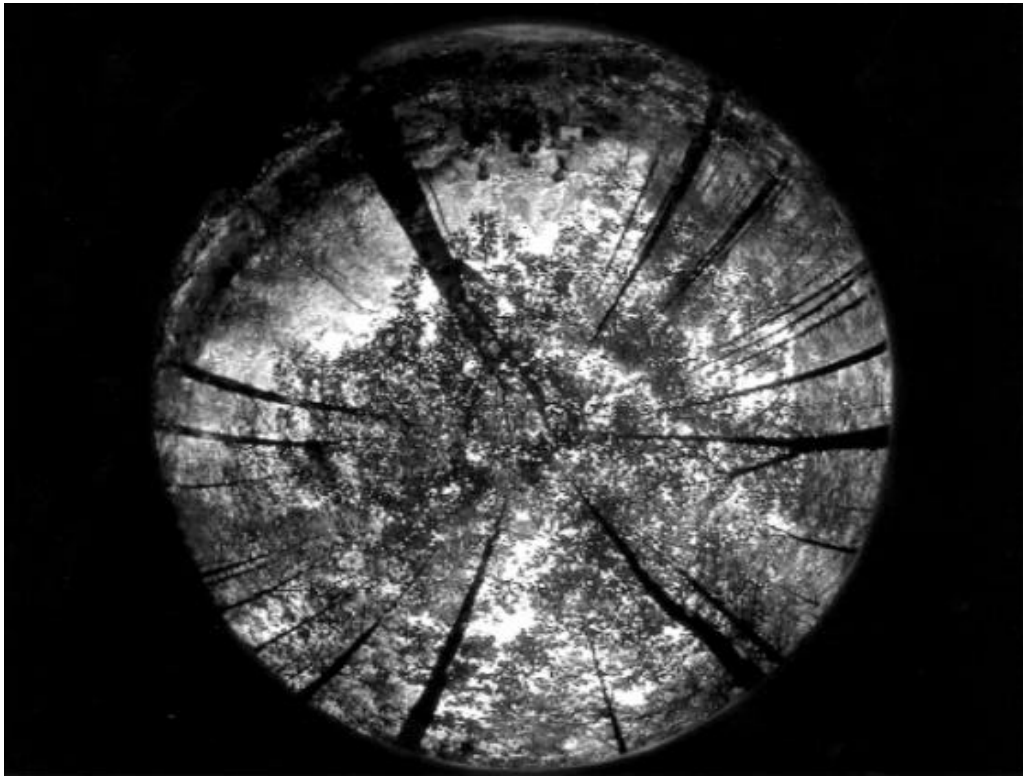
Figure 2.8. Hemispherical photograph taken in *Michelia maclurei* plantation in Xiaoping, Xiamen, China on 5 March 1999 (Photo by Prof.. T. Totsuka).
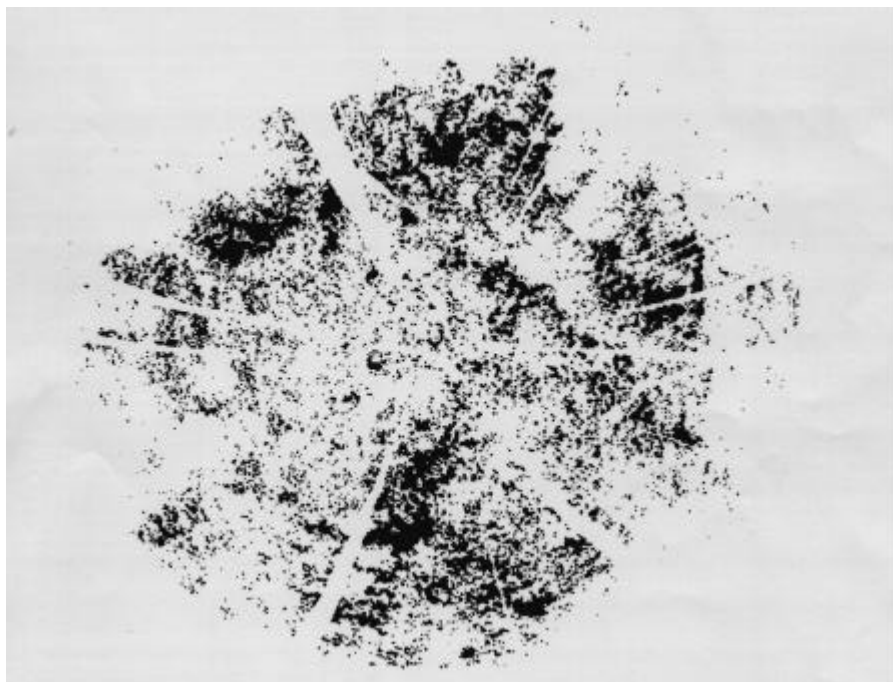

Figure 2.9. Hemispherical photograph taken in *Michelia maclurei* plantation in Xiaoping, Xiamen, China on 5 March 1999 (Black area corresponds to open space of the forest) (Photo by Prof.. T. Totsuka).
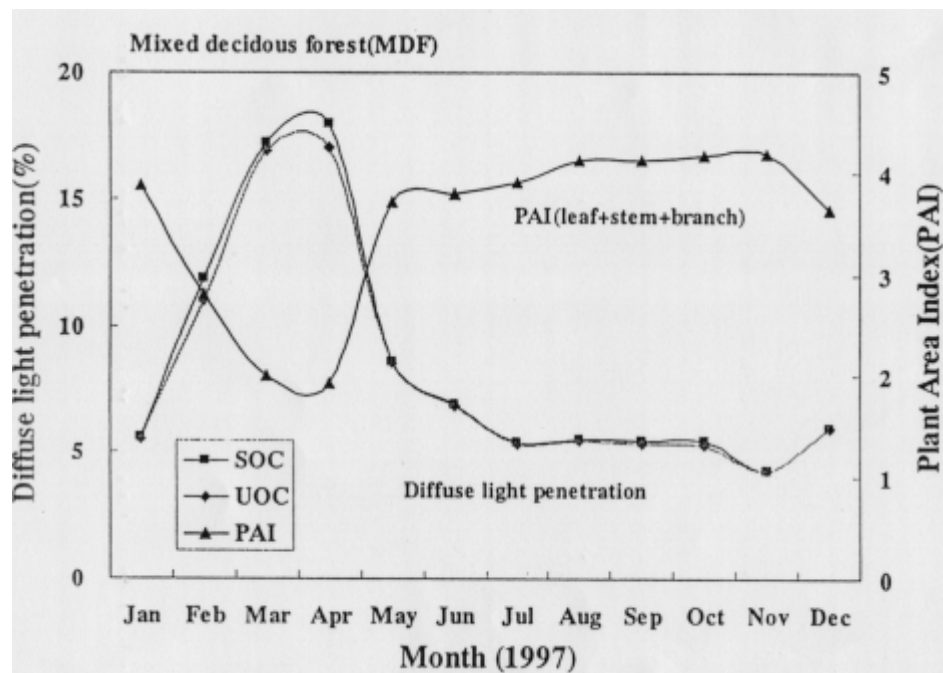
Figure 2.10. An example of seasonal changes of canopy structure of tropical deciduous forest based on photographic estimation from 1.3 m high (Ishizuka *et al.*, 2000).
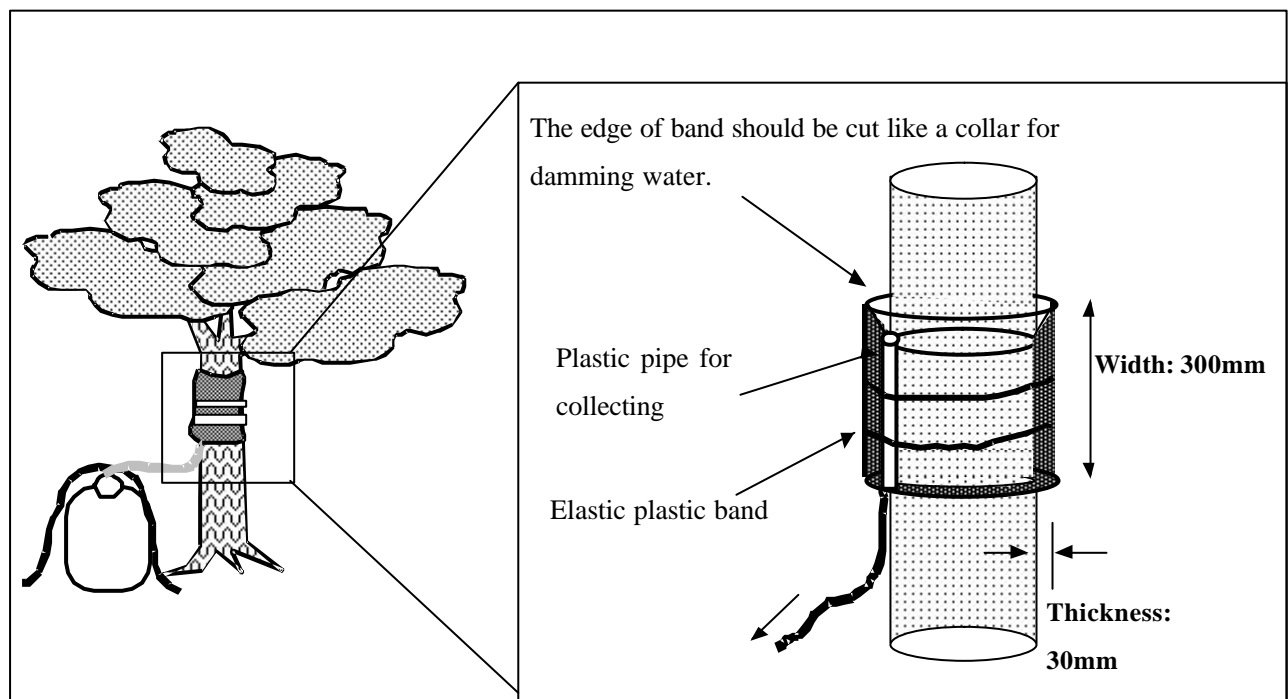

Figure 2.11. Equipment for collecting stem flow