Sub-Manual on Forest Vegetation Monitoring in EANET

Prepared by Task Force on Soil and Vegetation Monitoring of EANET
Endorsed by the Scientific Advisory Committee of EANET at its Sixth Session

Network Center for EANET
Acid Deposition and Oxidant Research Center

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1 Introduction

1.1 History

The development and improvement of methodologies for forest vegetation monitoring were described in the *Strategy Paper on Future Direction of Soil and Vegetation Monitoring of Acid Deposition Monitoring Network in East Asia (EANET)* (hereinafter referred to as "*Strategy Paper*") as one of the measures to be implemented for the early detection of possible impacts of acid deposition. To this end, it was anticipated that the Task Force on Soil and Vegetation Monitoring (TF) would prepare this sub-manual on forest vegetation monitoring.

EANET and the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests) jointly held a meeting entitled the Workshop on Elaboration and Development of Forest Monitoring in East Asia, in Seremban, Malaysia, on 16–19 December 2002. The workshop was a milestone that provided the basis for development of this sub-manual. Important technical issues were identified based on the Chairperson's Summary of that workshop and described in a document entitled "Technical subjects to be discussed for sub-manual on forest monitoring in East Asia." After further work, a document entitled "Procedures and schedule for preparing sub-manual on forest monitoring in East Asia" was released in 2003.

Discussions by the Task Force and the Network of Soil and Vegetation Monitoring Specialists (Network of Specialists) on possible contents and authors, based on technical issues and the latest scientific information, resulted in a document in March 2004, entitled "Contents and Authors for the Sub-manual on Forest Monitoring in East Asia." Lead authors were mainly members of this Task Force and the Network of Specialists. Other experts were designated as lead or contributing authors for some of the content, particularly regarding advanced techniques. The lead authors had prepared manuscripts for the respective chapters by early 2005. Drafts were posted on the EANET website in October 2005 and reviewed by members of the Task Force and the EANET's Scientific Advisory Committee (SAC), as well as experts involved in ICP Forests. The drafts were later revised and edited based on their comments and suggestions, resulting in this sub-manual.

The final draft of the sub-manual was presented and adopted in the sixth session of SAC in October 2006 after a few modification of terminology.

2.2 Outline of *Sub-Manual on EANET Forest Vegetation Monitoring*

Forest monitoring in basic surveys of EANET consists of (1) a general description of the condition of forests, and (2) a survey of tree decline (Table 1.1). A general description of forests in a country is included mainly to establish baseline data (one of EANET's basic objectives for soil and vegetation monitoring). Personnel conducting this work should refer to the *Technical Manual for Soil and Vegetation Monitoring in East Asia* (2000) (hereinafter referred to as "*Technical Manual*"). The survey of symptoms and phenomena of tree decline is carried out mainly to help with early detection of possible impacts of acid deposition (another of EANET's basic objectives). The current sub-manual was developed for this objective.

Table 1.1. Items and methodologies involved in EANET forest monitoring

	Survey item	Status	Purpose	Reference
General description of the forest	Description of trees (species name, diameter at breast height [DBH], and height)	Mandatory	Mainly for baseline data	*Technical Manual for Soil and Vegetation Monitoring* (2000)
	Understory vegetation	Mandatory		
Survey of tree decline	Observation	Mandatory	Mainly for early detection	*Technical Manual* and the *Sub-manual on Forest Monitoring* (this issue)
	Photographic record	Optional		
	Inference of possible cause(s)	Optional		

This sub-manual consists of two types of content: (1) the main text, and (2) reference material for future monitoring. The structure of the sub-manual and sources or references in other EANET documents are shown in Table 1.2. The main text is based on *Guidelines for Acid Deposition Monitoring in East Asia* (2000) (hereinafter referred to as "*Guidelines*") and the *Technical Manual*. Methodologies for survey of tree decline are based on recent experience in countries participating in EANET and described in Section 1.3.

Table 1.2. Structure of sub-manual and location of topics in EANET documents

Chapter		Content	Location in EANET documents
1		Introduction	History, structure of sub-manual, etc.
2		Additional methodologies for basic surveys of tree decline	Improved methodologies based on *Guidelines* and *Technical Manual*
Annex 2.1		Image analysis for assessment of tree crown condition	Modified methodologies based on *Technical Manual*
Annex 2.2		Hemispherical (fisheye) photography for assessment of canopy gaps and light penetration	Detailed methodologies based on suggestions in *Technical Manual*
3	3.1	Estimation of concentration and deposition of air pollutants in forest area	Detailed methodologies based on suggestions in *Guidelines* and *Technical Manual*
	3.2	Chemical analysis of needles and leaves	Modified methodologies based on *Technical Manual*
4		Quality assurance/quality control (QA/QC) in forest vegetation monitoring	Additional explanations for QA/QC program
5	5.1	Monitoring of lichens as bio-indicators of air pollution and acid deposition	Reference material
	5.2	Remote-sensing technologies	Reference material
Appendix		Information on research methodologies for determining plant sensitivity	Reference material

The reference materials describe methodologies that are new for EANET and suggested based on experience in Europe and several countries participating in EANET. The applicability of these methodologies in EANET monitoring will be discussed in future, but they are included here as information to consider in research activities in participating countries.

3.3 Practical use of the *sub-manual*

The methodologies described in the main text should be used for surveys of tree decline in forest monitoring. The surveys of tree decline include three components (Table 1.1): observation, photographic record, and inference of cause(s) of decline. Applicable methodologies for each component are shown in Table 1.3.

For observation of tree decline (mandatory), additional methodologies for surveying tree decline as part of the basic survey (Chapter 2) are applicable. Specific information for temperate, sub-arctic and (sub-)tropical zones is included in Sections 2.2 and 2.3, respectively. These improved methodologies should be used for observation of tree decline.

Table 1.3. Applicable methodologies for each item

Item	Reference chapter and content (methodology)		
Observation of tree decline (mandatory)	2		Additional methodologies for surveying tree decline as part of the basic survey
Photographic record of tree decline (optional)	The current *Technical Manual* can be used for recording the condition of the canopy		
Photographic record of tree decline (optional), especially for numerical analysis	Annex 2.1		Image analysis for assessment of tree crown condition
	Annex 2.2		Hemispherical photography for assessment of canopy gaps and light penetration
Inference of possible cause(s) of decline cause (optional)	3	3.1	Estimation of concentration and deposition of air pollutants in forest area
		3.2	Chemical analysis of needles and leaves
QA/QC program	4		QA/QC on monitoring forest vegetation

As for producing a photographic record of tree decline (optional), the current *Technical Manual* can be used for recording the condition of the canopy, but it is difficult to evaluate a photograph quantitatively. For numerical analysis of a photographic record, image analysis may be used to assess tree crown condition (Annex 2.1) and hemispherical photography may be used to assess canopy gaps and light penetration (Annex 2.2). These can be recommended as supplemental methodologies for photographic records. These forms of image analyses may also be helpful in quality control. Quantitative observation of tree decline may fluctuate due to the varied experience of surveyors and uncertainty of observation standard, however, so it is recommended to promote calibration of the standard and check observations by using other quantitative methodologies in order to control data quality. Computer-aided image analysis may be useful for crosschecking, although each analysis should also be checked under the QA/QC program. By comparing observations and image analyses, a more accurate condition of tree decline may be recorded. In fact, CROCO, a photographic image analysis system for determining tree crown condition (described in Annex 2.1) has been applied to the data of ICP Forests for QA/QC.

When the cause of forest decline is not known, documentation should include inferences of the cause(s) of decline (optional) and the connection to acid deposition. For this purpose, intensive surveys were suggested in the *Guidelines* and the *Technical Manual*, but, in practice, information on deposition and leaf chemistry should be accumulated through regular monitoring of the basic survey, if effects of acid deposition are anticipated. Methodologies described in Chapter 3 can be used for the optional components in this section. The methodologies may also be worth considering when conducting more intensive surveys of ecosystems.

The QA/QC Program for Soil and Vegetation Monitoring in East Asia was mainly focused on soil monitoring. Thus, a supplementary document for the QA/QC of forest monitoring was prepared (Chapter 4) for this sub-manual. The basic philosophy of the document is the same as the current QA/QC program, but supplementary descriptions of methodologies were added. The information in Chapter 4 should be utilized within the current QA/QC program.

4.4 Update of methodologies

Methodologies described in this sub-manual will be revised in the future based on the latest scientific information and the experiences of countries participating in EANET. The applicability of new methodologies introduced here through reference material will be discussed in the future process of revision of the *Guidelines* and *Technical Manual*.

2. Additional Methods to Identify Tree Decline as Part of the Basic Survey

The *Technical Manual* included a recommendation that certain tasks should be carried out to identify tree decline, a mandatory part of the basic survey (Table 2.1).

Table 2.1. Monitoring tasks to survey tree decline

Task	Classification
Observation of tree decline	Mandatory
Photographic record of tree decline	Optional
Estimation of decline cause(s)	Optional

Visual assessment of crown condition is the main method used to document tree decline, but there was not enough detailed information in the *Technical Manual* on procedures or instructions on observation techniques. Moreover, the methodologies described were based mainly on techniques developed for use in temperate zones such as Europe and Japan. It was therefore necessary to modify some of the procedures for the East Asian region to take its varied climatic zones and forests into account.

This chapter describes in more detail the basic procedures of the modified methodologies for observation of tree decline (Section 2.1), and provides some additional information on making observations in temperate, sub-arctic, tropical, and sub-tropical zones (sections 2.2 and 2.3, respectively). Surveyors conducting forest monitoring in all countries participating in EANET should follow the procedures described below. Those doing monitoring in Northeast Asian and Southeast Asian countries should also refer to sections 2.2 and 2.3, respectively.

2.1 Procedures of visual assessment of crown condition to identify tree decline

2.1.1 Basic concept

Visual assessment of tree crown condition is one of the principal methods used in forest monitoring. The goals of the assessment are to evaluate the condition of tree stands, by examining the state of the tree crowns, and attempt to determine the cause(s) of the observed disturbance (growth suppression) of trees; that is, identification of the negative factor(s) causing the damage, whether natural or anthropogenic. Several factors may be present simultaneously or only for certain time periods, such as air pollution, insect attacks, fungal disease, long-term drought, frost, intense winds, or physical damage caused by people or animals.

Tree crown condition is subject to fluctuations and changes over time, and varies around a benchmark state that is dependent on many factors, including tree species, age, and site condition. It is also dependent on the indicators/indices used to assess conditions. For example, defoliation and discoloration can have substantially different causes and may occur at the same time on the same trees. Thus, although it is difficult to say whether a defoliated tree is in better condition than one with discolored leaves or needles, it can be said that a tree showing both symptoms is in worse condition than a tree displaying, to the same extent, only one. When several factors are involved, it is then important to have a synthetic expression of crown condition status that can help identify any anomalous deviation from the benchmark state.

These two parameters of crown condition should be assessed annually:

- Defoliation/crown transparency
- Discoloration of leaves or needles

The next section provides information on the methodologies used to assess tree crown condition and evaluate the status of the sample trees selected, as well as to determine the potential causes of any defoliation and/or discoloration observed.

2.1.2 Preparation for the tree crown survey

a. Specialized evaluation team

The visual examination should be done by experts with experience in such work, as inexperienced people may overlook deviations or finer details in the state of the tree crown. It is recommended that two or three scientists specialized in different domains of forest studies—such as ecology, phytophysiology, forest entomology, or phytopathology—perform the examination.

b. Equipment

The following equipment is needed for the assessment of tree crown conditions:

- Vehicle
- Compass
- GPS-navigator
- Area maps
- Field binoculars
- Camera (digital recommended)

- Secateurs (tool for cutting branches in the tree crown)
- Measuring ribbon (tape) and/or diameter tape
- Height measurement instrument
- Paint and brush, plastic numbering tape
- Individual diaries and a field notebook

c. Reference tree stand

A typical stand of healthy trees should be selected as the reference (control) for the survey. The influence of air pollutants should be minimized by choosing reference tree stands that are far from any industrial center, are growing under the most favorable conditions for tree growth, and have a minimum of crown defoliation.

Note:

Researchers have so far failed to agree on a standard description of an optimal reference (control) tree stand to use for comparison to evaluate tree conditions. In multi-year studies in Russia, damaged (suppressed) trees were compared with relatively healthy ones—often called background trees.

2.1.3 Selection of trees for the visual assessment

a. Primary evaluation

The first step of the visual evaluation is to choose a monitoring plot to be used for a description of the trees present, which should be established according to Section 2.4.1.1 of the *Technical Manual*. The preferred plot size is 1,000 square meters (m^2), but 400 m^2 is acceptable in some cases.

All the trees belonging to the most prevalent (dominant) species that constitute the major layer of the tree stand in the monitoring plots should be evaluated.

b. Selection of trees for regular monitoring

Twenty dominant trees should be selected systematically, according to the procedures in the *Technical Manual*, as follows: five dominant trees should be randomly selected around the four compass points (North, South, East, and West) 12 meters (m) from the center of the permanent site (Figure 2.2.1). Trees sometimes have huge crowns, however, and it may be difficult to select five trees precisely at the compass points 12 m from the center, especially in tropical forests. Trees can be selected from a wider area (closer or farther from the center) in such a case. Even if it is difficult to select five trees at each compass point, at the least, two trees should be selected at each point, for a total of ten sample trees. Only predominant, dominant, and co-dominant trees (canopy classes 1, 2, and 3, as shown in Figure 2.2.2) without significant mechanical damage can qualify as sample trees.

The selected trees should be identifiable (e.g., permanently numbered) for re-assessment during subsequent inventories. <u>If trees are damaged or removed by management operations or blown over by wind later on, then new trees must be selected.</u> These newly selected trees must be labeled with new numbers that have not been previously assigned (Eichhorn et al. 2004).

<u>Note:</u>
Observations of the state of the trees should be documented by each surveyor in a special diary after first providing a general description of the monitoring plot, which should include the following basic information: location of the monitoring plot, its coordinates, height above sea level, slope steepness, any signs of fire or other impacts, forest type, tree species, species of understory vegetation, and soil type.

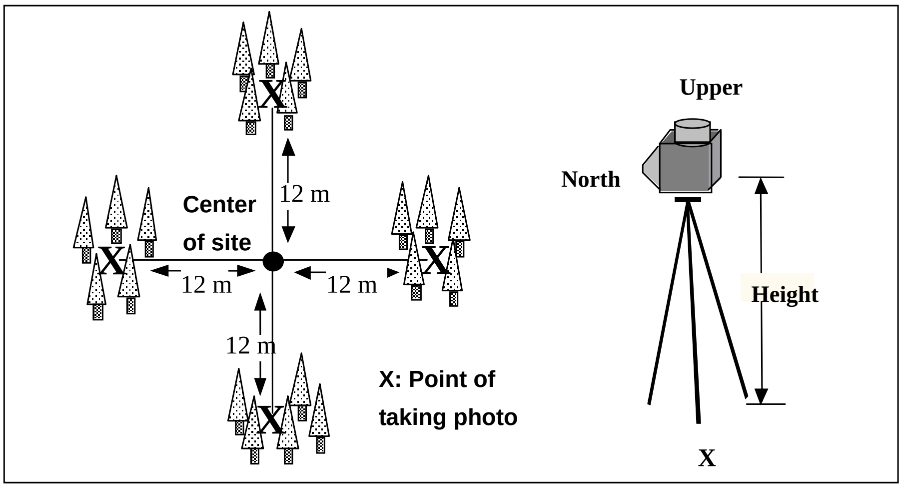

Figure 2.2.1. Selection of sample trees and position of the photographer

Figure 2.2.2. Illustration of crown canopy classes (from the *ICP Forests Manual*; Eichhorn et al. 2004).

Note: 1 = dominant; 2 = co-dominant; 3 = sub-dominant; 4 = suppressed; 5 = dying

2.1.4 Observation procedures

a. Recommended parameters for evaluating the condition of trees

To evaluate the decline class, the following items should be observed, assessed in 5% increments, and recorded according to the Decline Scale (Table 2.2) as follows:

1. Vitality of tree
2. Form of tree
3. Branch growth
4. Dieback of stem
5. Defoliation of crown
6. Deformation of leaves
7. Size of leaves
8. Discoloration of leaves
9. Injury to leaves

The most important parameters to be recorded are “defoliation of crown” and “discoloration of leaves.” For diagnostic purposes, it is also necessary to record which set of leaves is discolored, e.g., only leaves of the current year (0), leaves older than the current year (1), or all leaves regardless of age (3). The combination of defoliation and discoloration classes into a combined damage class is optional, but if chosen then the system outlined in Table 2.3 should be used.

Table 2.2. Decline scale

Items	Decline scale and observations	
Vitality of tree	0. No damage 1. Slightly damaged 2. Evidently damaged	3. Severely damaged 4. Dead
Form of tree	0. Normal 1. Some branches lacking 2. Evidently wanting	3. Severely wanting 4. Dead or almost dead
Branch growth	0. Normal growth 1. Somewhat reduced 2. Short and slender	3. Extremely short
Dieback of stem	0. Not found 1. Slight dieback 2. Evident dieback	3. Severe dieback 4. Completely dead
Defoliation of crown	0. Not defoliated (0–10%) 1. Slightly defoliated (>10–25%) 2. Moderately defoliated (>25–60%)	3. Severely defoliated (>60%) 4. Dead
Deformation of leaves	0. Normal 1. Slightly deformed 2. Evidently deformed	3. Completely deformed
Size of leaves	0. Normal 1. Somewhat small 2. Evidently small	3. Very small
Discoloration of leaves	0. None (0–10%) 1. Slight (>10–25%) 2. Moderate (>25–60%)	3. Severe (>60%)
Injury to leaves	0. No symptoms 1. Slightly injured 2. Evidently injured	3. Severely injured

Table 2.3. Damage class based combined defoliation and discoloration classes

Defoliation class	Discoloration class		
	1	2	3
	Resulting damage class		
0	**0**	**1**	**2**
1	**1**	**2**	**2**
2	**2**	**3**	**3**
3	**3**	**3**	**3**

When there is evidence of discoloration and/or defoliation, samples of branches with the symptomatic foliage should be collected carefully, followed by a hands-on or hand lens evaluation of the etiological agents (causes). Surface features of damaged leaves should also be examined.

b. Evaluation of tree crown defoliation and discoloration

Defoliation is generally estimated in 5% increments relative to a tree with full foliage. In dense stands this means that approximately the upper half of the tree crown could be assessed, and the whole tree crown in more open stands.

The reference tree could be either a healthy tree of the same species in the vicinity, a photograph representing a tree with full foliage, or the sample tree itself with imagined full foliage. If different classification schemes are used, the class intervals must be specified, i.e., the respective percentages of defoliation.

c. Assessment procedures

The following steps should be followed during the assessment:

- At least two trained observers should do the assessment using binoculars.
- Observers should have a satisfactory view of the tree from several observation points. On level ground, the optimal view is from a distance of one tree length. On slopes, trees should be observed at a distance of about one tree length above the tree or at least on the same level.
- Observers should stand at different positions from the tree and evaluate defoliation of the whole crown, and then their evaluations are brought together to form one general result to record in the field notebook. When the estimates produced by the two observers differ, both should change their observation position and re-evaluate until consensus is reached.

- Along with the level of defoliation, the observers should assess the level of discoloration, which is defined as loss of pigments or color change of leaves/needles to colorless, yellowing, or brown.
- Assessment should be done in full daylight, but it has to be recognized that the assessment, particularly of crown discoloration, may be affected by the quality of the light and the time of day.

Note:
Observers should have standard photographs of each tree species and of the different crown types representing the various defoliation classes with which to compare the trees to be assessed. Examples of various defoliation classes can also be used.

It is recommended, particularly for inexperienced surveyors, to use an atlas with photographs of trees showing different degrees of defoliation (photo guides of crown defoliation, e.g., Bosshard 1986). The development and publication of photographic documentation of tree species found in the region and the different crown types is a long-term vision of the Network, which would include developing and maintaining a database of such documentation by EANET.

d. Observation of other phenomena on leaves

When abnormal symptoms are found on leaves, a portion of branch samples containing the symptomatic foliage should be carefully collected, if necessary, and leaf condition should be described in detail in terms of the following aspects:

- Position and description of injured leaves (i.e., location in the tree crown in relation to dominant wind direction; sunny or shady; upper or bottom on crown; immature, mature, or old leaves)
- Pattern and position of the leaf injury (i.e., a spot between veins, small spots scattered entirely, discoloration from the pointed end of leaves, defoliation without discoloration, broken leaves, etc.) It is preferred that the Mansel color chart is used to match and record leaf color.
- Observation of dust on the leaves; a scanning electron microscope observation may be useful
- Existence of injured or dead trees or reduction of growth rate
- Date when the symptom(s) of damage is found

2.1.5 Frequency of assessment

The season when assessments are to be done, the number of replicates, and the frequency should be clearly indicated in the fieldbook. Assessments should be done at least once a year.

Nevertheless, as this could be quite a labor-consuming and expensive procedure, particularly in mountainous areas or territories remote from cities without proper roads, it would suffice to conduct an assessment at least once every three years.

The time of assessment should be between the end of the first flush of foliage (when the leaves and needles are fully developed) and the beginning of autumnal senescence. For most species, the most suitable time for assessment is mid to late summer. The value of assessments will be greatly increased if the assessments of particular plots are made during the same period each year (ideally, within ten days plus or minus).

2.1.6 Reporting procedures and formats

- The observation results should be verified by National Centers before reporting to the Network Center.
- The results should be reported on Form 2. Directions (N, S, W, or E), height (m), and diameter at breast height (DBH) of the respective monitored trees should also be recorded.
- The methods of assessment and the way the results are compiled must be explained in a supplementary statement. Observation of defoliation and discoloration can be reported in a combined assessment.
- In addition to reporting the results of assessments of respective monitoring plots, National Centers are also requested to submit a combined national report of all assessments, if possible. The same form should be used.
- A section of the national report should be devoted to the analysis of the possible causes of the observed damage, in particular with reference to atmospheric pollution.
- Results of any correlations between different types and stages of tree damage (defoliation, discoloration, other signs of damage) and parameters, such as site or stand characteristics, climatic data, etc., should be included in the annual report.
- Each National Center should also submit a written report annually describing the following:
  - Any deviations from the recommended procedures of assessment

- An evaluation of the observations made in the monitoring plots (e.g., unique, typical, unusual, etc.)
- A quality-control evaluation, whenever possible
- Copies of any publications arising from the work (optional)

➢ A digital copy of the report should also be sent to the Network Center.

2.1.7 Data processing

All the data on tree defoliation should be statistically processed and an arithmetic mean and its range of error calculated. The same procedure applies to the calculation of a discoloration arithmetic mean. These parameters are important for characterizing and comparing the specific monitoring plots.

2.2 Additional information for temperate and sub-arctic zones

2.2.1 Other signs of tree damage

Besides assessing defoliation and discoloration, the visual examination should focus on the crown, branches, leaves (needles), and trunk of each tree. The following peculiarities would indicate a certain negative impact: decrease in needle age; length reduction of needles and shoots; decrease in the number of pairs on individual needles; emergence of secondary needles; deformation and twisting of leaves; presence of micromicets ascocarps (fruiting bodies of a fungus)on leaves, needles, branches; presence of insect resin chambers on branches; chlorosis and necrosis of needles and leaves; signs of fire from previous years on tree stems.

2.2.2 Examples of visible damage (based on experience in Russia)

➢ An increased level of crown defoliation was observed as a symptom of negative impact, both on coniferous and deciduous tree species.

➢ In cases of observed impact caused by industrial emissions, a reduction of needle lifespan was practically always observed. For example, the natural lifespan of *Pinus sylvestris* needles in the Baikal region usually ranges from five to seven years, but in forests subjected to industrial emissions it may go down to one to two years.

➢ In cases of impacts caused by industrial emissions, insect attack, and diseases caused by fungus (micromicets), the following were observed: reduction of needle and shoot length, change of needle and leaf color, damaged leaves (i.e., presence of apertures, broken edges, necrotic sections, chlorosis spots, etc.).

- In cases of diseases caused by fungus (micromicets), needles might contain ascocarps and spores; twisted leaves, needle necroses, or underdevelopment (shortening) of the shoots might occur.
- In cases of insect attack, frequently observed effects included increased resin-release on branches and/or trunks; gnawed sections of leaves/needles, tissues on the surface of branches; new formations (galls, mines) in the needles and young shoots, as well as leaf deformation.
- After an intense incident of industrial emissions or insect attack, sometimes complete damage of needles/leaves was observed, that is, necrosis, die-off, and fall-off, with further growth of secondary needles (leaves) which were physiologically inferior, visibly smaller, and weighed less.

2.3 Additional information for tropical and sub-tropical zones

2.3.1 Basic concept

Monitoring tree crown conditions in forests is a valuable tool for detecting the status and trends of tree conditions in tropical zones. Therefore, it is essential to continue regular assessments over a long term. The basic concept of the methodology for assessing crown condition, however, was developed in temperate or boreal zones such as Europe. The structure of tropical rainforests is quite different from those of temperate/boreal forests, and it is sometimes difficult, for example, to observe crown condition from ground level in a dense tropical forest. Therefore, in the next section, additional ideas for observing the canopy are provided to supplement the basic method.

2.3.2 Structure of the forest canopy

The canopy is the combination of all leaves, twigs, and small branches in a stand of vegetation; it is the aggregate of all the tree crowns. The canopy is a region as well as a collection of objects. "Canopy structure" is the organization in space and time—including the position, extent, quantity, type, and connectivity—of the aboveground components of vegetation. It is often useful to consider the open spaces between canopy elements and the atmosphere contained within and between crowns as part of the canopy.

The proximate units of a canopy structure are the crowns of trees; the ultimate units are the leaves and twigs. As a hectare of closed canopy forest may have millions of leaves and many kilometers of twigs, however, most studies deal with these units statistically or focus on other levels of organization. Many scales of organization are evident; foliage may be clumped (often along branches and branch tips) and/or arranged in clusters, branch systems, and crownlets. Crowns are themselves sometimes grouped together.

Different forest types have different canopy structures. There is enormous diversity in canopy structure between latitudes. Tropical rainforests have greater annual leaf production, standing canopy biomass, and a higher leaf area index than other forests. Foliage-height profiles are rarely reported for tropical forest, but they are commonly presumed to be relatively uniform.

Rainforests tend to have a lower relative illuminance of visible light at the forest floor (often <1%), whereas temperate forest canopies tend to permit higher light transmittance (hardwoods in leaf, 1–3%; pines, >5%; hemlocks, <5%). Many of the bulk characteristics of temperate and tropical forest canopies—albedo, aerodynamic roughness, illuminance, interception capacity, and the behavior of stomatal conductance—may be roughly similar, however, given the reliability of the estimate. More important distinctions between forests of different latitudes arise in the interaction of structure and local microclimate. Although structures may not differ significantly, the manner by which structure affects energy partitioning, transport, and microclimate may be quite distinct.

The canopy is the primary site of interaction between the biosphere and atmosphere. The amount and spatial organization of aboveground plant parts influence both the atmospheric environment within canopies and the exchange of material and energy with the lower atmosphere. In the forest, atmospheric characteristics are strongly modified by canopy structure in two general ways. First, canopy surfaces act as passive drag elements and exchange surfaces for the absorption of wind energy, the dissipation of turbulence, and the exchange of radiation. Second, canopy surfaces actively participate in exchanges of biologically important compounds, such as carbon dioxide (CO_2) and water vapor.

Forest canopies are distinct from other forms of vegetation because they are "dense, extensive, tall, and perennial." They have more biomass, greater surface area, and lower average leaf area density. The structural complexity of forests makes them aerodynamically rougher than other forms of vegetation (except possibly woodlands).

This increases the effectiveness of daytime turbulent mixing, which in turn may reduce environmental gradients within the forest.

Forest structure is generally recognized as consisting of the amount and distribution in space of leaves, stems, twigs, and branches, but there are nearly as many distinct measures of canopy structure as there are canopy research programs. Because of the variation in attributes considered, comparisons are restricted to the most commonly measured attributes. Environmental variables are usually measured at only a few locations; spatial variation is rarely assessed.

The bulk of observations of canopy environment are average descriptions of processes and motivating structures. Quantification of structure has been inadequate, even in studies directly focused on forest-atmosphere interactions. This lack of detailed information limits our capacity to generalize about the importance of canopy structure and climate in affecting the canopy environment.

The theoretical basis of canopy-environment interactions is better developed than the observations used to test or validate them. Most current models deal with ideal cases and yield predictions about benchmark conditions. There is a need to extend these predictions to the understanding of particular environments. More empirical studies could be focused on transitional environments, transient regions such as within forest clearings (gaps), and environments that exhibit wide variation such as the outer forest canopy. The success in understanding benchmark conditions should be extended to appreciating the variation that organisms undoubtedly perceive.

Much forest canopy micrometeorology has focused on stands of simple structure, particularly single-species, single-aged forests, or crops with elevated, unimodel canopies. Studies of such situations have propelled the development of several useful descriptive and predictive models, but many forest stands are not so simple. There is a need to focus attention on the “non-ideal” canopies—mixed-species forests, forests of multiple-cohort stands, and those with partial or complete deciduous seasons (Parker 1987). Results obtained by the forest vegetation monitoring under the EANET program should help to fill these gaps. Over the years it is anticipated that a greater understanding of the dynamics of canopy structure as affected by acid deposition shall have been established as a consequence of these efforts.

2.3.3 Information on other methods to observe the canopy

a. Ground-based methods of canopy access

It is by no means necessary to climb into the canopy to conduct a canopy study. For collecting many kinds of data, climbing would be a waste of time. Ground-based methods are notably useful in studying species that are either extremely mobile or too sensitive to disturbance to be monitored from within the canopy, in gathering samples for museums or research (such as plant specimens or bird skins), or when the sampling protocol is so demanding that it is impractical to climb so often.

Some biologists actually get better information from a good ground-based vantage point. Fish-eye photographs of light flecks in the canopy can be analyzed by computer to determine the light regimes at specific understory positions. Canopy data can be gathered photographically from above the trees with the aid of balloons, ultra-light aircraft or—from still higher up—planes and satellites. With the aid of binoculars, most canopy trees can be identified quickly by an experienced eye.

Note:

Please refer to Annex 2.2, Hemispherical Photography for Assessment of Canopy Gaps and Light Penetration.

b. Climbing methods of canopy access

Many climbing techniques can be used for canopy access. The *peconha* is a technique originated by Brazilian Indians to climb the trunks of trees up to 40 centimeters in diameter. All that is required is a loop of webbing. Other direct trunk-climbing methods are avoided by responsible biologists whenever their use inflicts damage on trees (i.e., climbing spikes, tree surgeon's belt with spiked boots, tree bicycles or Swiss tree grippers, or boards with nails to create steps).

As an acceptable alternative, ladders can be lashed into place one above the other along the trunk with relatively few nails. Swiss tree grippers have been used to inchworm up trunks, although this method should only be used on hardwoods and trees with few epiphytes on the trunk so that they inflict little damage.

Climbing trees can be done without special equipment. Although the sheer physical strength required inhibits most large-bodied adults from free-climbing, native free-climbing specialists live in many parts of the tropics and work for years without injury.

A barehanded approach to tropical canopy access has serious safety drawbacks. Tropical forests can be impressively tall, and the selection of sound climbing trees is sometimes difficult except by a professional arborist or experienced researcher. Methods currently in favor today involve only the use of ropes or ladders. Platforms and walkways are of moderate cost, and they offer the additional advantage of creating a permanent structure, which can subsequently be used by many others (Moffett and Lowman 1987).

c. Canopy access by towers

Most observations of canopy lizards have been conducted from scaffold towers and a canopy walkway. Towers provide safe and easy access to the canopy except during the most severe weather conditions, but they are expensive and logistically difficult to construct.

d. Other canopy access methods

Other canopy access methods have been considered but not implemented. Among these are balloons (vulnerable to wind, difficult where there is steep topographic relief), cranes (expensive to operate, difficult to install), canopy cable networks (used in the 1960s, installation generally limited to the lower canopy), cables or fixed ladders, and cable walkways (difficult to install) (Reagan 1987).

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Form 2. Observation of tree decline

Available as an Excel file.

Form 2. Observation of tree decline

Name of plot:
Name of laboratory:
Name of reporter:

Individual No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Direction (E, W, S, or N)																				
Plant Name																				
Relative height																				
Tree height (m)																				
DBH (cm)																				
Vitality of tree																				
Form of tree																				
Branch growth																				
Dieback of stem																				
Defoliation of crown																				
Deformation of leaves																				
Size of leaves																				
Discoloration of leaves																				
Injury of leaves																				
Damage class*1																				

Estimated cause of decline:

Note: *1. Damage class will be decided based on combination of defoliation and discoloration classes

Annex 2.1 Using Image Analysis to Assess Tree Crown Condition

1. Objectives

Forest-monitoring programs, especially in Europe and North America, often include the intensive study of tree crown condition. One of the key parameters assessed in these studies is the transparency of tree crowns (the upper part of a tree formed by the branches and foliage), which is defined as the relative amount of light passing through the crown. Crown transparency, affected by needle or leaf loss (defoliation), is usually assessed visually from the ground by observers and compared to reference trees with full foliage, a process that makes assessments prone to observer error (e.g., Innes 1988). To reduce observer-caused variability, quality assurance procedures have been implemented in many countries (e.g., Ferretti 1994; Ferretti et al. 1999). In Switzerland, for example, reference photographs with various crown transparency values are used (Bosshard 1986; Müller and Stierlin 1990), and field-training courses are given annually to keep observer error low and to maintain consistency in assessment standards between inventories (Dobbertin and Brang 2001).

Repeated assessments by control observers have revealed significant differences, however, in the estimates between field teams within countries (Innes 1988; Ghosh et al. 1995; Solberg and Strand 1999; Wulff 2002). In addition, international cross-calibration courses, where the same trees are assessed by representatives from different countries, have revealed systematic differences between countries due to differing assessment methods (Innes 1993; Dobbertin et al. 1997; Ferretti 1998; Wulff 2002). These inconsistencies can lead to considerable differences in estimates of forest conditions along country borders (De Vries et al. 2000). Such observer-caused differences at national and international levels hamper accurate interpretation of the spatial patterns of crown transparency and the discovery of possible relationships between crown transparency and natural or anthropogenic stress factors (De Vries et al. 2000; Klap et al. 2000).

The discovery of such systematic differences led to studies to find methods to correct biases. Ghosh and Innes (1995) showed that combining field and control team assessments could produce unbiased estimates of the proportion of trees with a crown transparency above a fixed threshold, so long as the control team assessments were unbiased. Dobbertin et al. (1997) used data from cross-calibration courses to estimate correction factors for the differences between countries. These correction methods are limited, however, since significant differences may also exist between control observers within a country (Ghosh et al. 1995). Moreover, participants in international cross-calibration courses are not necessarily representative of the actual field teams within a country (Dobbertin et al. 1997; Ferretti 1998). These problems are also likely to increase over time because of the turnover of control observers and participants at the courses, a situation that cannot be avoided in long-term monitoring.

In order to address this problem of observer bias, Mizoue (2002) developed a semi-automatic image analysis system, called CROCO (which stands for crown condition), to assess tree crown transparency using photographs. The objective of using an image analysis system like CROCO is to provide reliable control assessments by which observer bias in assessing tree crown condition can be detected and corrected (theory and procedures are described below). It should be noted that image analysis of individual trees has a clear limitation in some applications due to poor visibility of the tree crown from the ground in many cases. This means that image analysis cannot be applied in all situations. Collecting and analyzing crown images of trees with good visibility, however, is helpful as a quality assurance and quality control (QA/QC) activity of assessments of tree crown condition.

2. Basic theory of CROCO

CROCO calculates a measure of crown transparency based on the dilation method, as follows: DSO = Ds – Do, where Ds and Do are the fractal dimensions of the silhouette of a tree crown and its outline, respectively (Mizoue 2001).

Transparency scores can be estimated from DSO values once a calibration curve between transparency scores and DSO values is established. It has been documented that DSO values decreased exponentially with increasing crown transparency scores when the Swiss (Bosshard 1986) and British (Innes 1990) photographic guides were used as references for visual crown transparency assessments (Mizoue 2001; Mizoue and Masutani 2003).

3. Equipment

The CROCO program is available for free in both Macintosh or Windows versions by contacting Nobuya Mizoue at Kyushu University, Japan (e-mail:

mizoue@ffp.kyushu-u.ac.jp). For the Macintosh version, NIH Image, a public domain software, is also needed (available at http://rsb.info.nih.gov/nih-image/). For the Windows version, free software called Scion Image is required (available at http://www.scioncorp.com/). As well, the widely used image software Adobe Photoshop or another similar type is required for use with both NIH Image and Scion Image, and a digital camera with a resolution of more than 300 dots per inch (DPI) should be used.

4. Procedures

Image processing involves four steps: (a) image acquisition, (b) pre-processing, (c) thresholding, and then (d) calculation of DSO and crown transparency (Mizoue 2002).

a. Image acquisition

The camera angle (CA) should be less than about 45 degrees (Figure A2-1-1) and the overlap with adjacent trees should be less than 50% of crown width (Figure A2-1-2) (Mizoue 2002). The leaves/needles of some species are sometimes very bright under sunny conditions, in which case photographing should be done under cloudy conditions, just after sunrise, or just before sunset to facilitate the thresholding procedure.

b. Pre-processing

Adobe Photoshop or a similar type of image software is used for pre-processing the original color images. First, a rectangular region of interest (ROI), where the crown of interest is enclosed but the parts overlapping the neighboring trees are excluded, should be selected using the computer mouse (Figure A2-1-2 and A2-1-3a), and all areas other than the ROI should be cropped out (Figure A2-1-3b) (Mizoue 2002; Mizoue et al. 2004). At the same time, the overlap rate with other trees should be classified using a system of eight classes (no overlap, 25%, 50%, 75%, or 100% overlap on one side of the crown, and 25%, 50%, or 75% overlap on both sides) (Figure A2-1-4).

To facilitate processing, the image should be re-scaled so that the larger side of the rectangle is 500 pixels. If there are other objects such as branch tips of neighboring trees within the ROI, these should be selected using the computer mouse and then deleted (Figure A2-1-3c). Then the image file needs to be converted from JPEG to TIFF format, since neither NIH Image nor Scion Image, used in the next steps, can deal with JPEG images. This re-scaling and converting of file format can be performed automatically for a series of images in a folder using Adobe Photoshop's batch-processing function. Last, the file names of the series of images should be changed so that they are composed of a common prefix and a suffix of a numerical sequence (i.e., photo1, photo2, photo3, etc.), since such file names are required for batch processing in the next steps.

c. Thresholding

The automatic thresholding algorithm based on between-class variance can be applied well to the blue-filtered gray images of tree crowns (Mizoue and Inoue 2001). After loading the CROCO program using the "Special" menu of NIH Image or Scion Image, the macro "Automatic Threshold" (AT) can be selected in the Special menu. AT subsequently implements automatic thresholding on the series of color images, and the original color images are converted to black-and-white (Figure A2-1-5). AT first displays dialog boxes. Once a common file name is entered at "File Name?" and the total number of image files with a common prefix are in a folder, silhouettes are automatically generated from the original color images. The converted silhouette images are saved with file names starting with "bin-" plus the common prefix chosen plus a numerical suffix (i.e., bin-photo1).

Since automatic thresholding may occasionally produce a clearly bad silhouette when foliage is brightly reflective (Mizoue and Inoue 2001), in such cases the bright parts have to be manually painted in with a dark color using the Photoshop software, and then the AT macro needs to be run again.

d. Calculation of DSO and crown transparency

After loading the CROCO program, the macro "Dilation" can be selected under the Special menu. It automatically calculates the DSO index as a measure of crown transparency from the silhouette generated by the AT function. Once a common prefix is entered (i.e., bin-photo) at the "File Name?" prompt and a number of files with a common prefix are selected, Dilation subsequently processes a series of silhouette images and displays the DSO results in a text file.

Exclusion of the outer parts of crowns that overlap with other trees results in a positive bias of the DSO values (underestimation of crown transparency), since the outer part of the two-dimensional silhouette of a crown is generally more transparent. Thus, the positive bias in DSO values for trees displaying overlap should be removed from the overlap class data and the linear regression, shown in Table A2-1-1 (Mizoue 2002). Samples classified as having a 75% overlap rate on both sides of the crown should be discarded for this study, however, since accurate corrections are not possible at such high rates of overlap (Mizoue 2002).

It is known that the DSO results of Swiss (Bosshard 1986) and British (Innes 1990) reference photographs decreased curvilinearly with increasing crown transparency and that the exponential function provided a good fit for this relationship (Mizoue 2001). In this case, crown transparency (CT) for a given tree can be estimated using the following calibration curve, where *a* and *b* are parameters fitted for each tree species (Mizoue and Dobbertin 2003):

$$CT = a \ln(DSO) + b$$

Figure A2-1-6 shows an example of this relationship for eight species in Switzerland (Mizoue and Dobbertin 2004). It is recommended that each assessment group using the same photographic references should make a reference curve using more than 20 sample trees with a wide range of crown transparency for each species.

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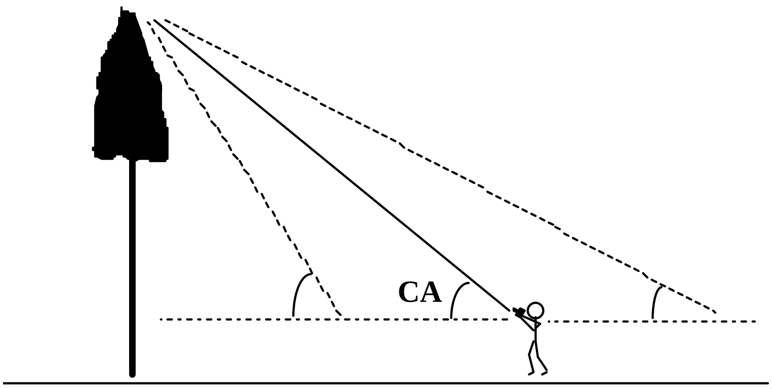

Figure A2-1-1. Example of recommended 45-degree camera angle (CA) when photographing a tree crown (Mizoue 2002)

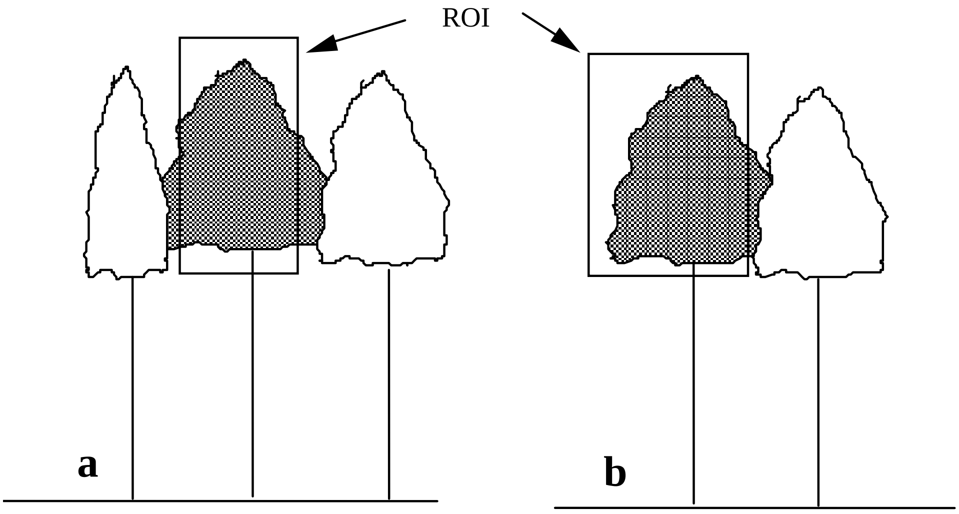

Figure A2-1-2. Crown overlapping with other trees on both sides (a) and one side (b). The region of interest (ROI) should be selected so as to exclude the overlapping portions (Mizoue 2002).

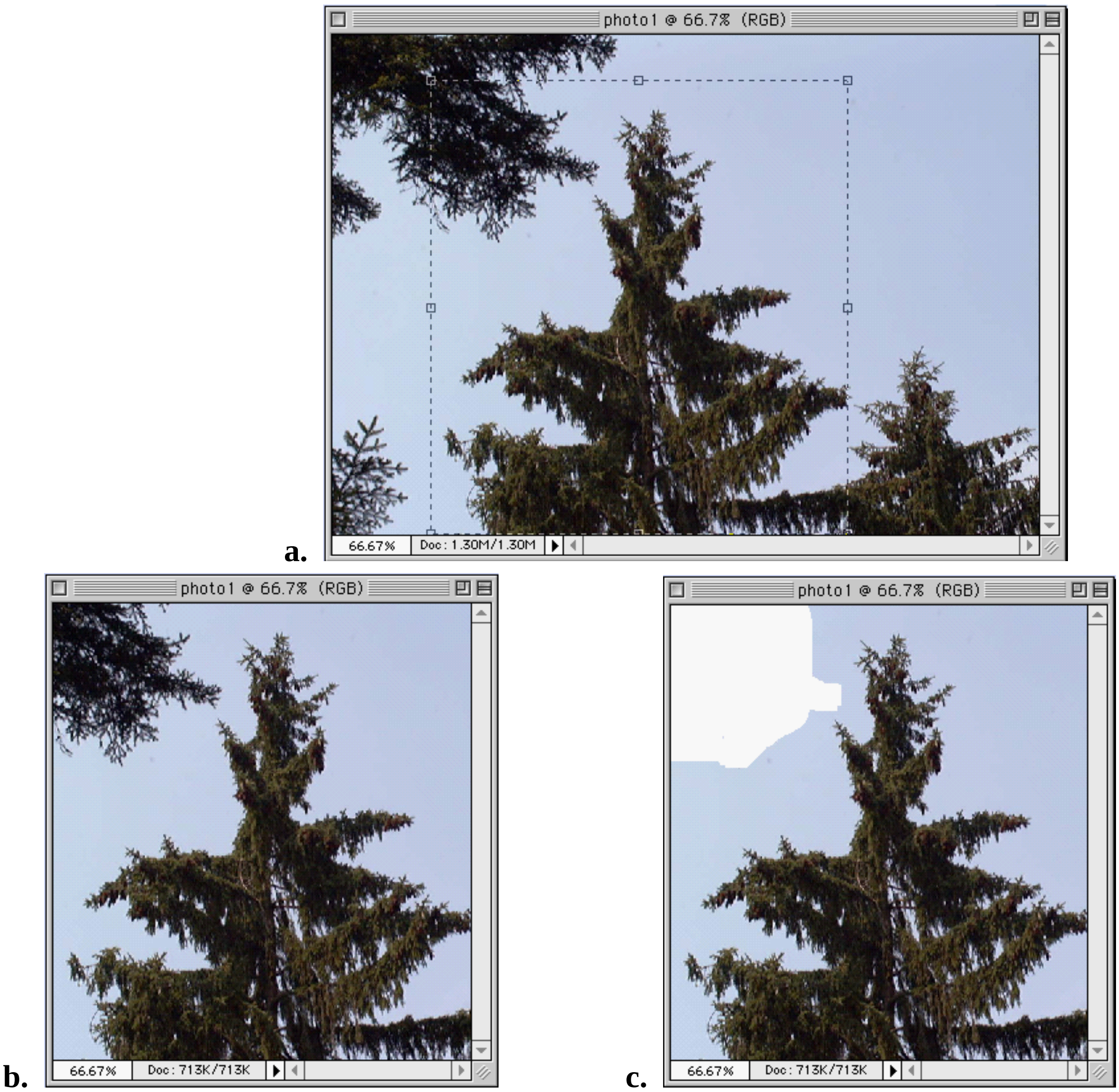

Figure A2-1-3. Steps in pre-processing an image using Adobe Photoshop: (a) select a region of interest (ROI), (b) crop away areas other than the ROI, and (c) erase other objects (Mizoue 2002)

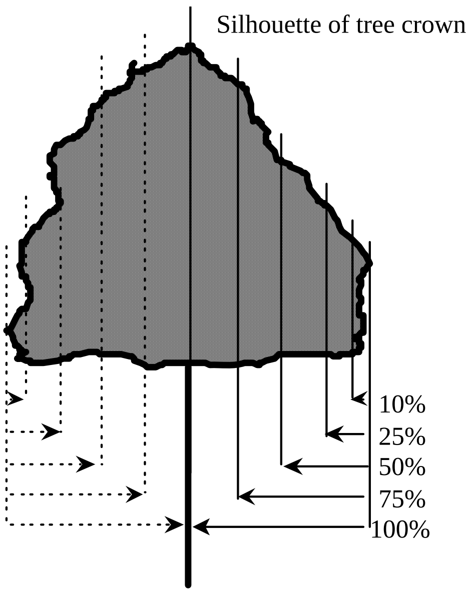

Figure A2-1-4. Classification of overlap rate (Mizoue 2002)

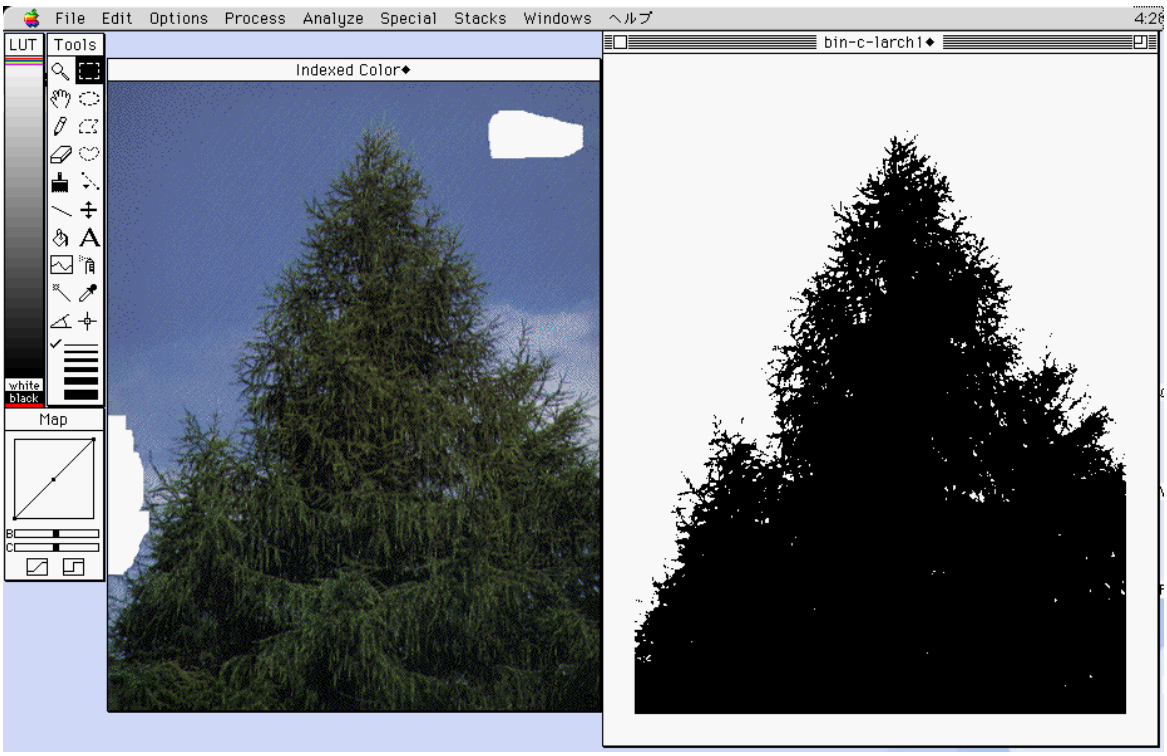

Figure A2-1-5. Example of implementing automatic thresholding (Mizoue 2002)

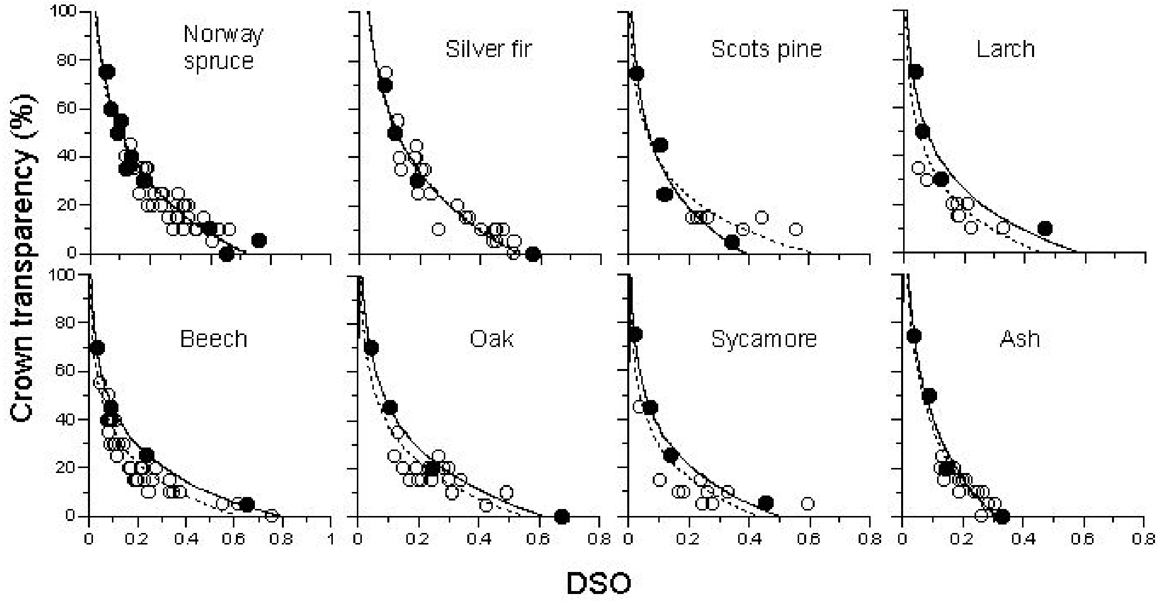

Figure A2-1-6. Calibration curves between DSO and tree crown transparency for various species

Note: Solid lines based only on the Swiss reference photographs (black circles); dotted lines are based on combined data from the first three sessions of the Swiss field-training courses (white circles) and the reference photographs (Mizoue and Dobbertin 2004).

Table A2-1-1. Parameters of regression lines of DSO from the entire crown (DSOn) against one from increased overlap rate (DSO_i)[DSO_n= a DSO_i+b] (Mizoue 2002)

Overlap rate (%)	Parameter a	Parameter b	R^2
Both sides			
25	0.8828	0.0088	0.9947
50	0.8091	–0.0110	0.9878
75	0.7334	–0.0583	0.8883
One side			
25	0.9465	0.0035	0.9984
50	0.9256	–0.0070	0.9968
75	0.9344	–0.0255	0.9876
100	0.9770	–0.0039	0.9718

Annex 2.2 Using hemispherical photography to assess canopy gaps and light penetration

1. Introduction

The light environment beneath a forest canopy is essential to the emergence, establishment, survival, and growth of understory trees and plants (Gray and Spies 1996; Lieffers et al. 1999; Nicotra et al. 1999). This is mainly regulated by the spatial and temporal distribution of tree leaf area within the forest, which is the crucial component in studies of regional and global phenomena such as acid rain and global warming (Running et al. 1989).

Since the light environment beneath a forest canopy also has spatially and temporally intensive variations (Rich et al. 1993; Nicotra et al. 1999; Mizunaga 2000; Kato and Komiyama 2002), it is important for forest scientists and ecologists to develop a simple, rapid, and inexpensive method to measure and/or estimate the canopy gaps and light penetration within a forest. Light sensors would directly provide the most accurate measurement at a specific point and time, but they are expensive and have a high maintenance cost (Barrie et al. 1990; Rich 1990; Jennings et al. 1999). The sensors would therefore be impractical for use in spatially and temporally intensive studies, so finding alternative methods is necessary (Machado and Reich 1999; Englund et al. 2000; Hale and Edwards 2002).

Hemispherical photography is an indirect technique for estimating canopy gaps and light penetration, which was first used for studying forests by Evans and Coombe (1959) and Anderson (1964), and its usefulness has been demonstrated in many studies (e.g., Chazdon and Field 1987; Barrie et al. 1990; Rich et al. 1993; Clearwater et al. 1999; Machado and Reich 1999; Englund et al. 2000; Frazer et al. 2001; Hale 2001, 2003; Hale and Edwards 2002; Inoue et al. 2002). The technique is widely used nowadays (recently, by Bellow and Nair 2003; Courbaud et al. 2003; Dignan and Bren 2003; Halverson et al. 2003). Furthermore, an inexpensive digital camera that can be equipped with an exclusive fish-eye lens has recently become available. Digital hemispherical photography should be useful in spatially and temporally intensive studies, since savings can be made on the cost, time, and labor usually involved in film processing and image scanning. For this reason, it is useful in estimating the light environment beneath a forest canopy (Englund et al. 2000; Frazer et al. 2001; Hale and Edwards 2002; Inoue et al. 2002).

Next, the three measures of canopy cover, canopy openness, and leaf area index, the typical index concerning canopy gaps and light penetration, are explained through the analysis of hemispherical photographs taken with a digital camera.

2. Principle

Hemispherical canopy photography is a technique for studying plant canopies via photographs acquired through a fisheye lens oriented towards the zenith (the point in the sky directly overhead) from beneath the canopy (Figure A2-2-1). A hemispherical photograph provides a permanent record and is therefore a valuable information source on the position, size, density, and distribution of canopy gaps (Jonckheere et al 2004). Hemispherical photographs generally provide a 180-degree field of view and a projection of a hemisphere onto a plane (Rich 1990). The lens geometry of most types of common and currently available hemispherical lens is known as the equi-distance or equi-solid angle projection (Herbert 1986; Frazer et al. 1997). In a perfect equi-distance or equi-solid angle projection of a 180-degree field of view, the resulting circular image shows a complete view of all sky directions, with the zenith in the center of the image and the horizons at the edges. Hemispherical photographs can be analyzed by hand or using automated digital image analysis to precisely measure the geometry and its implications. Once properly classified (i.e., by separating the pixels within an image into canopy cover and sky pixels) (Figure A2-2-2), hemispherical photographs literally provide a detailed map of sky visibility and obstruction.

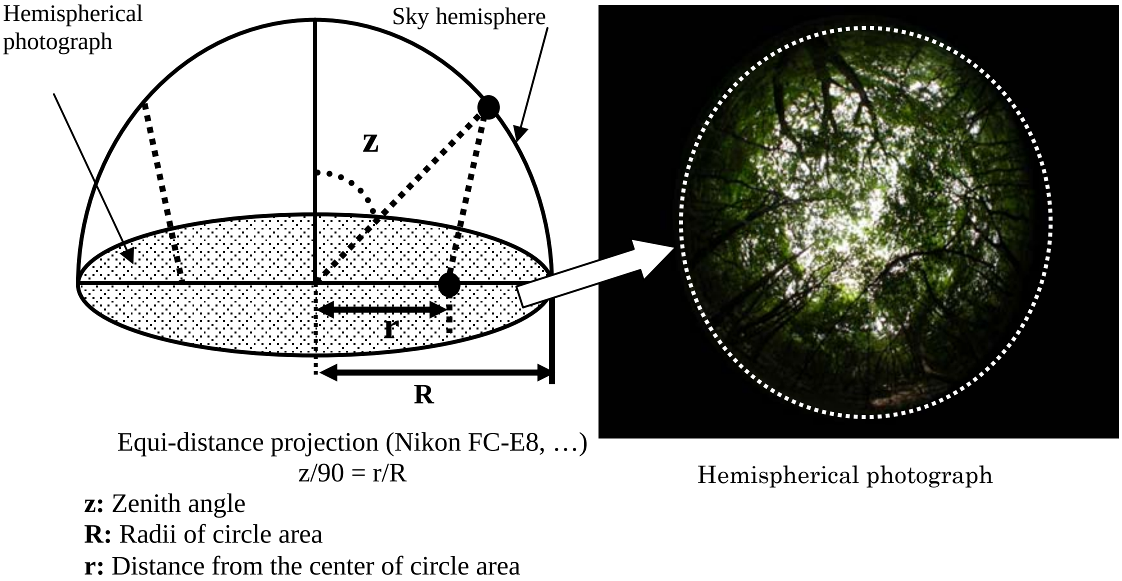

Figure A2-2-1. Principle of using a fisheye lens to produce a hemispherical photograph

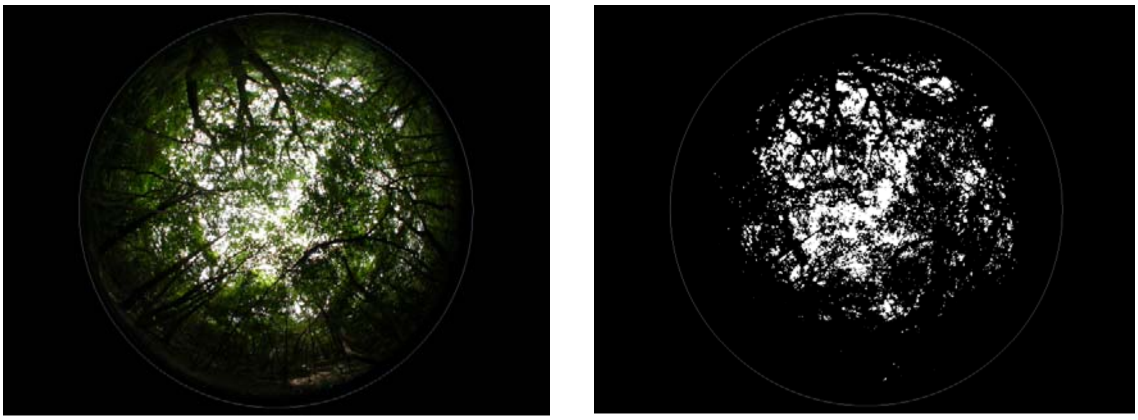

Figure A2-2-2. A hemispherical photograph (left) and the classified image (right), where black and white pixels, respectively, are considered as the canopy cover and the sky above. Classification is usually performed through binarization.

In turn, solar radiation regimes and canopy characteristics can be inferred from this map of sky geometry. In the case of plant canopies, a hemispherical photograph can be interpreted as a map of the directions of canopy openings relative to the location from which the photograph is taken. It can be inspected to provide insight into heterogeneity within a given canopy and to compare canopies at different sites (Jonckheere et al. 2004).

a. Canopy cover and canopy openness

The projection of the sky hemisphere can be thought to consist of 89 concentric rings, dividing the main radius into 89 parts (Figure A2-2-1 and A2-2-3). Each ring corresponds to a circular sphere segment in the sky hemisphere with an arc of one degree. The area of all segments is different and will be smaller on the segment nearer to the sky zenith. To obtain canopy cover from a hemispherical photograph, we can calculate cover for each of the 89 rings, but have to correct for the actual area of that segment on the sky hemisphere. The area on a sphere segment, defined by upper angle z_i and lower angle z_{i+1} (i = 0,...,89), is given by the following equation:

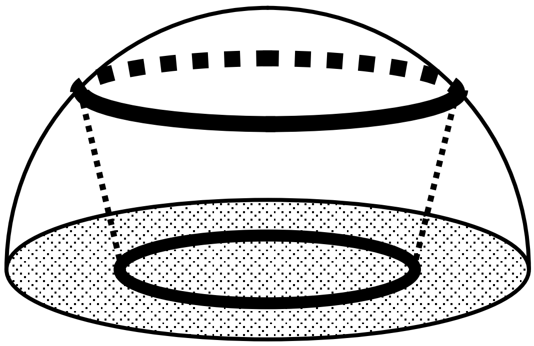

Figure A2-2-3. Concentric rings on hemisphere and hemispherical photograph

$$A_{z_i - z_{i+1}} = 2\pi R^2 \left(\cos z_i - \cos z_{i+1}\right)$$

In the forest this relation can be used to estimate the approximate canopy cover in gaps. Thus, to obtain the total canopy cover (*Cv*) and canopy openness (*Co*) of a site we have to obtain the sum of the cover fractions ($Tc_{z_i - z_{i+1}}$) and the gap fractions ($Tg_{z_i - z_{i+1}}$) per concentric ring multiplied by their part in the sky fraction, as follows:

$$Cv = \sum_{i=0}^{89} Tc_{z_i - z_{i+1}} \frac{A_{z_i - z_{i+1}}}{2\pi R}$$
$$Co = \sum_{i=0}^{89} Tg_{z_i - z_{i+1}} \frac{A_{z_i - z_{i+1}}}{2\pi R}$$
$$Cv = 1 - Co$$

In practice, $Tc_{z_i - z_{i+1}}$ and $Tg_{z_i - z_{i+1}}$ are obtained using the following formulae:

$$Tc_{z_i - z_{i+1}} = \frac{Nc_{z_i - z_{i+1}}}{N_{z_i - z_{i+1}}},$$
$$Tg_{z_i - z_{i+1}} = \frac{Ng_{z_i - z_{i+1}}}{N_{z_i - z_{i+1}}}$$

where $N_{z_i - z_{i+1}}$, $Nc_{z_i - z_{i+1}}$, and $Ng_{z_i - z_{i+1}}$ are the numbers representing the total, canopy cover, and sky pixels, respectively, within a concentric ring of a hemispherical photograph.

b. Leaf area index

The leaf area index (LAI) is directly related to forest productivity, specifically photosynthesis, because of its influence on stomatal area and absorbed, photosynthetically active radiation. LAI is therefore a key state variable of some well-known process models of forest growth that provide tools for studying the effects of nutrient cycling, water balance, and management practices on forest ecosystem function (Pocewicz et al. 2004).

The LAI of a plant is defined as the amount of leaf area per unit of ground area. Since this is difficult to measure directly, many indirect measurement methods to estimate LAI have been developed and are mainly based upon the determination of gap fractions in the foliage (Steege 1993). Light has a chance to be intercepted by leaves as it passes through the vegetation. The chance of being intercepted depends on the path length through the vegetation, the foliage density, and foliage orientation. With the assumptions that leaves are small, randomly distributed, have no azimuthal preference (horizontal component of a direction), and do not transmit light, the gap fraction in the zenithal view angle z can be related to LAI.

On the analysis of a hemispherical photograph, a method described by Welles and Norman (1991), which is used by the LI-2000 Plant Canopy Analyzer, is implemented in scientific image processing software (e.g., HEMIPHOT, GLA, etc.). In the bands of 15 degrees around the five viewing angles (7, 23, 38, 53, 68) as that of the Plant Canopy Analyzer, the gap fraction (openness, Tg) is calculated using similar methods as calculating canopy openness, and total LAI is then calculated as

$$LAI = 2\sum_{z=7}^{z=58}\left[-\ln(Tg)\frac{W_z}{S_z}\right]$$

where z takes the five values mentioned above, W_z are weights to account for area correction, and S_z are the reciprocal path length corrections, $\frac{1}{\cos z}$. For restrictions using this method, see Welles and Norman (1991).

3. Equipment

The equipment needed to take hemispherical photographs and some manufacturers are listed in Table A2-2-1 and shown in Figure A2-2-4.

Table A2-2-1. Example of equipment used in hemispherical photography

Equipment	Manufacturer	Name
1. Lens	Nikon	Fisheye Converter (FC-E8)
2. Digital camera	Nikon	Coolpix 4500
3. Spirit (bubble) level	Hakuba	KPA-04RT
4. Camera platform	MANFROTTO	Magnesium 3D Head (460Mg)
5. Tripod	MANFROTTO	PRO Tripod W/O Head (055PRO)

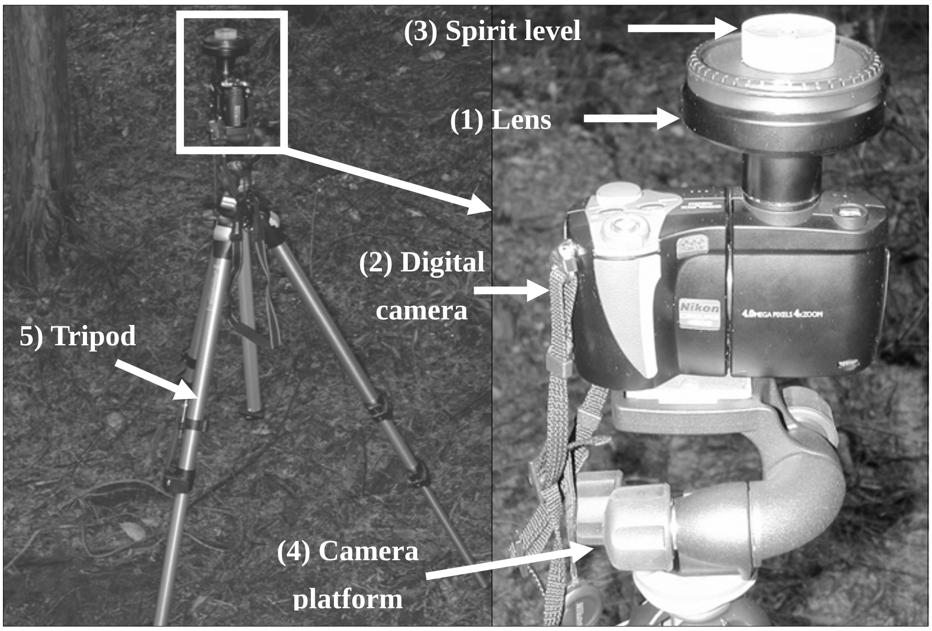

Figure A2-2-4. Overview of equipment

a. Fisheye lens

Finding a fisheye lens for a film camera is getting difficult these days. For example, one of the most popular types of fisheye lens for film cameras was Nikon's 8-mm fisheye lens, but it is no longer being produced. Nikon's fisheye converter (FC-E8), listed in the table above, is an accessory for Nikon's digital camera (but not for all cameras). It has a 180-degree field of view with perfect equi-distance projection, and is currently available at a relatively low price. Some researchers (Englund et al. 2000; Frazer et al., 2001; Hale and Edwards 2002; Inoue et al. 2002) have tested it and compared it with a fisheye lens for a film camera in terms of their respective analytical features. Based on these comparisons, this product is recommended, because the software used for analyzing hemispherical photographs supports those taken with the lens with an equi-distance projection. (Other products can be used as long as they have a 180-degree field of view with perfect equi-distance projection.)

b. Digital camera

The product listed in the above table is just an example. If the purpose is only to estimate canopy gaps and light penetration, the resolution of the camera is not important (Inoue et al. 2004). Any camera that is compatible with the fisheye lens mentioned above can be used.

c. Spirit (bubble) level

The product listed in the table above is just an example. Any product that does the same job can be used.

d. Camera platform

For studying plant canopies using photographs, the digital camera fitted with a fisheye lens must be correctly oriented towards the zenith from beneath the canopy, which requires careful preparation and set-up. There is a product that automatically performs this process (e.g., the Self Leveling Mount from Delta-T Devices Ltd.), but it is very expensive. The product listed in the table is much cheaper and (based on experience) will still save time and labor.

e. Tripod

The product listed in the table is also just an example. A strong but lightweight tripod is best, since it needs to be transported and used in the forest. In addition, since the estimates obtained through the analysis of hemispherical photographs is that for the portion above the height at which the photograph is taken, the maximum height that the tripod elevates the camera should depend on the state of the forest to be studied. If the height of the forest understory is one meter, the photograph should be taken at a height higher than one meter above the ground.

4. Procedures

a. Number of measurements

As suggested by Weiss et al. (2004), if the spatial domain to be represented is a large area, then the local measurements could be extended to the whole area, thanks to remote-sensing measurements (Baret et al. 2003; Cohen et al. 2003; Chen 1999; Tian et al. 2002) or the use of geostatistical techniques such as kriging (a regression technique used to approximate or interpolate data) (Goovaerts 1997). The researchers also reviewed the number of measurements on spatial domains corresponding to a few tens of meters where only one canopy type was represented, as follows:

"The number of individual measurements could be reduced to 10 per ESU (elementary sampling unit) for both wheat and pine canopies. This is in good agreement with the methodology described earlier where between 5 and 15 individual measurements were taken. More recently Leblanc et al. (2002) proposed using only four hemispherical photographs to get a good sampling of temperate and boreal forests."

Considering the recommendations of previous reports, between five and ten individual measurements where only one canopy type is represented would be required on spatial domains corresponding to a few tens of meters. To choose the locations where to take the photographs from, a line or grid transect should first be set in the spatial domain. After that the photographs should be taken at nearly constant intervals on the line transect or at the intersecting point of the grid transect.

b. Conditions required for the best measurements

When taking hemispherical photographs in the field, the basic goal is to obtain the best photographs suitable for analysis. Although a high-contrast image with even sky lighting is best, this is difficult to do and is the primary weakness of this technique. In practice, it is best to take photographs under conditions with even backlighting, in particular just before dawn or just after sunset, and <u>on days with evenly overcast skies</u>. Under the light of a clear day, patches of light usually appear in a hemispherical photograph, which presents difficulties for analysis because it is usually classified as sky by the image processing software (Figure A2-2-5).

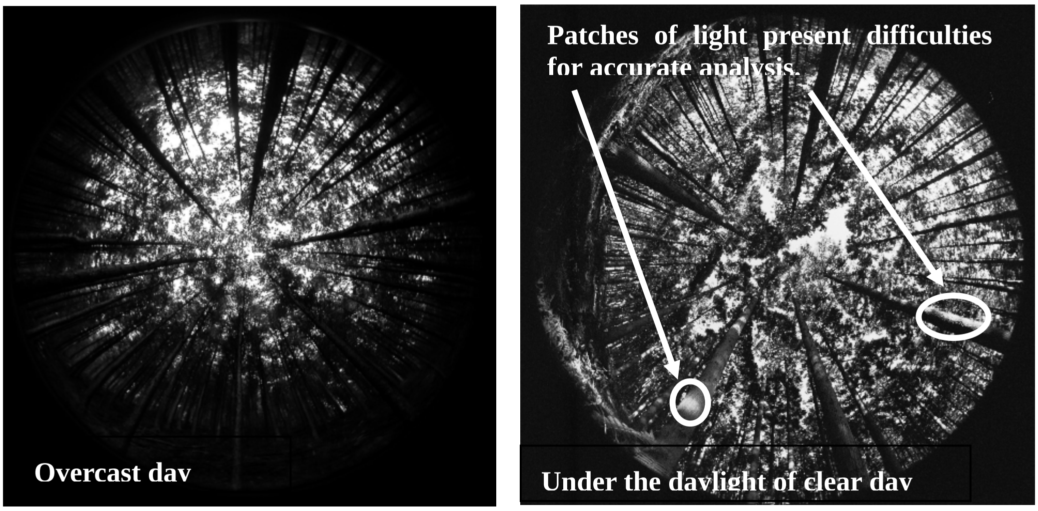

Figure A2-2-5. Photographs taken under different light conditions

c. Measurement steps

Before conducting the following process, the height above ground for taking the photographs must first be decided, which should be greater than that of the forest floor, as mentioned above. Note that a fixed height is desirable for the analysis. In previous studies, a height of one meter or 1.3 meters (diameter at breast height) is usually used when the forest understory is not higher than that.

1. Attach the digital camera to the tripod.
2. Place a spirit level on the lens cap and set the tripod so that the surface of the lens cap is level. (The lens cap of the FC-E8 is only placed on the lens. Be careful when handling.)
3. Turn on the digital camera.
4. Check whether the image on the camera monitor is a 180-degree field of view. If not, zoom out.
5. Take the photographs.
6. Mark the spot where the tripod is positioned with a stake for the next time measurements are taken.

d. Field note on data processing

In the field, recording the following information is required for the subsequent data processing. At the least, recording the five components of date, name of location, point number, forest cover type, and the number of photographs is required to match information about the photography point with the image taken. Other information (i.e., longitude and latitude) can be obtained from the map.

Date	Location name	Point number	Longitude	Latitude	Forest cover type	Number of photographs
:	:	:	:	:	:	:

e. Manpower

Based on experience, if a global positioning system (GPS) is available, only one researcher is required for taking the measurements in the field. If a GPS is not available, then at least two researchers are required.

5. Reporting procedures and formats

As mentioned above, a hemispherical photograph provides a valuable record of the state of a forest when the photograph was taken, not just the results of the analysis (Jonckheere et al. 2004). Therefore, after taking the photographs, it is recommended that a document also be created with the following information, as follows:

File name	Photography date	Location name	Point number	Longitude	Latitude	Forest cover type
:	:	:	:	:	:	:

The openness of the canopy and the LAI obtained from the analysis of a hemispherical photograph are only two types of information that can be obtained within the forest where the photograph was taken. Therefore, the results obtained from the analysis of hemispherical photographs should be averaged on spatial domains corresponding to a few tens of meters within which only one canopy type is represented. Thus, the reporting format should be as follows:

Photography date	Location name	Longitude	Latitude	Forest cover type	Averaged LAI	Averaged canopy cover
:	:	:	:			:

In this format, the longitude and latitude recorded should be at the center or representative point of spatial domains where the hemispherical photographs were averaged. The values of LAI and canopy cover for averaging are obtained from those of LAI (Plant Canopy Analyzer) and cover (%), shown in List A2-2-1 .

6. Data processing

a. Software

LIA for Win32 (LIA32), which is freeware (not free software) that anyone can freely use and copy, is available online at http://hp.vector.co.jp/authors/VA008416/index-e.html (Figure A2-2-6).

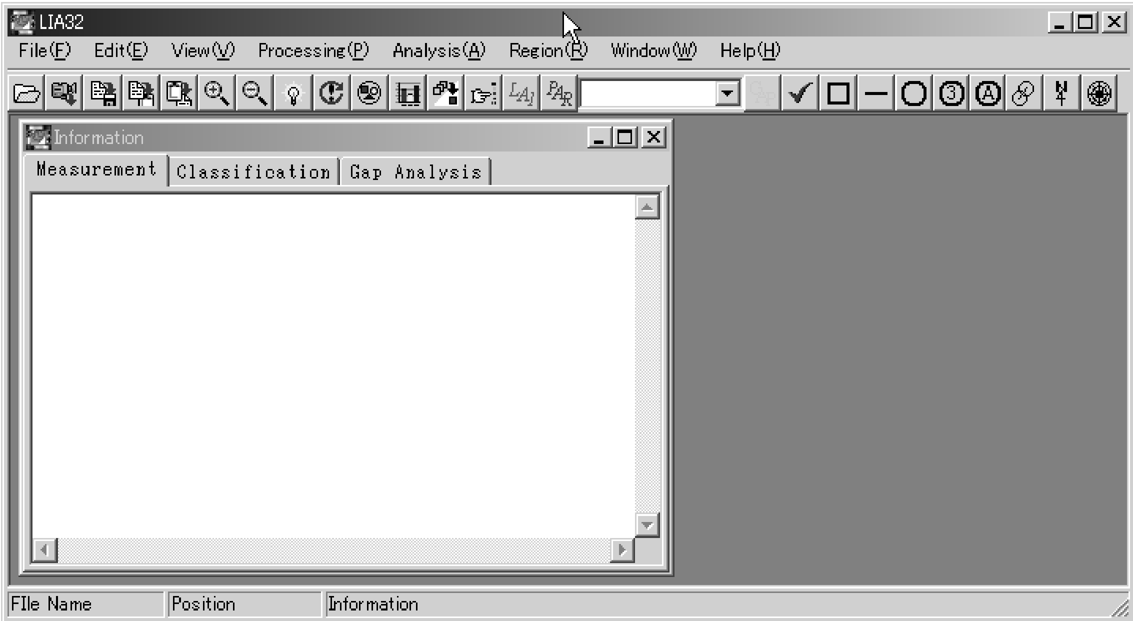

Figure A2-2-6. Screen capture of the main menu of the LIA32 software

- System requirements
  - Operating system: Microsoft Windows Me/2000 or later version
  - Memory (RAM): A minimum of 64MB RAM is required, but larger images require more RAM.
  - Storage (hard disk): Minimum of 1.5 MB of free space
  - Video display: Minimum is 24-bit true color depth with a display adapter for hardware acceleration
- Installing or removing LIA32

- LIA32 does not have an installer, so first unzip all the files in the downloaded file into the same folder. To remove LIA32, just delete the folder. If you want to use an image format other than bitmap (*.BMP), you must download and install the Susie plug-in (image viewer) for the image format you want to use (e.g., JPEG, TIFF, ...). Please decompress the *.SPI

file within the archive file of the plug-in into the same folder as LIA32. The official Susie Plug-in Library is available at
http://www.agr.nagoya-u.ac.jp/%7Eshinkan/LIA32/index-e.html
or http://homepage3.nifty.com/~takechin/archives/spi32008.lzh
(JPEG /GIF /TIFF /Pi/ PIC/ Pic2/ XLD4/ MAG/PICT/ LHA/Zip).

In addition, the latest official plug-in for JPEG format, required for digital camera images, is available at
http://homepage3.nifty.com/~takechin/archives/ifjpg033.lzh.

Since these files are compressed, unzip them first to access the plug-in files (*.SPI). Although the format (LZH) of these files may not be familiar outside Japan, the software available to unpack files in the LZH format, Archive Converter Ver.0.31, is available at
http://www.ponsoftware.jp/archiver/explzh/arcc0310.exe.

b. Steps for analyzing a hemispherical photograph

Before analyzing, the hemispherical image taken by the digital camera must be downloaded to a computer. Once this is done, launch LIA32 and carry out the following steps:

1. Under the main menu, select [File], [Open], and then choose the image.
2. Select [Region], [Hemispherical Image], [Set Area], [Automatic], and then an automatically selected circle area is drawn on the image. If the selected circle area does not fit in the circle area within the image, then go back and select [Region], [Hemispherical Image], [Set Area], [From Three Points], and then click at three points on the edge of the circle area of the image.
3. Select [Analysis], [Options], and then set up each option (binarization and fish-eye lens type settings) (Figure A2-2-7). In LAI estimation, the binarization setting is usually selected as greater or equal (>=), since it is usually brighter than other portions in the leaf gap, and the luminosity value is large.
4. Select [Analysis], [LAI Measurement], and then the results are output into the information window, as shown in List A2-2-1.

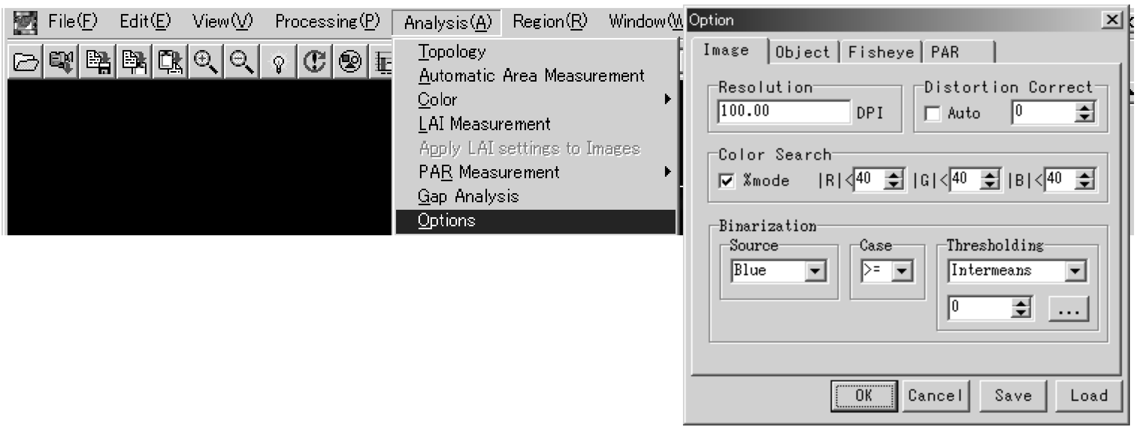

Figure A2-2-7. Example of the Analysis menu in LIA32

Estimated by openness using intermeans cover (%): 87.417

Sky factor: 21.180

Zenith angle	Total pixel	Sky pixel	openness
7(0 - 14)	64348	37507	0.583
22(15 - 29)	189184	74366	0.393
38(30 - 44)	303668	60799	0.200
52(45 - 59)	403396	49911	0.124
68(60 - 74)	486556	18112	0.037

LAI (PCA): 2.362
LAI (N&C): 2.321
V/H: 0.559
MLA: 70.262

List A2-2-1. Example of analysis results generated by LIA32

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3. Basic Survey: Optional Items

3.1 Estimating the concentration and deposition of air pollutants in forest areas

Measuring the concentration and amount of deposition of air pollutants in forest areas is an effective way to evaluate the actual or potential effects of acid deposition on trees, but most EANET wet and dry deposition monitoring sites have so far been established outside forest areas and are separate from soil and forest monitoring sites. This subchapter describes methodologies for estimating the air concentration of gaseous pollutants as well as the amount of acid deposition in forests areas. The resulting data will be useful for discussion of the connections between acid deposition/air pollution and symptoms of tree decline, and their implications.

3.1.1 Estimating concentrations of air pollutants in forest areas

1) Basic concept

The direct effects of air pollution on trees, such as visible damage caused by ozone (O_3), for example, have been reported in Europe. Since deposition amounts cannot be estimated using other approaches alone such as measuring throughfall (precipitation passing through the tree canopy) or stemflow (precipitation flowing down tree branches and stems), estimating O_3 concentration in forest areas may be very important for risk assessments relating to O_3. Moreover, the measurement of other pollutants, such as sulfur dioxide (SO_2), nitrous oxide (NO_X) (nitrogen dioxide [NO_2] and/or nitrogen oxide [NO]), and ammonia (NH_3), will also be useful for discussion of the possible effects of air pollution on forests. The use of passive samplers is a simple, low-cost way to monitor forest areas, and collecting air concentration measurements by passive sampling can be used to pre-screen areas to be intensively monitored.

2) Equipment/apparatuses/instruments

a) Passive samplers

Several types of passive sampler are used in Europe, Africa, Australia, East Asia, and the United States. The ones profiled in Table 3-1-1-1 are from the IVL Swedish Environment Research Institute (IVL) (e.g., Ferm and Svanberg 1998; Ayers et al. 1998; Carmichael et al. 2003) and Ogawa & Company USA, Inc. (e.g., Saito et al. 1997; Bytnerowicz et al. 2004). (See Annex 1 for a description of the structural components of these two samplers.)

Table 3-1-1-1. Overview of passive samplers from Ogawa and IVL

Compound	Reagents for filters	Analytical method	Supplier
SO_2	NaOH	SO_4^{2-} by ion chromatograph (IC)	IVL
	10% TEA		Ogawa
NO_2	NaOH + NaI	Colorimetry	IVL
	10% TEA		Ogawa
NO_X	10% TEA + PTIO	Colorimetry	Ogawa
NH_3	Citric acid	NH_4^+ by IC	IVL/Ogawa
O_3	$NONO_2 + K_2CO_3$	NO_3^- by IC (O₃ will oxidize NO_2)	IVL
	$Na_2NO_2 + K_2CO_3$		Ogawa

Note: NaOH = sodium hydroxide; TEA = triethanolamine; PTIO = 2-phenyl 1-4, 4, 5, 5-tetramethlimidazoline 3-oxide 1-oxyl; K_2CO_3 = potassium carbonate; SO_4^{2-} = sulfate anion; NH_4^+ = ammonium ion; NO_3^- = nitrate

Filters impregnated with reagents are placed in the passive sampler. Then, based on the property of molecular diffusion, gaseous pollutants are absorbed on the filters. Later, back in the laboratory, the pollutants are extracted in deionized water and analyzed using an ion chromatograph or spectrophotometer.

An appropriate sampler should be selected after taking the experiences and information from various countries into account. Further information on the samplers and relevant research programs can be obtained on the following webpages:

- IVL's diffusive samplers for air monitoring:
  http://www.ivl.se/en/business/monitoring/diffusive_samplers.asp
- IDAF Programme (IGAC/DEBITS/Africa):
  http://medias.obs-mip.fr/idaf/
- CSIRO Marine and Atmospheric Research:
  http://www.dar.csiro.au/information/samplers.htm
- Ogawa passive sampler:
  http://ogawausa.com/passive.html
- US National Park Service Ozone Passive Sampler Monitoring Program:
  http://www2.nature.nps.gov/air/studies/passives.htm

The European standard for selection of diffusive samplers is available in a document of the European Committee for Standardization (CEN/TC 264/WG11, EN 13528-3: 2003) entitled “Ambient Air Quality—Diffusive samplers for the determination of concentrations of gases and vapours—Requirements and test methods—Part 3: Guide to selection, use and maintenance.”

b) Reagents for colorimetric analysis

Procedures for preparing reagents for colorimetric analysis may be available in the instructions of the respective samplers, but the representative reagents are listed below for reference:

- Sulfanilamide solution: Prepare by dissolving 80 grams (g) of reagent grade sulfanilamide in a mixture of 200 milliliters (ml) of concentrated phosphoric acid and 700 ml of water. The solution is then diluted with water to make a total of 1,000 ml.
- NEDA solution: Prepare by dissolving 0.56 g of N-(1-Naphthyl)-ethylenediamine dihydrochloride (NEDA) into 100 ml of water. The solution should be stored in a refrigerator.
- Color-producing reagent: Prepare immediately before use. The sulfanilamide solution and the NEDA solution should be mixed in a 10:1 ratio (i.e., ten parts sulfanilamide solution to one part NEDA solution).
- Nitrite standard solution

c) Analytical instruments

- Spectrophotometer: colorimetric analysis of NO_2 and NO_X
- Ion chromatograph for anions: SO_2 and O_3
- Ion chromatograph for cations: NH_3

3) Procedures

a) Establishment of monitoring plots

The following local criteria should be considered:

- Air pollution concentrations should be measured near but outside the forest where the basic survey sites of soil and vegetation monitoring are located. An open field near the forest would be suitable.
- The horizontal distance between a large obstruction and the sampler should

be at least twice the obstruction's height, or the top of the obstruction as viewed from the sampler should be less than 30 degrees above the horizon.

- The sampling site should be located away from local sources of emissions or contamination such as waste disposal sites, incinerators, parking lots, open storage sites of agricultural products, and household furnaces and stoves. Any site within 100 meters (m) of these sources of emissions and contamination should not be considered a candidate as a sampling site.

Note:
Meteorological conditions, such as wind direction/speed, temperature, humidity, amount of precipitation, and solar radiation, should be measured in the plot for future evaluation. If this is not feasible, then the data collected at the nearest meteorological observatory can be used.

b) Installation of samplers

Basically, five impregnated filters (duplicate samplings and three blanks) should be prepared for the respective pollutants for a regularly scheduled measurement period. Two filters should be set in the samplers in the laboratory using clean tweezers and plastic gloves to avoid contamination. The samplers should then be packed in plastic bags to avoid being exposed to the ambient air before installation and then stored in a refrigerator. Three blank filters should be kept in a refrigerator for comparison before analysis.

The samplers should be delivered to the sampling plot in a cooler bag or box. Two sets of the sampler (two filters) for each pollutant should be installed at a height of between two and four meters above the ground on a metal or wood pole, and then covered by a shelter to shield them from rain and direct sunlight. The samplers should then be exposed to the ambient air for a predetermined period.

Note:
To ensure that filters are not affected by exposure to outside conditions, such as heat and sunlight, blank field values should first be established through testing. In this case, samplers with blank filters should be installed alongside the actual samplers under the same conditions. Plastic films can be used to wrap the blank samplers to avoid exposure to gaseous pollutants.

c) Measurement period

Measurement should be carried out on a 14-day basis (every two weeks). At remote sites, the measurement period can be extended to four weeks if necessary, and at highly polluted sites, shortened to one week. Measurement of ozone can be limited to the leafed period for deciduous species, but measurement of other pollutants should be ongoing throughout the year.

d) Collection of samplers

The exposed samplers should be collected and packed in plastic bags in the field. New samplers with unexposed filters should be installed at the same position for ongoing measurement. The collected samplers should be taken back to the laboratory and stored in a refrigerator before analysis.

e) Chemical analysis

In the laboratory, air pollutants absorbed on the filters should be dissolved in deionized water and analyzed according to the instructions that come with the respective samplers. In the case of SO_2, it should be oxidized to SO_4^{2-} by using hydrogen peroxide (H_2O_2) before analysis (Ogawa & Co. 1998). The three blank filters for the respective pollutants should be extracted the same way.

SO_2, NH_3, and O_3 should be analyzed with an ion chromatograph as SO_4^{2-}, NH_4^+, and NO_3^-, respectively. This analysis should be carried out according to the EANET *Technical Manual for Wet Deposition Monitoring*.

NO_2 and NO_X ($NO + NO_2$) should be analyzed with a spectrophotometer (e.g., see Annex 2). The amount of NO can be estimated as differences between NO_2 and NO_X.

f) Calculation of air concentrations of pollutants

Average air concentration for the measurement period should be calculated based on the amount of air pollutants collected on the filters. Formulas for converting the amounts collected to the concentrations present were reported for the respective samplers based on the diffusion theory and practices. (See Annex 3 for the calculation of air concentration using the Ogawa passive sampler.)

4) Reporting procedures and formats

The following information and sampling data should be reported on Form 3-1-1 (see below).

a) Information required on monitoring sites and laboratory

- Country
- Name of the plot: name of the forest for the basic soil and forest survey
- Latitude: (North or South) DDMMSS
- Longitude: DDMMSS
- Altitude: in meters
- Name of laboratory
- Name of reporter

b) Information required on sampling and chemical analysis

- Start date and time of the measurement period: year/month/day/hour:minute (e.g., 1:50 AM, July 10, 2005 or 2005/7/10 13:50)
- End date and time of the measurement period: year/month/day/hour:minute
- Measurement period: minutes
- Average temperature for the period: degrees Celsius (°C)
- Relative humidity: percent (%)
- Water vapor pressure at the average temperature: millimeters of mercury (mmHg)
- Amounts collected on filters: nanograms (ng)
- Average concentration during the measurement period: parts per billion (ppb)
- Type of passive sampler

5) Data processing

The average and variation of the replicate samplers should be calculated for the respective sampling periods. Seasonal changes in concentrations and average concentrations over the whole year and/or leafed period (growing season) should be evaluated on the basis of the periodical data.

Accumulated over the threshold of 40 ppb (AOT40) is used in the risk assessment of O_3 on European plant species, but hourly data is necessary for the calculation. Recent studies have introduced the possibility of estimating AOT40 by using the data generated from passive samplers and a simulation model (Gerosa et al. 2003).

The latest techniques such as this should be considered for evaluation.

Dry deposition velocities can be estimated using the inferential method if meteorological data is available. Calculation of dry deposition amounts in forest areas should also be considered for future evaluation.

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Annex 3.1. Structural design of the IVL and Ogawa passive samplers

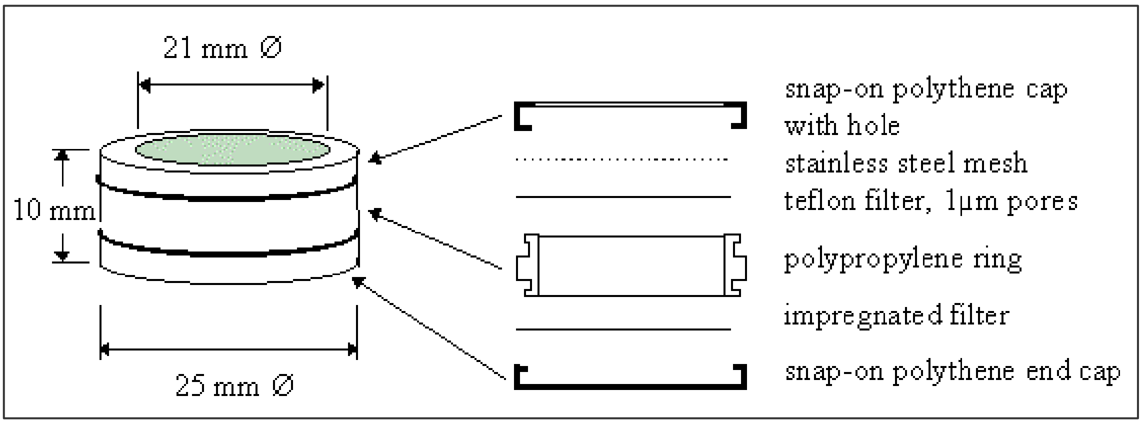

Figure 3-1-1-1. IVL passive sampler (Ayers et al. 1998)

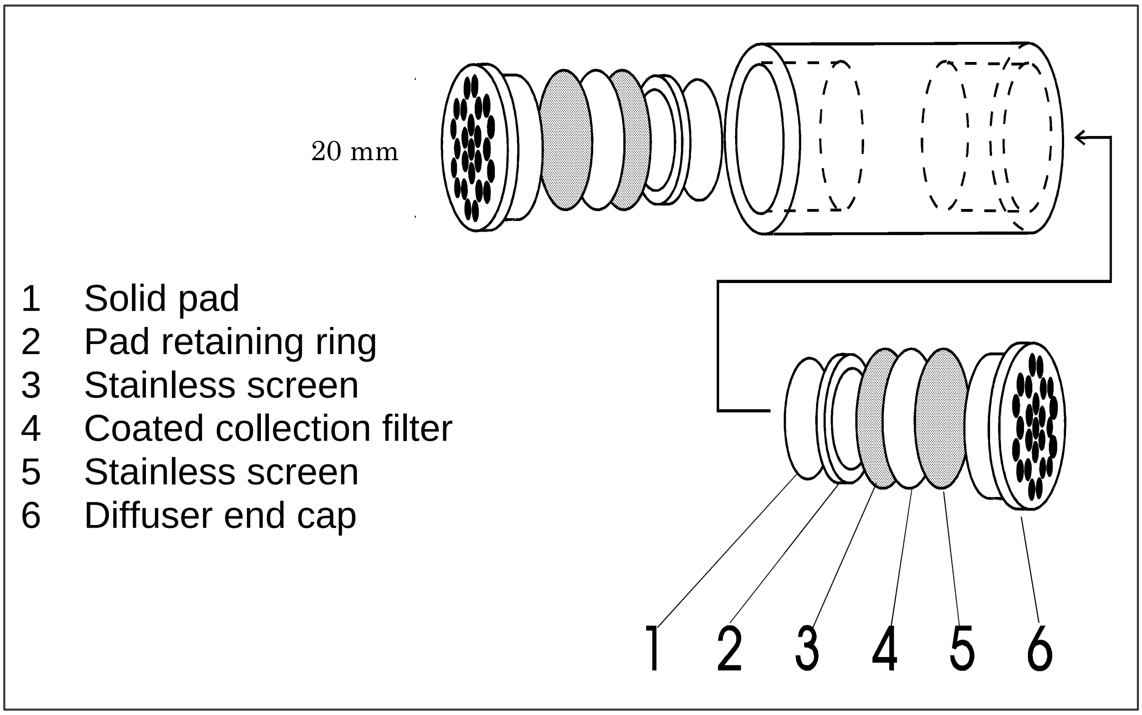

Figure 3-1-1-2. Ogawa passive sampler (Ogawa & Co. 1998)

Note: The sampler has two separate chambers, allowing the measurement of two pollutants at the same time.

Annex 3.2. Colorimetric analysis of NO_2 and NO_X

Suggested basic procedures for the colorimetric analysis of NO_2 and NO_X are as follows (Ogawa & Co. 1998):

1) Extraction of filters
   Exposed filters should be placed in a 25 ml glass or plastic vial containing 8 ml of water, and then shaken immediately. Over the next 30 minutes, the vials should be shaken occasionally.

2) Color producing
   At the end of the first 30-minute period, the vials should be cooled to 2–6ºC and 2 ml of color-producing reagent should be added. Then the vials should be shaken quickly and kept cool for an additional 30 minutes.

3) Colorimetric analysis
   The vials should be allowed to equilibrate at room temperature for about 20 minutes, and then the amount of colored derivative can be determined with a spectrophotometer at a wavelength of 545 nanometers (nm).

   Unexposed filters should be put through the same procedure to determine a blank value baseline.

Annex 3.3. Calculation of air concentration using the Ogawa passive sampler

Average air concentration during the measurement period should be calculated based on the amount of air pollutants collected on the filters. The following formulas for converting from the amounts collected to the concentrations present were reported based on the diffusion theory and practices by Ogawa & Co. (1998) and Hirano et al. (2002).

SO_2 (ppbv) = $\alpha_{SO2} * W_{SO2} / t$

NO (ppbv) = $\alpha_{NO} * (W_{NOX} - W_{NO2}) / t$

NO_2 (ppbv) = $\alpha_{NO2} * W_{NO2} / t$

NH_3 (ppbv) = $\alpha_{NH3} * W_{NH3} / t$

O_3 (ppbv) = $\alpha_{O3} * W_{O3} / t$

where

W_{SO2}, W_{NOX}, W_{NO2}, W_{NH3}, and W_{O3} = collection volume (ng);

α_{SO2}, α_{NO}, α_{NO2}, α_{NH3}, and α_{O3} = conversion coefficients (ppbv*minutes/ng);

and t = exposed time (min).

Conversion coefficients depend on temperature, humidity, water vapor pressure, and/or exposure time. The following coefficients were reported at 20ºC, 70% relative humidity, and 1 atm:

$\alpha_{SO2} = 39.4$

$\alpha_{NO} = 56$

$\alpha_{NO2} = 57$

$\alpha_{NH3} = 43.8$

$\alpha_{O3} = 46.2 * 10^2/(9.94*\ln(t) – 6.53)$

For other conditions, coefficients can be calculated using the following equations:

$\alpha_{SO2} = 39.4*(293/(273+T))^{1.83}$

$\alpha_{NO} = 45.3*(-0.046*T+219.94)/(-0.439*P*RH+208.16)$

$\alpha_{NO2} = 77.2*(2.003*T+89.41)/(0.637*P*RH+131.47)$

$\alpha_{NH3} = 43.8*(293/(273+T))^{1.83}$

$\alpha_{O3} = 46.2 * 10^2*(293/(273+T))^{1.83}/(9.94*\ln(t) – 6.53)$

where

T = average temperature during the measurement period (ºC)

RH = relative humidity

$P = (2*P_N/(P_T+P_N))^{2/3}$

P_N = water vapor pressure at 20ºC (17.535 mmHg)

P_T = water vapor pressure at the average temperature (mmHg)

3.1.2 Estimation of deposition amounts in forest areas

1) Basic concept

This subchapter describes methodologies for estimating the total amount of acid deposition in a forest area using stemflow/throughfall collection and analysis for more precise evaluation.

2) Equipment

a) Rain sampler for rainfall and throughfall

A simple rain sampler consisting of a plastic funnel (20 centimeters [cm] in diameter) connected to a 20–30-liter plastic tank can be used, as shown in Figure 3-1-2-1. The mouth of the funnel should be set more than 1.3 m high. Polyethylene or polypropylene may be used as the material for the sampler. It is recommended that a membrane filter (e.g., 0.8 micrometer [µm] pore size) and/or plastic mesh can be placed between the funnel and the bottle of the sampler to avoid contamination by dust and fallen leaves/branches.

Note:

If gutters are used to sample throughfall, a relatively large plastic gutter should be used (e.g., 0.1 m x 1.8 m or 0.1 m x 3.6 m; Working Group on Acidic Deposition for JCUEF 1999).

In areas where considerable snowfall is expected in winter, snowfall collectors should be prepared. A cylindrical polyethylene container (i.e., a polyethylene bucket) of 20–30 cm in diameter can be used as a sampler. The depth of the container should be at least twice the diameter to prevent collected snow from being blown out (Second ISAG 2000).

The forest type should be taken into account when deciding the height of the sampler. For example, if low branches will hinder collection, then the height should be approximately one meter off the ground.

b) Stemflow sampler: urethane rubber method (plastic foam method)

A plastic band (i.e., 3 cm thick and 30 cm wide) made from flexible plastic foam (i.e., polyethylene foam) should be rolled around the tree trunk like a collar to dam the stemflow of water, as shown in Figure 3-1-2-2. The water dammed by the plastic collar should then be fed into a plastic tank using a plastic tube (hose).

Note:
A tank with a capacity of over 100 liters (L) may be needed for weekly, biweekly, or monthly collection.

c) Components and instruments required for chemical analysis

- **Components for fieldwork**
  - Several liters of distilled water carried in a bottle for washing the samplers in the field
  - Cylinder for measuring water volume in the field (a plastic one with a capacity of more than 1 L is useful)
  - Plastic bottles for collecting samples (more than 1 L capacity each)
  - Replacement materials for the samplers: membrane filters, plastic mesh
  - Repair parts for the sampler: plastic tubes, silicone paste, etc.

- **Components/instruments for chemical analysis**
  - Membrane filter (pore size smaller than 0.45 μm) for filtration of samples
  - Plastic bottles for storing samples
  - pH meter with a glass electrode
  - Electrical conductivity (EC) meter
  - Ion chromatograph for anions
  - Ion chromatograph for cations

3) Procedures

a) Establishment of plots

The forest site selected for soil and vegetation monitoring may be more than 1 ha in area, according to the site criteria listed in the EANET *Technical Manual for Soil and Vegetation Monitoring* (Second ISAG for EANET 2000). A plot measuring 50 x 50 m should be established in the forest for measuring throughfall/stemflow.

b) Installation of samplers

- Rainfall

At least one sampler, preferably three, should be installed out in the open near the forest being monitored, and more than 500 m from any road with heavy traffic. The horizontal distance between any large obstruction and the collector should be at least twice the height of the obstruction, or the top of the obstruction should be less than 30 degrees above the horizon as viewed from the sampler position. The EANET criteria for wet deposition monitoring should be referred to before installation.

- Throughfall

  Several sets of the same type of sampler should be installed on the forest floor under the canopy, and should not be placed near any tree trunks. The sampling bottles should be kept cool and in the dark to prevent any chemical reaction or algae growth. At least three sets of the sampler should be installed in a plot, but it is recommended ten sets would be best in order to obtain sufficient representative data.

  Note:

  Sampling bottles can be kept cool and in the dark by placing them in a pit dug in the ground. At the least, however, each bottle should be wrapped with an appropriate cover such as aluminum foil to shade it from sunlight. Adding a biocide in the sampling bottles, such as thymol (2-isopropyl-5-methyl phenol), should be considered to prevent microbial activities during the sunny and warm season and in tropical regions. The *Technical Manual for Wet Deposition Monitoring in East Asia* (Second Interim Scientific Advisory Group for Acid Deposition Monitoring Network in East Asia 2000) should be referred to for use of biocides.

  The number and density of samplers in the forest should be discussed in advance to cover the variability of deposition amounts by throughfall. Forest properties should be taken into account at the same time (e.g., uniformity of the forest, dominant species, and tree density). In case gutters are used, the number and length of gutters should also be discussed to take variability into account. It is recommended that a pre-study of the variation within the plot should be done before the final sampling plan is decided.

- Stemflow

Several sets of sampler should be installed at a height of 1.3 m on the trunks of dominant tree species. At least three sets of the sampler should be installed for each species, but it is recommended that five to ten sets of the sampler should be installed to obtain representative data. Silicone paste should be used to seal any gaps between the bark and the plastic collar, and plastic mesh should be installed on the mouth of the tube at the collar to trap large debris such as fallen branches and leaves.

Note:
When the plastic collar is installed on a tree trunk, it is important to be careful not to injure the cambium, and the use of any adhesive is not allowed (except silicone paste) to seal gaps between the bark and the plastic collar.

c) Basic field parameters

The following basic field parameters should be recorded during installation of the samplers for reference in future evaluations.

- Stand density: the number of trees in the unit area (e.g., trees/hectare)
- Area of canopy projection of each tree selected for stemflow sampling

Note:
Knowing the percentage of canopy gap may be useful for evaluation of stemflow data. This can be estimated by using the hemispherical photography technique described in Annex 2.2.

d) Sample collection

The plastic tanks used in the field to store rain/throughfall/stemflow water must be emptied at regular intervals. Water volume can be measured by using a measuring cylinder (capacity of more than one liter suggested). A portion of the water samples (1–2 L) should be transferred to plastic bottles at the same time. Samplers should then be washed with deionized water before installation of new filters and other parts.

The samples should then be brought back to laboratory where EC and pH should be measured before filtration is done. When these measurements are completed, filtrate the sample with a membrane filter with a pore size smaller than 0.45 µm to remove any particulate. Store filtered water in 500 ml plastic bottles in a refrigerator until chemical analysis is done.

e) Sampling frequency

The same sampling interval should be established for rainfall, throughfall, and stemflow. Weekly collection is preferable, but at least monthly collection (four-week interval) should be adopted in order to evaluate seasonal changes in deposition amounts. Decide on the frequency of collection once logistics and feasibility, including cost and/or manpower, are taken into account.

Note:

Daily sampling (when it rains) at a fixed time (e.g., 9:00 a.m.) is preferable if a wet-only sampler is available.

f) Chemical analysis

Required measurement parameters and recommended instruments for chemical analysis of rainfall/throughfall/stemflow samples are shown in Table 3-1-2-1.

Table 3-1-2-1. Required measurement parameters and recommended instruments

Measurement parameter	Recommended instrument
Electrical conductivity (EC)	EC meter
Acidity (pH)	pH meter with glass electrode
Cations: Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}	Ion chromatograph Atomic absorption spectrometer ICP-AES
Cations: NH_4^{+}	Ion chromatograph Spectrophotometer
Anions: NO_3^{-}, SO_4^{2-}, and Cl^{-}	Ion chromatograph Spectrophotometer

Note: Refer to the *Technical Manual for Wet Deposition Monitoring in East Asia* (2000) for more details.

4) Reporting procedures and formats

Data and other relevant information must be reported using the following three forms in Excel format: (1) Form 3-1-2 A: Rainfall; (2) Form 3-1-2 B: Throughfall;

and (3) Form 3-1-2 C: Stemflow. The following information should be reported:

a) Information on monitoring sites and laboratory

- Name of site
- Name of plot (and/or plot number) for throughfall and stemflow
- Funnel area for rainfall and throughfall (unit: cm^2)
- Projection area for stemflow (unit: m^2)
- Name of laboratory
- Name of reporter

b) Information on sampling and chemical analysis

- Sample number
- Start and end of sampling: date and time
- Anion concentrations: SO_4^{2-}, NO_3^-, Cl^- (micromole [μmol]/L)
- Cation concentrations: NH_4^+, Na^+, K^+, Ca^{2+}, Mg^{2+} (μmol/L)
- Electrical conductivity (millisiemens/meter [mS/m])
- pH (0.01 unit)
- Sample amount (ml or g)
- End date of analysis
- Notes: any other pertinent information
- Precipitation amount (automatically calculated in Excel)
- R1: cation and anion balance (automatically calculated in Excel)
- R2: conductivity agreement (automatically calculated in Excel)

5) Data processing

By using the forms in the Excel format, some parameters, such as precipitation amount, hydrogen ion (H^+) concentration, deposition amount, R1, and R2, can be calculated automatically. These parameters are useful for primary evaluation and quality control, and are defined as follows:

- Definition of precipitation amount:

  Precipitation amount (mm) = S * 10 / F

  where S = sample amount (ml or g)

  F = funnel area (cm^2)
- Definition of H^+ concentrations:

  $[H^+] = 10^{(6-pH)} / 1.008$ (μmol/L)

- Definition of deposition amount for the sampling period:
  Deposition amount = Conc * Pa (μmol/m^2)
  Deposition amount = Conc * Pa * V / 100 (mol_c/ha)
  where Conc = precipitation concentration (μmol/L)
  Pa = precipitation amount (mm)
  V = ion valence
- Definition of R1:
  R1 = (C - A) / (C + A) * 100 (%)
  where C = total cations equivalent concentration (mol_c/L) (including H^+)
  A = total anions equivalent concentration (mol_c/L)
- Definition of R2:
  R2 = (ECcalc – ECmeas) / (ECcalc + ECmeas) * 100 (%)
  where ECcalc = the calculated conductivity (mS/m)
  ECmeas = the measured conductivity (mS/m)
  ECclac = {349.7 * $10^{(6\text{-pH})}$ + 80.0 * 2c(SO_4^{2-}) + 71.5c(NO_3^-) +76.3c(Cl^-) + 73.5c(NH_4^+) + 50.1c(Na^+) + 73.5c(K^+) + 59.8 * 2c(Ca^{2+}) + 53.3 * 2c(Mg^{2+})} / 10,000
  c( ): ionic concentrations (μmol/L)
  The constants are the molar conductivity of the individual ion at an infinite dilution at 25^0C (Scm^2/mol) (Table 3-1-2-2).

Table 3-1-2-2. Basic constants

Ion	*Molecular Weight (M)*	*Molar Conductivity (Scm² /mol)*
H^+	1.008	349.7
NH_4^+	18.04	73.5
Ca^{2+}	40.08	59.8 ×2
K^+	39.10	73.5
Mg^{2+}	24.31	53.3×2
Na^+	22.99	50.1
NO_3^+	62.01	71.5
SO42-	96.06	80.0×2
Cl^-	35.45	76.3
HCO_3^-	61.02	44.5
$HCOO^-$	45.0	54.6
$CH3COO^-$	59.1	40.9
F^-	19.00	55.5
Br^-	79.90	78.2
NO_2^-	46.01	71.8
PO_4^{3-}	94.97	69.0×3

Source: Kagaku Binran, p. II-460–461. 3rd ed., 1984, Maruzen, Tokyo.

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UN ECE 2004. Sampling and analysis of deposition, *In:* Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests, Part VI.

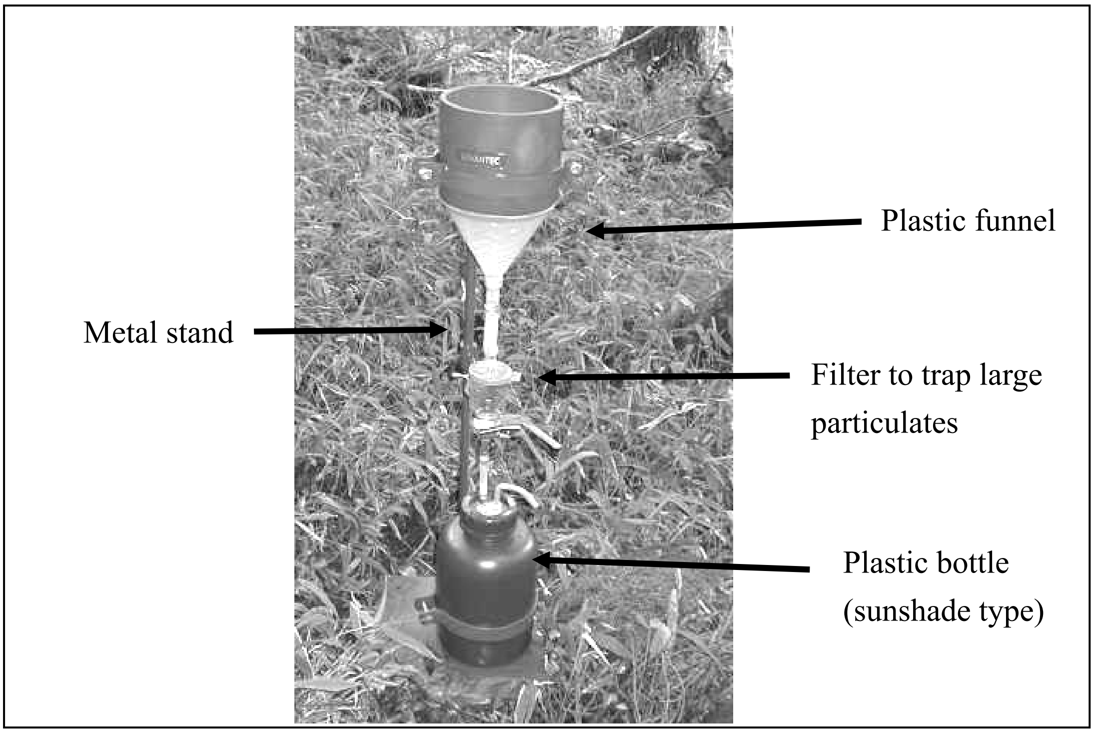

Figure 3-1-2-1. An example of equipment used to collect rainfall/throughfall

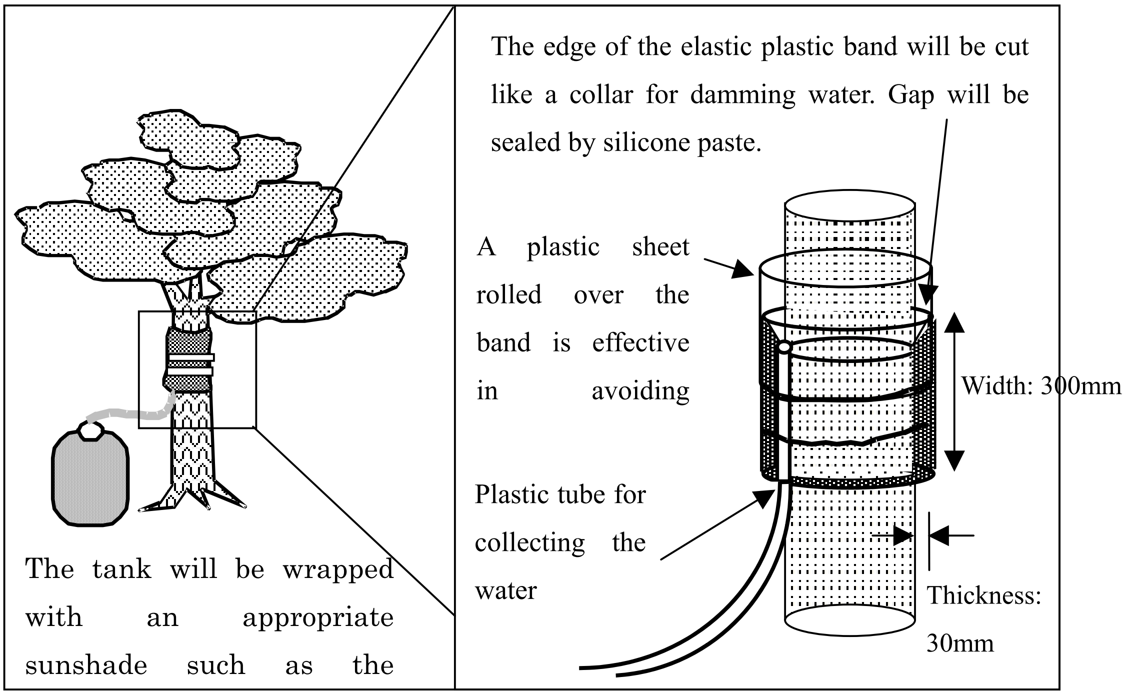

Figure 3-1-2-2. Equipment used in the plastic foam method to collect stemflow from a tree

Form 3-1-1

The form is available as an Excel file.

Form 3-1-1 Results of air concentration measurements by the passive diffusion sampler

Information about sites

Country	Name of the plot	Latitude	Longitude	Altitude
		(N or S) DDMMSS	DDMMSS	m

Name of Laboratory: **Name of Reporter:**

Information about sampling and chemical analysis

Start date	End date	Measurement period	Average temperature	Relative humidity	Water-vapor pressure	Compound	Sampler No.	Collected amounts	Average conc.
time/dd/mm/yy		min.	°C	%	mmHg			ng	ppb
						SO_2	S-1		
							S-2		
						NO_2	N-1		
							N-2		
						NOx	N-3		
							N-4		
						NH_3	N-5		
							N-6		
						O_3	O-1		
							O-2		

Type of the passive sampler:

Form 3-1-2 A

This form is available as an Excel file. The R_1 and R_2 values and deposition amounts will be calculated automatically.

Form 3-1-2 A Results of Rainfall

Site name :

Funnel area (cm^2): 314

Name of Laboratory :

Name of reporter :

Sample No.	Sampling period				SO_4^{2-}	NO_3^-	Cl^-	NH_4^+	Na^+	K^+	Ca^{2+}	Mg^{2+}	EC	pH	Sample amount	Precipitation amount	R1	R2	Date of analysis	Note
	Start		End																	
	Date	Time	Date	Time	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L	mS/m		g or mL	mm				
ex.	2003/4/2	11:00	2003/4/30	11:00	16.6	21.4	45.1	3.9	43.5	2.6	20.0	8.2	1.91	5.00	5940.0	189.2	7.7	-5.7		

Form 3-1-2 B

This form is available as an Excel file. The R_1 and R_2 values and deposition amounts will be calculated automatically.

Form 3-1-2 B Results of Throughfall (1)

Site name :

Area of collector (cm^2 314

Plot No.

Name of Laboratory :

Name of reporter :

Sample No.	Sampling period				SO_4^{2-}	NO_3^-	Cl^-	NH_4^+	Na^+	K^+	Ca^{2+}	Mg^{2+}	EC	pH	Sample amount	Precipitation amount	R1	R2	Date of analysis	Note
	Start		End																	
	Date	Time	Date	Time	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L	mS/m		g or mL	mm				
ex.	2003/4/2	11:00	2003/4/30	11:00	70.8	142.9	456.3	55.6	413.0	87.0	69.8	61.7	10.01	4.90	850.0	27.1	5.7	3.3		

Form 3-1-2 C

This form is available as an Excel file. The R_1 and R_2 values and deposition amounts will be calculated automatically.

Form 3-1-2 C Results of Stemflow (1)

Site name :

Projection area (m^2): 10.26

Plot No.

Name of Laboratory :

Name of reporter :

Sample No.	Sampling period				SO_4^{2-}	NO_3^-	Cl^-	NH_4^+	Na^+	K^+	Ca^{2+}	Mg^{2+}	EC	pH	Sample amount	Precipitation amount	R1	R2	Date of analysis	Note
	Start		End																	
	Date	Time	Date	Time	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L	mS/m		g or mL	mm				
ex.	2003/4/2	11:00	2003/4/30	11:00	133.2	50.0	878.9	2.2	673.9	66.5	79.8	74.1	18.05	3.90	9300.0	0.9	-0.8	3.1		

3.2 Chemical analysis of needles and leaves to determine sulfur content

1) Basic concept

Not all participants of the EANET program are in a position yet to use chemical analysis of needles (leaves) as part of forest condition monitoring, but active development of this aspect of monitoring is encouraged, as it will provide very useful information. Analysis of the sulfur content in the needles/leaves of trees is recommended as one of the first steps in this direction. Gaseous sulfur in the form of sulfur dioxide (SO_2) is emitted pervasively into the atmosphere by many sources: heat power plants, metal producing plants, chemical industries, transportation exhaust, etc. As SO_2 is so widely distributed, it plays a large part in the process of acidification of the environment and is responsible for a large share of specific negative impacts on forests. In order to monitor forest conditions, it is therefore important to determine the sulfur content in the needles and leaves of trees in forested areas, as these are the assimilation organs that perform the most active gas exchange involving SO_2 absorption. After field and laboratory testing was conducted on the most appropriate method of determining sulfur content in tree needles and leaves, the methodology described below was selected (Mochalova, 1975; Yermakov, 1987;Rowell, 1994). It is sufficiently precise and does not require overly expensive equipment.

Determining sulfur content in biological compounds involves transferring the sulfur from the various organic compounds into one concrete form (e.g., sulfate) and conducting a quantitative assessment. This method of sulfur determination is based on the mineralization of plant material through dry aching and further sulfur recovery from the mineralizate using barium chloride. Sulfur from the mineralizate is directly analyzed in the form of barium sulfate as a precipitate, and gum arabic and hydroxilamin are used as additives to stabilize it. Quantitative content is then spectrophotometrically assessed by acquiring the optical density of the suspension.

2) Reagents and instruments

a) Reagents

- Gum arabic (0.5% glue): 3.62 ml of the glue is dissolved in 100 ml of water. (The powder type of the gum arabic can be used.)
- Hydroxylamine hydrochloride: 25 g of $NH_2OH \cdot HCl$ is dissolved in 500 ml of water.

- 2 M hydrogen chloride (HCl): 164 ml is dissolved in 1 L of water.

b) Instruments

- Muffle furnace
- Spectrophotometer

3) Sampling procedures

a) Selection of sample trees

The five trees required for sampling should be selected according to the following criteria:

- The trees should be distributed over the total plot area or around the plot if the stand is homogeneous over a larger area.
- They should belong to the predominant and dominant classes (forest with closed canopy) or to the trees with average height plus or minus 20% (forest with open canopy).
- They should be in the vicinity of the soil sampling plots. Care must be taken, however, not to damage the main roots of sample trees by soil sampling.
- They should be representative of the mean defoliation level of the plot (within 5%).

b) Collection of leaf samples

Samples of 500 g of fresh leaves (needles) should be collected from the crown top or outer canopy of the selected trees. Mature and current-year leaves (needles) (three or four months after leaves come out) should be collected from the sunny side in the upper canopy. Second- or third-year leaves (needles) can be collected as the reference to the current year when comparing dry deposition. Take note that leaves should be classified by their age and this should be recorded in a field note. The leaves collected from the five trees should then be mixed together. Sampling should not be done during continuous times of rain or during a long drought.

c) Pretreatment

The leaf (needles) samples should be oven-dried for two days at 80°C and then ground into particles smaller than 0.5 mm using a Willey mill. Gather about 100 g and store in a plastic bottle.

4) Analytical procedures

a) Place one gram of finely ground, absolutely dry plant material in a quartz tank or porcelain crucible, add 2 ml of ethyl alcohol for better burning, and then set the material on fire.

b) Place the tank in a muffle furnace and roast until the mass is completely white. The furnace should reach a temperature of 750–800°C and burn the sample for more than 24 hours. After that, weigh the crucibles and their contents to determine ash content.

c) Dissolve burnt needles in 20 ml of 2 M hot hydrochloric acid and filter through a folded filter into a 50 ml retort or volumetric flask. Then bring the acquired ash solution (mineralizate) to 50 ml.

d) To determine sulfur content, select 20 ml of the mineralizate from the retort and add the following components: 20 ml of deionized water, 1 ml of hydroxilamin chlorhydrate, 2 ml of gum arabic and 250 mg of barium chloride. Mix the solution for one minute on the electrical mixer (agitator).

e) Determination is then conducted on the spectrophotometer at a wavelength of 460 nm.

f) Construction of the calibration curve:

Prepare a sample solution containing 50 μg of sulfur in 1 ml by dissolving 272 mg of dry potassium sulfate (K_2SO_4) in 1 L of deionized water.

Working calibration solutions are prepared in accordance with the following table:

Retort number	1	2	3	4	5	6
Quantity of sample solution, ml	1	2	4	8	10	20
Sulfur content in the solution, μg/ml	50	100	200	400	500	1,000

Collect 25 ml of the sample solution in the retort, where all the necessary reagents are added as follows: add 1 ml of hydroxilamin, 2 ml of gum Arabic, and 250 mg of barium chloride; dilute the contents of the retort with deionized water to reach the mark; shake for one minute, then determine the results with the spectrophotometer at a wavelength of 460 nm.

5) Data processing and reporting procedures

Total sulfur content in the sample is calculated in percent from absolutely dry matter using the following formula:

$X = (a \cdot V \cdot 100)/(H \cdot c)$

where

X = sulfur content

a = sulfur quantity (g) found through the calibration curve (μg)

V = total mineralizate volume (ml)

100 = coefficient of transfer to percent

H = mass of dry sample (g)

c = volume of mineralizate taken for the analysis (ml)

Please use Form 3-2 for reporting the results.

Form 3-2

Site name:
Name of laboratory:
Name of reporter:

Plot no.	Species name	Total sulfur content (% for dry leaves)	Notes

References

Mochalova, A.D. 1975. Spectrometric method of sulphur determination in plants. Agriculture Abroad: **4:** 17 (in Russian).

Yermakov, A.I. (ed.). 1987. Spectrophotometric determination of sulphur content. In: Methods of biochemical studies of plants. Leningrad: Agropromizdat, Leningrad division, p. 390–391 (in Russian).

Rowell, D.L. 1994. Soil science: Methods and applications. Longman Scientific & Technical, Longman Group UK Limited.

4. Quality Assurance and Quality Control (QA/QC) in Forest Vegetation Monitoring

The QA/QC Program for Soil and Vegetation Monitoring in East Asia—one of several QA/QC programs focusing on specific areas of monitoring operations—was adopted at the First Interim Scientific Advisory Group (ISAG) Meeting in October 1998. It was based mainly on the *Monitoring Guidelines* and *Technical Manuals for Acid Deposition Monitoring in East Asia*, which were adopted at the Expert Meetings between 1993 and 1997, and then revised after the current *Technical Manuals* were adopted at the Second ISAG in March 2000.

The following is a list of primary measures that were recommended for implementation in all QA/QC programs, which together cover all monitoring activities, including those of the Network Center (NC), the National Centers, and the organizations conducting sampling and chemical analysis:

- Each country participating in EANET should develop a national QA/QC program.
- Responsibilities should be clearly assigned to national QA/QC managers, supervisors in sampling or analytical organizations, and personnel in charge of data management and reporting, etc.
- All organizations involved in sampling and chemical analysis (laboratory) should prepare standard operating procedures (SOPs) that meet the actual needs and capabilities of participating organizations, but that still harmonize with the *Technical Manuals* and the other national QA/QC programs.
- Data quality objectives (DQOs) should be set and followed.

A statistical model of a multi-stage sampling system was also proposed in the current *Technical Manual*, and detailed procedures were discussed, but mainly on soil monitoring. Because comparatively little has been discussed before specifically on QA/QC in forest vegetation monitoring, the aim of this chapter is to elaborate on some basic concepts and applicable models.

4.1 Objectives

This *Sub-Manual for Forest Vegetation Monitoring* was developed mainly to establish baseline data and early detection of the possible impacts of acid deposition on forest ecosystems and their components, one of the main objectives of EANET soil and vegetation monitoring.

According to the *Strategy Paper for Future Direction of Soil and Vegetation Monitoring of EANET*, the objective of any QA/QC activity should be to manage all monitoring activities so as to obtain reliable data and accurately evaluate the effects of acid deposition on ecosystems and their components in all countries participating in EANET throughout the East Asian region.

One of the concepts that can be applied in the area of environmental monitoring is total quality management (TQM), a methodology that applies industrial management concepts to improving the production of goods and services. TQM is a combination of quality and management tools aimed at increasing business while reducing losses due to wasteful practices, and it promotes the Plan-Do-Check-Act cycle that encompasses measurements, statistical process control, and organizational learning.

4.2 Management approach and QA/QC programs

According to the TQM concept, a well-constructed strategy is necessary for effective management of forest monitoring, and it should be aimed at managing all steps of monitoring, from the selection of monitoring sites to the evaluation of data.

Generally speaking, the main part of any management strategy can be systematically constructed as a data model, a statistical model, or a mathematical model. For example, in the instance of a data matrix of measurements multiplied by calendar days, often what can be used is a fixed effect ANOVA (analysis of variance) model to conduct comparisons (e.g., between test site data and control data), a random effect ANOVA model to evaluate a sample mean and variances from each level of a sampling hierarchy (see note below for descriptions of both models), an attenuation equation, an advection-dispersion model, or system models such as MAGIC (Model for Acidification of Groundwater in Catchments) or ILWAS (Integrated Lake Watershed Acidification Study), etc. QA/QC programs can usually be described as sets of such models, accompanied by technical descriptions of the whole process and each monitoring procedure, and they can help to ensure the production of high-quality data throughout all monitoring processes.

Note:
In the random effect model, for example, the sulfur content found for determination j from leaf i is written as

$X_{ij} = \mu + A_i + \varepsilon_{ij}$ $(i = 1,\ldots, a; j = 1,\ldots,n)$
where $A_i = N(0, \sigma^2_A)$, $\varepsilon_{ij} = N(0, \sigma^2)$.
$N(0, \sigma^2)$ means normally distributed with a mean 0 and variance σ^2.

In a fixed effect model, this is written as
$X_{ij} = \mu + \alpha_i + \varepsilon_{ij}$ $[\alpha_i$ fixed, $\varepsilon_{ij} = N(0, \sigma^2)$]
where $\Sigma\alpha_i = 0$.

A major reason for using a random effect model is to know, for instance, the data variances from each level of a nested sampling hierarchy (see Section 4.3), while a fixed effect model is used to compare data from a certain level against control data.

4.3 Management approach to EANET forest monitoring

Monitoring is often begun by first conducting a sample survey. As is typical for an environmental survey, there is a statistical nested design for sampling in which a mean value and a variance component are estimated as a representative value of each level of a hierarchical system. The benefits are information about the precision of estimations and ways to improve that precision.

The following levels of hierarchy are used for sampling in EANET's forest monitoring system:

- Entire EANET region
- Member countries
- Specific areas
- Specific plots
- Trees/canopies
- Leaves
- Etc.

This hierarchy can be used as a guide for constructing management strategies and a QA/QC program for forest monitoring. In other words, based on the objectives of monitoring, it can be said that baseline data must be obtained from each level of the hierarchy in order to detect impacts of acid deposition as early as possible.

4.4 Models of acute and chronic impacts

The impacts of acid deposition, caused by sources of air pollutants and acid precipitation, can be divided into two categories: acute or chronic. Note that a model of acute impacts is naturally different from one of chronic impacts. For instance, an attenuation equation can be fitted to a relationship between leaf sulfur concentration and proximity to a large source of emissions to evaluate the acute impacts of pollutants on a forest, but more complicated system models are needed, for example, to analyze sulfur cycling in a forest ecosystem to evaluate chronic impacts.

4.5 Models for managing forest monitoring

The following three models have been prepared for monitoring the methods used in forest monitoring:

a. ANOVA model for evaluating a hierarchical system of sampling
Representative data from each level of the hierarchical system should be known for each parameter concerned with evaluating baseline data and pollution/acid deposition impacts, usually using a random effect ANOVA model.

An inter-laboratory comparison program should then be conducted, in which between-lab variance, within-lab variance, and residual variance are evaluated using a fixed ANOVA model.

Combining the results from both models, that is, in a mixed ANOVA model, for example, baseline data of forest conditions in EANET countries can be evaluated, including information on laboratory performance.

An example of this can be seen in EANET's QA/QC Program for Soil and Vegetation Monitoring in East Asia.

b. Attenuation equation model/advection-dispersion model
An attenuation relationship, for example, between the sulfur content in a tree leaf and the distance from an emission source in a small area is often described by an attenuation equation. A simple attenuation model is an exponential equation, as follows:

$$I(t) = I_0 \exp(-\mu t)$$

where I is sulfur content in a tree leaf, t is distance from the emission source, and μ is the attenuation constant.

An advection-dispersion model, on the other hand, could be used to analyze the relationship between air pollution and forest ecosystems. A real-world model including the attenuation type of mathematical equation model should be developed through experimental research.

c. System model

The process of cycling chemical species is often described by a complicated system model like MAGIC, ILVAS, etc., and much of the data for such a model is usually obtained from field monitoring. Although it is not anticipated that a chemical species cycling model will be constructed soon within EANET, this will be critical with the accumulation of high-quality data and related information for the construction of some possible system models.

d. Models for EANET forest monitoring

In principle, forest monitoring systems in EANET use a random effect ANOVA model. Such a model is used to evaluate data on visual assessment, image analysis of crown condition, and photographic assessment of canopy gaps and light penetration, following the levels of hierarchy in EANET's forest monitoring system mentioned above.

For example, several photographic methods, including remote sensing, can be considered for the assessment of forest condition. These methods are related to data from each level of the hierarchy, combined with some parameters from visual, chemical, or biological monitoring, as follows in Table 4.1.

Table 4.1. Photographic methods for each level of sampling hierarchy

	Area	Plot	Tree/canopy	Leaf
Photographic method:	R/S	R/S	I/H	M/S

Note: R/S = remote sensing; I/H = image analysis/hemispherical photography; M/S = microscopy and photonics micro-measurement such as positron emission tomography (PET).

In this case, visual, chemical, or biological monitoring data can be considered as ground truthing data for remote sensing.

Second, some types of statistical models have been prepared for inter-laboratory comparison tests. Such models are described in EANET's *Technical Manual for Soil and Vegetation Monitoring in East Asia* and *Textbook, Quality Assurance and Quality Control in Soil Monitoring System*.

Third, in comparatively small areas, an attenuation equation can be used to analyze the relationship between an emission source and the sulfur content in tree leaves, crown condition, canopy gaps, or lichens indicators. Data should be monitored on a transect drawn toward the source for this purpose or a concentric circle. Discriminant criteria may be useful to evaluate impacts. An advection-dispersion model may be applied to the analysis of the much more complicated and/or wide-ranging relationship between emission sources and each monitoring plot.

Fourth, it must be emphasized that good system models should be designed to have coordination between a systematic field survey (in which data precision is estimated) and system analysis (in which a possible system model is well assumed).

4.6 Selection of sampling sites

When selecting sampling sites for in-country forest monitoring according to the management strategy, please refer to Section 2.2 "Selection of basic survey site" in the *Technical Documents for Soil and Vegetation Monitoring in East Asia*. Besides the description of sampling site selection, however, the following materials and information should be considered and analyzed to effectively establish sites within the context of this QA/QC program:

- a) Maps of important emission sources
- b) Related information on artificial sources
- c) Related information on natural sources
- d) Predominant wind direction
- e) Concentric contour map of pollution
- f) Country-wide land-use map
- g) Country-wide vegetation map
- h) Country-wide soil map
- i) Critical loads map
- j) Wind diagram
- k) Statistics and estimation of emissions

Knowing specific information about a candidate monitoring site is critical to creating an effective strategy and model for the best management of monitoring. For example, is there a large pollution source (e.g., a power station, smelter, industrial area, or a road with heavy traffic) that might affect the monitoring area? Which direction does the wind mainly come from throughout the year, for example, when considering trans-boundary pollution? Such questions should be carefully considered before deciding on monitoring sites that fit with the central strategy.

4.7 QA/QC of each monitoring operation

4.7.1 QA/QC of visual assessments

a) Model: ANOVA model with one of the following two structures

(a) All data/year in each country
= 2 to 3 observers * 5 (2 at least) trees * 4 directions * some plots * 9 observation items

(b) All data/year in each country
= 2 to 3 observers * 20 (8) trees (= 5[2 at least] trees * 4 directions) * some plots * 9 observation items

b) Basic features of the models

- Structure “a” can be considered as a mixed model, as shown in Table 4.2. The location of a certain stand of trees in a forest may sometimes be one of the most important factors affecting tree growth in terms of availability of sunlight, seasonal winds, etc.
- Structure “b” can be considered as a random effect model, as shown in Table 4.3. When a forest area is large enough and the topography is relatively simple, the effects of directions may be negligible and the structure may be more accurate.
- Assessment should be done by at least two trained observers, as described in Chapter 2, and it is useful to submit the raw results of the two separate observations to the National Centers and NC for QA/QC of EANET’s entire monitoring operation.
- Dominant and co-dominant tree species should be selected for observation, as described in Chapter 2.
- It is preferable that observations are carried out while referring to standard photographs of each tree species, as recommended in Chapter 2.

Table 4.2. ANOVA table (a) for each item in a country

Source of variance	d.f.	Type of effect
Plots	Some–1	Random
Directions	3	Fixed
Trees	4 (1)	Random
Observers	1–2	Random

Note: d.f. = degree of freedom

Table 4.3. ANOVA table (b) for each item in a country

Source of variance	d.f.	Type of effect
Plots	Some–1	Random
Trees	19 (7)	Random
Observers	1–2	Random

Note: d.f. = degrees of freedom

4.7.2 QA/QC for estimates of air pollutant concentrations

a) Concentrations of air pollutants

(a) Objective: Estimation of the concentrations of gaseous pollutants based on measurements gathered using a passive sampler.

(b) Pollutants: sulfur dioxide (SO_2), nitrogen dioxide (NO_2), nitrogen oxide (NO_x), ammonium (NH_4), ozone (O_3)

(c) Plot: An open field near but outside of the forest being monitored and more than 100 meters (m) far from any emission source.

(d) Model: This depends basically on the siting/sampling methods used in the forest monitoring, but the following should be included:

5 filters (2 samples, 3 blanks) * every week/2 weeks/4 weeks.

(e) Basic requirements:

- Two sample filters for each pollutant should be set in the samplers. Exposed samples should be stored in a refrigerator until analysis of the two filters and three blanks can be done (at the same time).
- An average of the two sets of data for the measurement periods should be used for analysis of seasonal change and annual mean.
- Three blank filters should be stored in a refrigerator before analysis, as described in Chapter 2.
- If blank values are changed by exposure to outside conditions, such as heat

and sunlight, then blank field values should be checked for analysis. In this case, samplers with blank filters should be installed with the actual samplers under the same conditions. Plastic sheets should be used to wrap the blank samplers to avoid exposure to gaseous pollutants.

b) Deposition amounts

(a) Objective: Estimating total deposition in a forest area

(b) Items: Chemical parameters of rainfall, throughfall, and stemflow

(c) Plot: A 50 x 50 m plot for measuring stemflow and throughfall in an area larger than 1 ha

(d) Model: The ANOVA model depends basically on the siting methods employed in the forest sampling, but the following should be included:

- Rainfall: One sampler set should be installed in the forest more than 500 m from any road that has heavy traffic and 50 m from any tall trees or buildings.
- Throughfall: At least three sampler sets, preferably more than ten sets, should be installed, depending on the variability of the amount of deposition from throughfall.
- Stemflow: At least three sampler sets should be installed
- Sampling frequency: This should be the same for the three items (rainfall, throughfall, and stemflow) preferably weekly, but at least monthly (daily for wet-only sampler).

(e) The data quality of R1 and R2 should be evaluated, especially for rainfall samples.

4.7.3 QA/QC of chemical analysis

Inter-laboratory comparison should be conducted as is done in soil chemical analysis.

4.7.4 QA/QC of image analysis of tree crown condition and canopy structure

Image analysis may be the best method to evaluate tree crown condition and canopy structure so as to provide more objective or explicit data for the assessment, that is, better representative data in each country or estimation along, for example, a transect from a source. These can be complementary tools to assess tree crown condition and canopy structure, as described above. If so, the same QA/QC program should also be applied to these methods.

4.8 Advanced QA/QC methods

4.8.1 QA/QC for monitoring of lichens

(a) Objective: Early detection of air pollution/acid deposition impact through the monitoring of chlorophyll content and chlorophyll degradation of lichens.

(b) Items: Chlorophyll content and chlorophyll degradation

(c) Plot: The goal is to sample any lichen thalli and thalli fragments that extend beyond the upper edge of a measuring tape from 4 to 10 model trees, in 7 to 50 plots at the forest monitoring site, in different seasons (e.g., wet and dry seasons) for at least three to five years. Select mature trees of the same age and/or same size over the whole site, and sample at a fixed height (1.5 m is recommended). As far as possible, conditions should be the same in all the sample plots, and plots should be distributed as evenly as possible in each level of the sampling hierarchy.

(d) Model: ANOVA model and/or attenuation model, depending on the siting tactics of the forest sampling. The following should be included:
7 to 50 plots of 4 to 10 model trees and 5 to 7 aliquots (replicates) at each site using a random model.

(e) Requirements: A lichen taxonomist should be involved in this work. The thalli must be washed with a calcium carbonate ($CaCO_3$) saturated acetone.

4.8.2 QA/QC of remote sensing

Ground truthing procedures should be conducted to improve accuracy of assessments by remote sensing. Spatial data from accurate remote sensing can often provide a powerful tool for distributing the point information to cover a larger area. Ground truthing procedures are described in many field textbooks.

5. Advanced Methodologies for Future Monitoring and Evaluation

This chapter describes several monitoring methodologies new to discussion and application in EANET, which are offered for consideration on the basis of experience in Europe and/or several countries participating in EANET. While their application in EANET monitoring will be discussed further in the future, this review will still be informative for existing research activities in EANET countries.

5.1 Monitoring lichens as bio-indicators of air pollution/acid deposition: Analysis of chlorophyll degradation and changes in lichen communities

5.1.1 Monitoring chlorophyll content and degradation in lichens

1) Principles

Lichens are very sensitive to air pollutants and are used as indicators of air pollution worldwide (Boonpragob and Nash III 1991; Balaguer and Manrique 1991). They grow on various substrates such as bark, rock, soil, concrete, and metal by metabolizing atmospheric water in the form of rain, fog, and dew for growth. Having no protective layer on their outer surface, such as wax or cuticle as vascular plants do, they are therefore very sensitive to air pollutants, including acid deposition. The following are the two main methods of using lichens for biomonitoring:

1) The first involves analyzing chlorophyll content in lichens and monitoring them for any chlorophyll degradation in order to detect early damage before severe damage occurs, which can cause degradation and even extinction of lichen communities. In the event that the original lichens have already disappeared from the impacted location, lichen materials can be transplanted, monitored, and then analyzed.
2) The second method is to monitor the species composition of a lichen community at a particular location at regular intervals, which may vary from three to five years, and assess changes over time. This allows the slow response of lichens to pollutants to be observed, but it usually requires expertise in lichen taxonomy to make identifications at the genus and species levels. The advantage of this method is that it only requires simple, inexpensive equipment.

Chlorophyll in plants absorbs light energy for the photosynthetic production of organic matter. By dissolving that matter in dimethyl sulfoxide (DMSO), peaks of absorption show at 435 and 665 nanometers (nm) (Hiscox and Israelstam 1979; Ronen and Galun 1984). As chlorophyll is sensitive to acid, heavy metals, and other pollutants (Nash III 1976; Puckett 1976; Rao and Le Blanc 1966), when exposed to these it degrades into phaeophytin, and the absorption peaks shift to 415 nm and decline at 665 nm. Therefore, the ratio of chlorophyll absorption at 435/415 nm can be used to estimate the percent of phaeophytin, the degradation product, compared to chlorophyll content (Ronen and Galun 1984).

Summary of the methods:

Lichens are efficient interceptors of heavy metals and particulate pollutants. Analysis of lichen thalli provides information on concentrations of heavy metals and other pollutants in the air or in precipitation.

2) Objectives

a) Monitor air quality and the effects of acid deposition on lichens, a sensitive bioindicator, before damage occurs to whole ecosystems.
b) Provide alternative methodologies that use less complicated equipment and simple techniques more appropriate for use in developing countries.

3) Equipment and chemicals required

a) Equipment
- Ultraviolet (UV)-visible spectrophotometer
- Weighing scale
- Incubator set at 65 degrees Celsius (^oC)
- Test tubes

b) Chemicals
- Dimethyl sulfoxide (DMSO)
- Calcium carbonate ($CaCO_3$)
- Acetone
- Polyvinylpolypyrrolidone
- Hydrogen chloride (HCl)
- Magnesium carbonate ($MgCO_3$)

4) Procedures

a) Field observation and sample collection

i) The same site should be used for observation and sampling (or in the vicinity) as any existing wet and dry deposition or soil and vegetation monitoring site.
ii) Collection and transplantation of lichens can be performed, according to Boonpragob (2002), as follows:
1. Lichen collection:
Select fruticose or foliose lichens, preferably a species without lichen substances or natural products that may mask chlorophyll absorption. Note, however, that natural lichen substances can be removed by washing samples in a $CaCO_3$-saturated acetone solution (Silberstein and Galun 1988).
2. Transplanting lichens:
If there are no lichens in the study area, then transplantation from another area can be done. It is recommended that lichen should be transplanted to a control site that has similar topography (i.e., altitude) and climate in order to assess the effect of transplantation on the lichens.

b) Laboratory analysis

i) Chlorophyll and phaeophytin extraction

1. Wash 20 milligrams (mg) of air-dried thalli six times with 3 milliliters (ml) of $CaCO_3$-saturated acetone to remove lichen acids, and then allow the residual acetone to evaporate.
2. Immerse the dry thalli in 5 ml of DMSO containing 2.5 mg/ml of polyvinylpolypyrrolidone.
3. Incubate at 65°C for 45 minutes in the dark to allow the chlorophyll and phaeophytin to be extracted.
4. Allow the extract to cool to the ambient temperature and then add 5 ml of DMSO.
5. Remove an aliquot (a portion of the total amount of solution) from the extract and measure the optical density (OD) of the solution at 665 nm and 648 nm.
6. Measure the OD at 435 nm and 415 nm to estimate the degradation of chlorophyll into phaeophytin.

ii) Determining chlorophyll content:

This can be done, according to Barnes et al. (1992), by using the following equations:

$$C_{total}\ (mg/L) = 20.34\ OD_{648} + 7.49\ OD_{665}$$
$$C_a\ (mg/L) = 14.85\ OD_{665} - 5.14\ OD_{648}$$
$$C_b\ (mg/L) = 25.48\ OD_{648} - 7.63\ OD_{665}$$

iii) Preparing a standard curve used to estimate chlorophyll degradation:

1. Wash 220 mg of air-dry thalli with 100% $CaCO_3$-saturated acetone, which involves six 1-minute rinses with 30 ml of the bathing medium, then allow the residual acetone to evaporate from the thalli.
2. Immerse the dry thalli in 55 ml of DMSO containing 2.5 mg/L of polyvinylpolypyrrolidone.
3. Incubate it at 65°C for 45 minutes in the dark.
4. Allow it to cool to the ambient temperature, and then add 55 ml of DMSO.
5. Divide the solution into two portions.
   5.1. Keep one portion intact as the solution of the chlorophyll.
   5.2. Acidify the other portion with about 330 microliters (µl), or 0.3 ml, of 1N HCl for ten minutes in the dark. This portion is the equivalent solution of phaeophytin.
6. Neutralize the acidified portion with solid $MgCO_3$.
7. Prepare a series of mixtures by volume, starting from a 100% chlorophyll solution plus 0% phaeophytin solution, 90% chlorophyll plus 10% phaeophytin, and so on, to 0% chlorophyll plus 100% phaeophytin.
8. Measure the OD of the mixtures at 435 nm and 415 nm.
9. Calculate the ratios of OD435/OD415.
10. Construct a standard calibration graph of the ratio of chlorophyll/phaeophytin in the mixtures plotted against the ratio of OD435/OD415. This graph is then used as a standard to estimate the

extent of degradation of chlorophyll into phaeophytin in the lichens at the polluted site.

iv) Remarks on quality control:
Some natural lichen substances absorb light in the UV region, which interferes with absorption of chlorophyll in the blue region. Therefore, it is necessary to completely remove lichen substances by washing samples in $CaCO_3$-saturated acetone (as described above). This can be checked by running the whole spectrum of chlorophyll absorption at 280–760 nm.

c) Season, number of replicates, and sampling frequency

i) Season:
Air pollution has different seasonal effects. For example, they seem to be more severe in the dry season than the rainy season; while wet deposition is an efficient remover of acid pollutants. As chlorophyll responds quickly to seasonal change and can be readily observed, it is therefore recommended that sampling should be done in both dry and wet seasons during the first and subsequent samplings.

ii) Number of replicates:
Chlorophyll content in lichens and plants varies, which is normal for any biological system, so it is recommended that at least five to seven samples are taken for measuring chlorophyll content. Phaeophytin, which is a degradation product of chlorophyll, can be measured using the same samples.

iii) Personnel:
One person is enough to employ the chlorophyll technique, while one to two field assistants are required for lichen transplantation. Note that a lichen taxonomist may be required to identify lichens at the start of the project.

5) Reporting procedures and format

a) Data to be reported

i) Site description:

- Location
- Grid reference (GPS)
- Map indicating industrial and urban areas
- Date(s) of collection and transplantation
- Scientific name of host trees
- Type of forest (floristic region)
- Compass direction of lichen sample on host tree(s)

ii) Chlorophyll data:

- Scientific names of lichens present
- Number of samples
- In table form: OD415, OD435, OD648, and OD665 nm
- Contents of calculated total chlorophyll and chlorophyll *a* and *b*

- Standard curve for estimating percent of phaeophytin (only once at the beginning of the monitoring process)
- Regression equation for estimating the percent of phaeophytin
- Percent of phaeophytin

b) Reporting format
See the form below.

6) Data processing

a) Compare the amounts of chlorophyll content and proportion of phaeophytin (in percent of chlorophyll and phaeophytin present in the samples at the beginning of observation). A high percentage of phaeophytin indicates more degradation of chlorophyll by acid or other pollutants.

b) Use statistical analysis to find the correlation between the amounts of chlorophyll and/or phaeophytin with data on wet and dry acid deposition.

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Reporting format for chlorophyll/phaeophytin analysis

Site name ……………………… Site classification …………………Location ………………….. Grid references (GPS) ………..
Forest type …………………….. Host trees ………………………. Scientific name of lichens …………………………
Name of laboratory ……………………………………. Name of reporter ………………………………..

Date	Sample no.	Optical density (OD)				Chlorophyll (mg g^{-1})			OD 435/415	% phaeophytin	Remarks
		415 nm	435 nm	648 nm	665 nm	Total	a	b			

5.1.2 Monitoring epiphytic lichens in the context of air pollution/acid deposition studies of forest ecosystems in the East Asian region

1) Introduction

During the 1960s and 1970s, growing industrialization and transport development in Europe and North America led to increasing levels of anthropogenic air pollution. The resulting side-effects on human health, forests, agriculture, vegetation, rivers and lakes, ecosystems, and materials, including historic and cultural monuments, caused great concern among both the public and various levels of government. As many pollutants can be transported in the atmosphere for hundreds, even thousands of kilometers, the international aspect of the problem was also recognized. As a result, the Long-range Transboundary Air Pollution Convention of the United Nations Economic Commission for Europe was adopted in 1979 (UNECE 2003), with most European countries, the United States, and Canada participating. The convention addresses monitoring strategies, including, among others, the International Cooperative Programme on Integrated Monitoring of Air Pollution Effects on Ecosystems (ICP IM) (Kleemola and Forsius 2003). Lichen monitoring is a part of the ICP IM's activities (Finnish Environment Institute 2004).

Environmental monitoring involves the observation and assessment of changes in ecosystems and their components caused by anthropogenic influence (Izrael et al. 1978). An effective monitoring system makes it possible to quantify the present state of the environment and subsequent changes. An important function of monitoring is to assess the environmental quality of areas that are not affected by local anthropogenic impacts, i.e., establishing a control baseline of background levels of pollutants. According to the size of a territory, one can specify regional and global background levels. At these levels, terrestrial ecosystems are mainly affected by anthropogenic factors such as air pollution and global climate change. The assessment of biota responses to altered climatic and atmospheric conditions provided by monitoring forms an important basis for ecosystem management and environmental decision-making.

Lichens have been monitored as indicators of local air pollution for more than 100 years. They are choice organisms to use to monitor the ecological effects of air pollution and climate change, because their reaction to these stresses is stronger and their "intrinsic" variability is less than that of other organisms (Insarov et al. 1999; Insarov and Insarova 2001). A background lichen monitoring system should include two main parts: an observation system, and assessment/evaluation/trend detection. The following section mainly describes the first part, observation.

2) Objectives

- To establish a network of sites and sample plots for long-term lichen monitoring in the East Asian region.
- To provide a baseline against which changes in epiphytic lichen communities influenced by background levels of air pollution/acid deposition can be detected and measured.

3) Selection of sites, plots, and trees

a) Monitoring plots should be chosen within a protected area (national park, nature reserve, etc.), where human impacts, such as forestry, agriculture, or construction, are non-existent or restricted, and as far as possible from emission sources, in order to detect the effects of changes in large-scale air pollution on lichen communities without being masked by other effects.

b) Define groups of trees as sample plots, with considerably less distance between members of the group than between plots. The distance between trees within each plot should be about the same for all plots in the site. Avoid forest edges, sheltered depressions, steep topography, wind-exposed heights, and maritime areas. Sample plots within the site should belong to as narrow a stratum as possible in order to reduce the effects of spatial variation in lichen communities due to substrate, altitude, slope, aspect, shading, or moisture conditions. As far as possible, conditions should be the same at all sample plots. While selecting plots, no *a priori* information on lichen abundance should be taken into account.

c) Select a number of plots per site and trees per plot, depending on the availability of substrata, variability of lichen community characteristics throughout the site, expected changes to be detected, and logistical issues. The same number of trees for all plots is recommended. In practice, however, the number of plots per site may vary from 7 to 50, and the number of trees per plot may vary from 4 to 10.

d) Select mature trees with nearly vertical trunks, while omitting trees with severe visible damage to their stem or crown. Within these parameters, select trees randomly without *a priori* information on the presence and abundance of lichens. To exclude the effects of varying tree age, trees of the same age and/or size should be selected throughout the whole site for the first and subsequent observations.

4) Sampling lichens on tree trunks

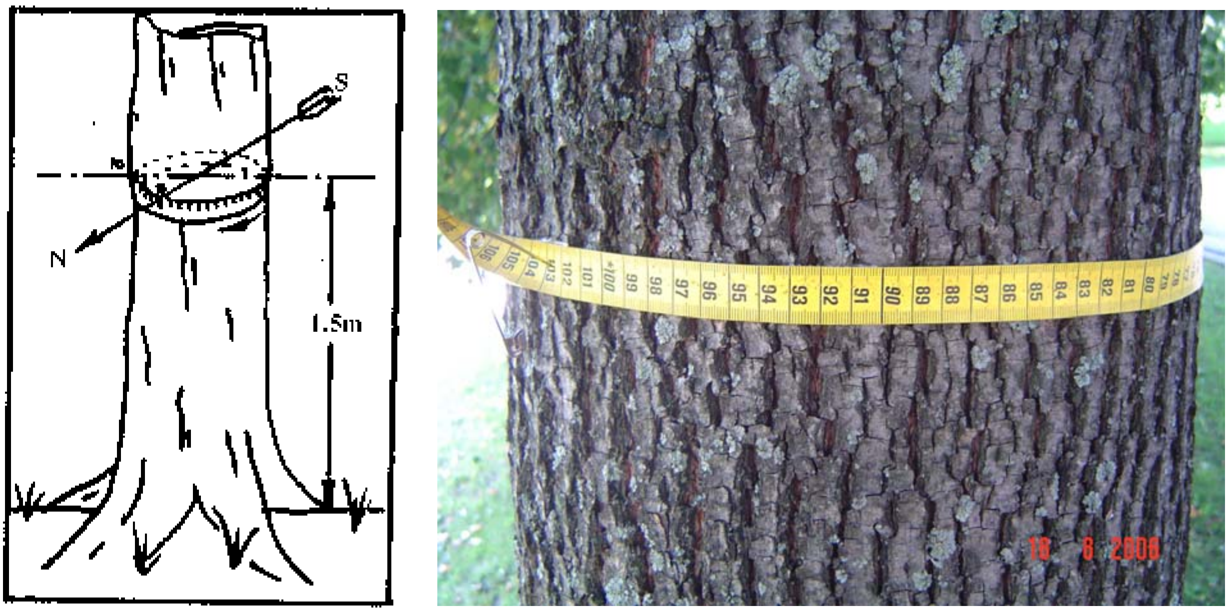
a) Sampling of lichens should be done at a fixed height from the tree base. First, place a measuring tape around the tree in a clockwise direction from north (zero) at a recommended height of 1.5 meters (m), although 0.5–1.0 m can be used for trees with numerous dry snags or on steep slopes.

b) Starting at zero, record the length of all lichen thalli and thalli fragments intersected by the upper edge of the measuring tape. Record the projective cover as the linear distance occupied by each thallus or thallus fragment (see Figure 5.1.2). For fruticose lichens with a branch width less then one millimeter (mm), note the number of branches crossing each millimeter on the scale (Insarov and Pchiolkin 1988).

c) Take samples of unidentified species in the field for later identification in the laboratory.

5) Repeat observations

a) The observation interval depends on the variation of lichen communities across the site, expected changes to be detected, and logistical issues. In practice, the interval is between one and ten years, although three to five years is recommended.
b) For repeat observations at the same site, select plots and trees as described in point 3 above. Note that trees should be of the same age and/or size for all observations. (In practice, this means that different trees may be selected for future observations.)



a. b.

Figure 5.1.2. Measuring lichen thalli on a tree trunk
a. Sampling scheme
b. Measuring tape on the tree trunk

6) Data reporting

a) Provide the following information for each site, sample plot, model tree (tree selected for lichen sampling), and measured lichen thallus, or thallus fragment:

➢ **Site**
- Name of the site
- Latitude and longitude of the site
- Floristic zone (according to Takhtajan 1986)
- Height above sea level (if the same across all sample plots)
- Measurement tape height on tree trunk
- Number of plots within the site

- **Plot**
  - Sample plot number within the site
  - Sample plot coordinates
  - Height above sea level (if not the same across all sample plots)
  - Slope angle
  - Direction of slope exposure
  - Crown cover
  - Collector number (if the same across all trees in the sample plot)
  - Average diameter of trees in the sample plot
  - Basal area, in square meters per hectare (ha)*
  - Tree species selected for lichen sampling
  - Other tree species
  - Number of model trees within the plot
- **Model tree**
  - Tree number within the sample plot
  - Circumference length
  - Tree height, m
  - Diameter at breast height (DBH)*
  - Tree age*
  - Last five-year radial increment, measured in 0.1 mm*
  - Next to last five-year radial increment, measured in 0.1 mm*
  - Number of thalli*
- **Lichens**
  - Number of lichen species on the tree
  - Latin name of each lichen species**
  - Lichen species number in the full glossary (see below)**
  - Beginning of intersection with measuring tape
  - End of intersection with measuring tape
  - Presence in Red Data Books (lists of species threatened with extinction)*
  - Thallome type (crustose, foliose, fruticose, or squamulose)*
  - Number of twigs (for lichens less than 1 mm wide)
  - Lichen width correction***
  - Twig width standard error***

* Optional.
** Can be added after final identification in the lab.
*** Can be added after measuring twig width in the lab (Insarov and Pchiolkin 1988).

b) Provide a list of the numbered lichen species recorded throughout the site in alphabetic order (full glossary).

7) Quality assurance and quality control (QA/QC)

A major source of errors in lichen monitoring is the misidentification of species, so a lichen expert is needed for reliable identification.

Data quality control should be provided by computer database tools.

8) Limitations

a) It may be difficult to locate sites not affected by human impacts that provide an even distribution of suitable substrata and without a significant gradient in environmental conditions. In mountain regions, initial stratum and plot selection can be done using forest inventory, land management, topographical maps, and satellite images. Preliminary inspection of candidate sites and plots is recommended.

b) Lichen sampling can be conducted in any season and in any conditions under which lichens are dry. Avoid sampling in rainy or foggy conditions, or shortly after rain or fog.

c) This method was tested and approved for monitoring in the boreal and temperate zones of the East Asian region. For the tropical zone, testing and approval is still needed.

9) Next steps

a) Collect information on the sensitivity of lichens to major atmospheric pollutants in the East Asian region.

b) Develop a database for storage and processing lichen monitoring data. This database should have a QA/QC plan for computer data.

c) Conduct subsequent observations.

d) Elaborate data evaluation and trend detection procedures.

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Annex 5.1. Personnel required

Two to six people are required to conduct monitoring of epiphytic lichens, and should include a lichen expert(s). Training in the field is recommended.

Annex 5.2. Equipment required

The equipment required includes a 4x4 vehicle; GPS equipment; magnifying lenses of x20, x10, and x5; measuring tapes with the scale marked in millimeters; compasses; headlamps; plastic and craft paper packs; adhesive labels; permanent markers; and color bands. For collecting epiphytic lichens, be sure to have a fixed-blade knife, a one-inch wide chisel or smaller, and a geologist’s hammer with a pick head.

5.2 Remote sensing technologies: A planted forest management system in Japan using satellite remote sensing

5.2.1 Basic concepts

Approximately 25.15 million ha, or 67% of Japan's land area, is forested, making it the third most forested country in the world after Finland (76%) and Sweden (70%). Of this total, in 1995, 10.40 million ha were man-made forest (planted forest) and 13.38 million ha were natural forest. From 1966 to 1995 the total forest area changed by only a small amount, but the proportion of planted forest increased by 31% and that of natural forest decreased by 14% (Japan FAO Association 1997). Figure 5-2-1 shows the primary types of planted forest in Japan in 1995. The species are all coniferous varieties, including Sugi, or Japanese cedar (*Cryptomeria japonica*), Hinoki (*Chamaecyparis obtusa*), Karamatsu (*Larix leptolepis*), and Akamatsu or Japanese red pine (*Pinus densiflora*). Sugi and Hinoki produce high-quality timber used in house construction and are the main trees planted in Japan, but increasing quantities of cheaper timber have been imported in recent years. In 1995, for example, 89% of Japan's industrial wood supply came from imported wood.

This increase in the use of imported timber, in addition to an aging and decreasing forestry workforce, has had a significant effect on Japan's forest industry. The main effect has been an increase in the amount of poorly maintained forests, because planted forests require constant maintenance, including weeding, removal of climbers, pruning, and thinning. Figure 5-2-2 shows examples of poorly maintained planted forests in Wakayama prefecture, Japan. These forests are not only uneconomical but also have a reduced ability to resist natural disasters, including landslides and droughts, such as the one that occurred in the summer of 1994. In addition, they have a reduced capacity to fix carbon. These negative effects can be minimized, however, through the use of effective management systems. The purpose of the study described below was to propose a technique for establishing biomass energy supply systems incorporating forest management systems based on satellite data. An examination of regions in which planted forests were blighted was conducted using Landsat thematic mapper (TM) imagery captured in 1995 and 2000, which included an assessment of possible biomass energy supply systems using such forests.

5.2.2 Instruments

<u>Software:</u>
LEICA Geo-systems / ERDAS IMAGINE 8.7 Professional
ATOCOR 3 for ERDAS IMAGINE (see http://gis.leica-geosystems.com/)
<u>PC:</u>
CPU: Pentium 4 / 2GHz; RAM: 1 GB; OS: Windows 2000 Professional
<u>Data:</u>
LANDSAT-5/TM Obs. Date: 1995/08/04   Path-Row: 110-36, 110-37
LANDSAT-5/TM Obs. Date: 2000/10/04   Path-Row: 110-36, 110-37
Digital elevation model (DEM) data for the area

5.2.3 Procedures

The following are some useful links for basic instructions on remote sensing:

NASA: National Aeronautics and Space Administration, Remote Sensing Tutorial, http://rst.gsfc.nasa.gov
University of Maryland: Global Land Cover Facility, http://glcf.umiacs.umd.edu
ESA: European Space Agency, http://www.esa.int/

Figure 5-2-4 shows the workflow followed during this study. In the first stage of the process, four images from the Landsat/TM satellite were processed using ERDAS Imagine software (version 8.7). This step involved rectification using ground control points (140 points in a full scene) from Digital Mapimage (Geographical Survey Institute 1998), atmospheric correction and haze removal, topographic normalization for the slope surface using a non-Lambertian Reflection Model, and the creation of a full mosaic. After being processed, they were compared to the TM images captured in 2000 to quantify changes in land cover. The forest area in this study is mountainous, so some errors in the satellite images needed to be removed using a three-dimensional model. Note that the atmospheric/topographic correction and haze removal were based on the MODTRAN computer program (Geosystems 1998) using ATCOR3 software (for more details, see http://gis.leica-geosystems.com/). A simpler correction model can be used, however, if the study area is flat.

The planted forests were classified according to “vitality” by using on-site data that were stepped in the study area and the Normalized Difference Vegetation Index (NDVI)—the ratio between the difference and sum of two spectral bands—calculated from the satellite imagery. In addition, the forest stock was quantified by estimating the speed of tree growth. Note that forest “vitality” must be carefully defined, as are the definitions of sound and blighted man-made forests. The Forestry Agency in Japan defines a sound planted forest as one that has no blighted trees, has wood production under way, has high resistance to snow, wind, and insect damage, and has maintenance being performed (Forestry Agency). On the other hand, a blighted forest is defined as one where there is no maintenance plan in operation and which contains crowded, discolored trees (Osumi 1987), as shown in Figure 5-2-2. The undergrowth does not grow well in such forests because little sunlight reaches the ground, so topsoil is not well protected, thereby increasing the possibility of landslides. To obtain high-quality data for differentiating every tree, a spectroradiometer can be used, such as the one made by Field-Spec (ASD Inc. at http://www.asdi.com). Note that it is more effective to use a spectroradiometer in combination with aerial remote sensing data and high-resolution satellite remote sensing data.

It is normally easier to extract the differences in forest vitality by using satellite data taken at the season of high plant photosynthesis. In terms of manpower, it took about two person-months to process our study area. (The total forest area in this study was 82 square kilometers [km^2].)

5.2.4 Quantification of vitality for managing planted forests

The test areas were sampled in order to classify them by vitality level using satellite imagery. The condition of the forest was investigated in 1995 and 2000. In these investigations, the forests were classified into the following four categories:

Sound forest: No blighted trees

Lightly blighted: Less than 20% of trees suffering from blight
Medium blighted: Between 20% and 70% of trees suffering from blight
Heavily blighted: Between 70% and 100% of trees suffering from blight

Ten typical areas (each measuring approximately 10,000 square meters [m^2]) were selected as sample areas and examined. Figure 5-2-5 shows the areas and classifications and Figure 5-2-6 shows the 1995 TM image of the study area.

To specify the classified forest in the ten study areas, the NDVI results and site investigation data were compared. Figure 5-2-7 shows the NDVI for each area. The area of classified forest was estimated using the NDVI. Here, the four classifications (sound, lightly blighted, medium blight, heavily blighted) were weighted according to the size of the sampling areas. Table 5-2-1 shows the areas classified using the four ranges of weighted NDVI. The total area of sound planted forest is estimated to be 18.19 km^2, or 52% of the planted forest, but 48% of the forest has some level of blight and 8.4% is classified as heavily blighted. Figure 5-2-8 shows the land use in the study region, Figure 5-2-9 shows the location of planted forests, and Figure 5-2-10 shows the man-made forest classified into the four levels of vitality.

The results of this classification were verified using two methods: (a) comparison with TM imagery captured in 2000 and (b) further site investigation.

(a) Comparison with 2000 TM imagery: The quality of the forest blighted in 1995 is unlikely to have improved by 2000 due to the lack of maintenance. Therefore, the five-year change was confirmed using TM imagery captured in 2000. Figure 5-2-11 shows the results of the comparison of the two sets of imagery. It can be seen that 98% of the forest specified as blighted in 1995 was in the same condition in 2000, with the remainder changing to grass or barren land.

(b) Further site investigation: To confirm the above situation, an additional site investigation was performed at 97 points in 2001. These points were composed of 23 points of light blight in 1995, 45 points of medium blight, and 29 points of heavy blight. In this investigation, 79% of the points were confirmed as being at the same vitality level of the estimate result (lightly blighted: 17 points; middle blighted: 39 points; heavily blighted: 21 points).

The above is a description of a system for quantifying the stock of man-made forests and classifying them by vitality using Landsat TM images taken in 1995 and 2000. The information supplied by this system is useful for planning a sustainable timber supply, a biomass energy system, quantification of carbon dioxide (CO_2) fixation, and evaluating effects of acid rain on forest vegetation, among others.

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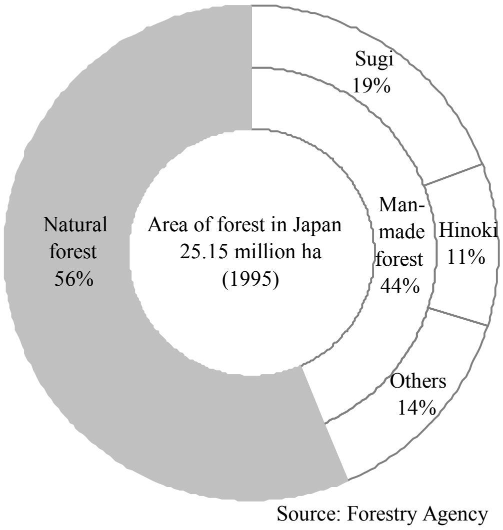

Figure 5-2-1. The state of Japan's forests, 1995

Figure 5-2-2. Poorly maintained man-made forest
Note: Sugi (*Cryptomeria japonica*), Wakayama Pref., Japan, 2002.

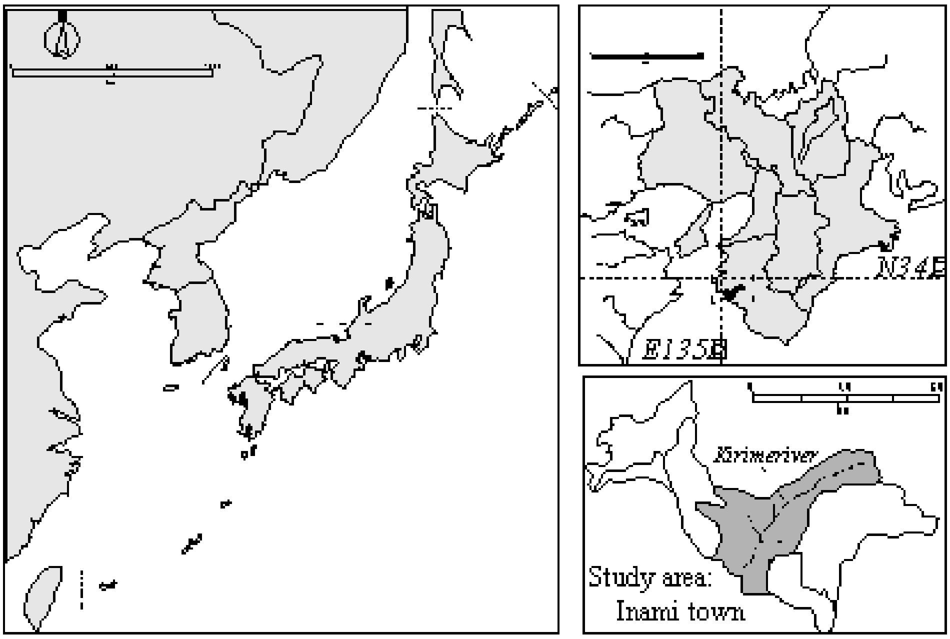

Figure. 5-2-3. Study area: Inami town, Wakayama prefecture, Japan

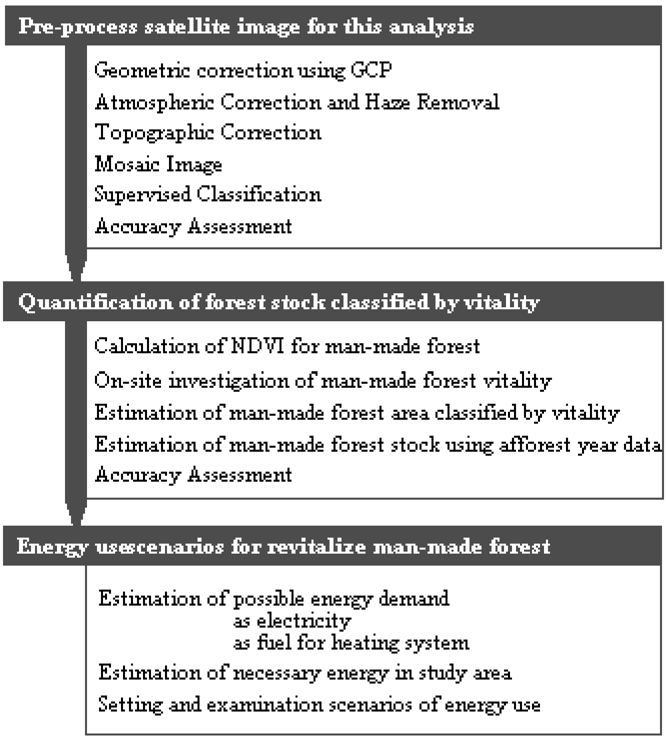

Figure 5-2-4. Study overview

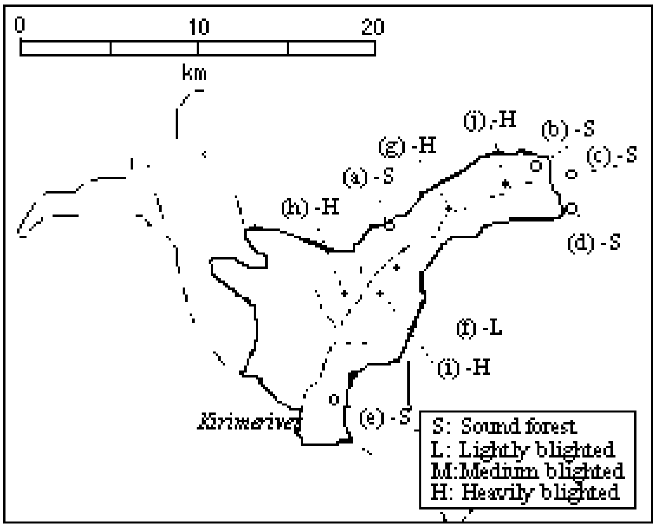

Figure 5-2-5. Sampling points

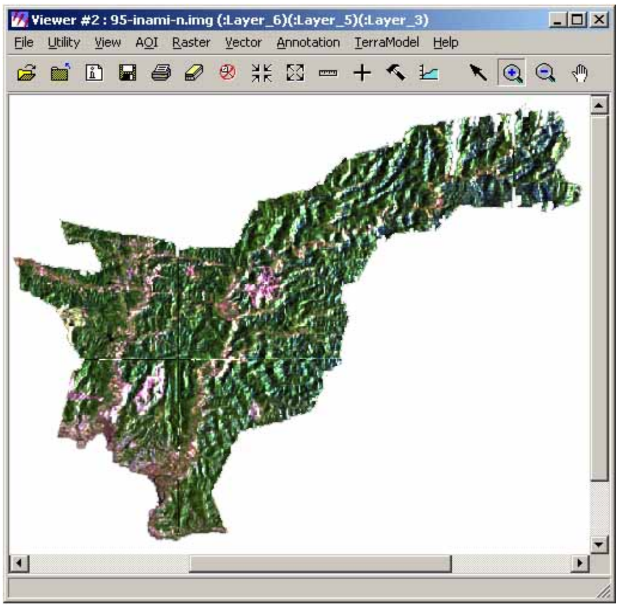

Figure 5-2-6. TM image of study area, 1995

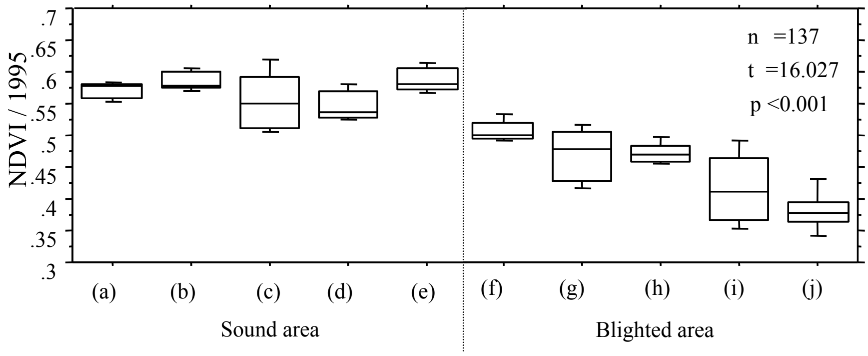

Figure 5-2-7. NDVI of the ten study areas

Table 5-2-1. Man-made forest classified by vitality level

		NDVI Min.	NDVI Max.	Area (km^2)	Area (%)
All man-made forest		-	-	34.98	-
	Sound forest	0.551	1.000	18.19	52.0%
	Lightly blighted forest (blighted under 20%)	0.499	0.511	3.71	10.6%
	Medium blighted forest (blighted 20% - 70%)	0.432	0.499	10.14	29.0%
	Heavily blighted forest (blighted over 70%)	0.373	0.432	2.94	8.4%

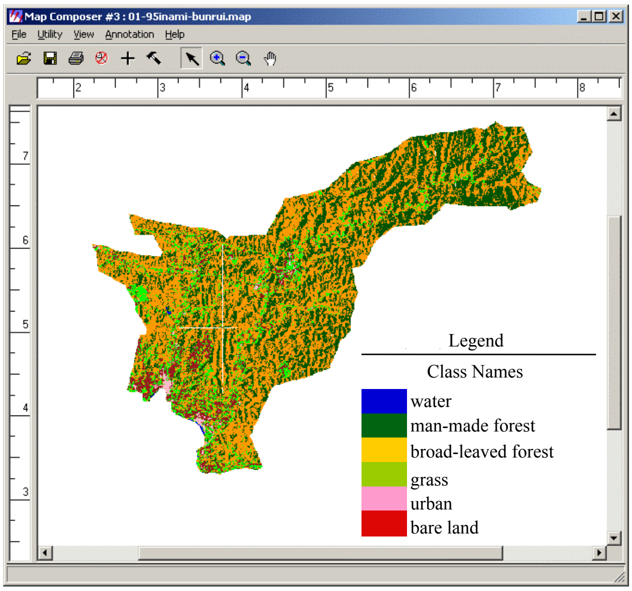

Figure 5-2-8. Land use in Inami town, 1995

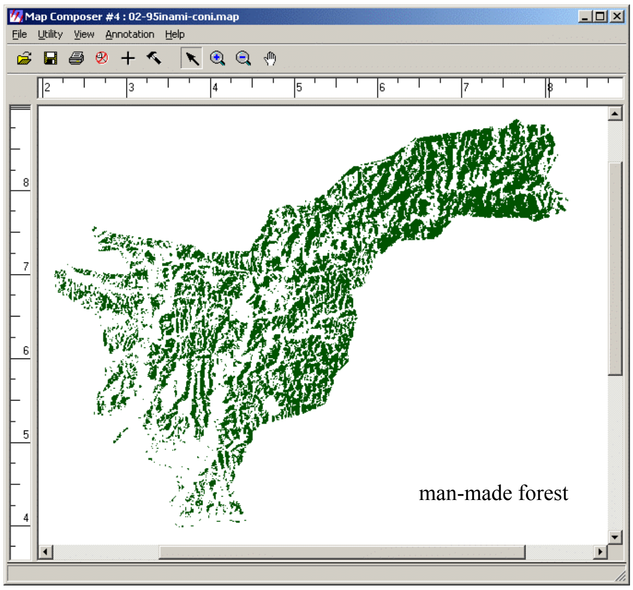

Figure 5-2-9. Man-made forest in Inami town, 1995

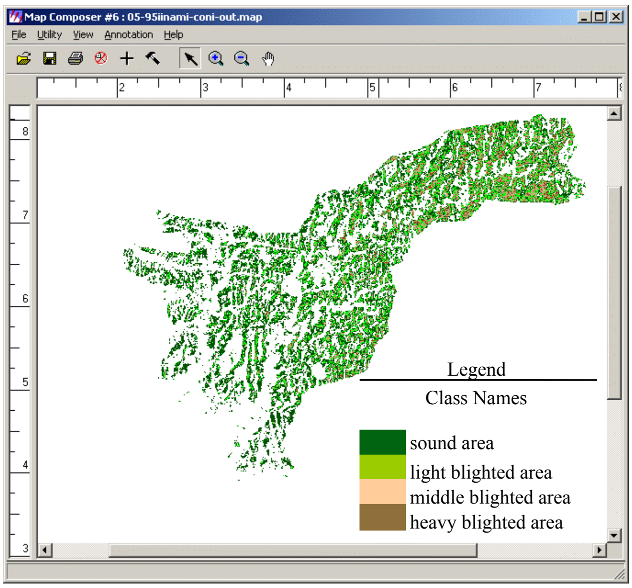

Figure 5-2-10. Man-made forest classified into four levels of vitality

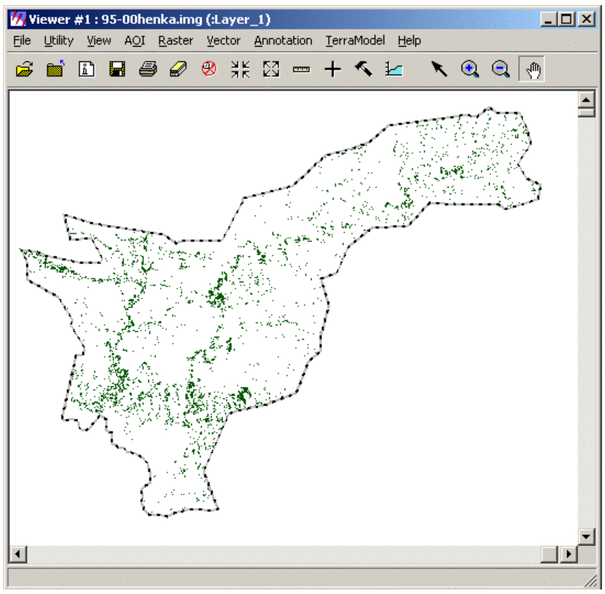

Figure 5-2-11. Change of man-made forest, 1995–2000

Appendix: Methodologies to Research Plant Sensitivities to Acid Deposition

Introduction

There is some evidence in Japan that the dieback of trees and deterioration of forests is related to acid deposition. A dieback of Japanese cedar (*Cryptomeria japonica*), for example, has been observed in the wider area of the Kanto district in central Japan. Also, in the northwestern regions of the district, the deterioration of white birch (*Betula platyphyla*) and Japanese larch (*Larix leptolepis*) can be seen at an elevation of 1,800 meters (m) on Mt. Akagi, about 120 kilometers (km) northwest of Tokyo. On Mt. Ohyama, around 50 km southwest of Tokyo, many dead tree trunks of Japanese fir (*Abies firma*) can be seen at an elevation of 1,200 m. In southwestern Honshu, deterioration of Japanese cedar and red pine forests (*Pinus densiflora*) can be seen along the Seto Inland Sea. As well, in Fukuoka prefecture, Kyushu district (southern Japan), some dieback of Japanese fir trees has been seen in the higher elevations of mountains near Fukuoka. It has been suggested that acid rain or acid fog is one of the causes of the observed forest deterioration and die-offs.

Effects of acid deposition on trees in Japan

The effects of acid rain or acid deposition on vegetation can be classified into two categories of effects: direct and indirect. As a symptom of direct effects, it has been reported that visible damage of crop plants can be detected when rain with an acidity level below a pH of 3.0 is sprayed on the above-ground parts of plants. It is very hard, however, to detect the suppression of tree growth by exposure to simulated acid rain above a pH of 3.5.

According to Izuta et al. (1993), young Japanese fir trees exposed to acid rain for 150 days showed some increase in defoliation rate at a pH of 4.0. In this study, the simulated acid rain consisted of a 1:1 mixture of 1 M of sulfuric acid (H_2SO_4) and 1 M of nitric acid (HNO_3). The seedlings were treated with simulated acid rain of pH 2.0, 3.0, and 4.0, as well as deionized water (the control at pH 6.7), twice a week for 30 weeks, from 8 May through 4 December 1991. Visible red-brown foliar damage was observed in the seedlings treated with rain with a pH of 2.0. At the middle sampling (July 31), the total fresh weight of seedlings treated with rain at pH 2.0, 3.0, and 4.0 was significantly less than that of the control. The total dry weights of seedlings treated with rain at pH 2.0 and 3.0 were significantly reduced by 28% and 20%, respectively, compared with the control. At the final sampling (4 December), the total fresh weight and total dry weight of seedlings treated with rain at a pH 2.0 were significantly reduced (by 23% and 29%, respectively) compared with the control. The relative growth rate and net assimilation rate on a dry plant weight basis during the growth period of 30 weeks were reduced in proportion to the reduction of rain pH. These results suggest that fir seedlings are sensitive to acid rain of pH 3.0 and above (Izuta et al. 1993).

Izuta et al. (1998) reported the symptoms of direct effects of acid rain on several Japanese forest tree species (see Annex 1). Two- or three-year-old seedlings of Japanese white birch (*Betula platyphylla*) and Japanese beech (*Fagus crenata*), two-year-old Japanese cedar (*Cryptomeria japonica*), and eight-year-old Nikko fir (*Abies homolepis*) were used as plant materials. The heights of seedlings of Japanese cedar, Nikko fir, Japanese white birch, and Japanese beech were about 9 centimeters (cm), 17 cm, 59 cm, and 16 cm, respectively. In the greenhouse, seedlings were exposed to simulated acid rain (SAR) of pH 4.0, 3.0, 2.5, and 2.0, and deionized water of pH 5.6 (control rain) three times per week for 20 weeks, from May to September in 1995. The exposure to SAR or deionized water was conducted at a rate of 20 millimeters (mm) per day during the nighttime in the greenhouse. The SAR was a mixture of deionized water, sulfate, nitrate, and chloride (SO_4^{2-} : NO_3^- : Cl^- = 5 : 2 : 3 equivalent ratio). The total amount of precipitation during the exposure period of 20 weeks was about 1,100 mm. The average air temperature and daily cumulative solar radiation in the greenhouse during the exposure period were 20.5 degrees Celsius (°C) (maximum of 29.3°C, minimum of 12.9°C) and 7.3 megajoules (MJ) m^{-2}, respectively. The evaluation of visible foliar injuries, conducted immediately after the completion of 20 weeks of exposure, revealed that when Japanese cedar seedlings were exposed to a SAR of pH 2.0 for 20 weeks, visible necrotic injuries appeared at the tip of the needles. Chlorotic and necrotic injuries were observed at the tip of the needles in Nikko fir seedlings exposed to pH 2.5 and 2.0. In Japanese white birch and beech seedlings, exposure to a SAR of pH 2.5 and 2.0 caused visible foliar injuries such as marginal necrosis and necrotic spots. From these results, the threshold pH of SAR for the appearance of visible foliar injuries is considered to be 2.5 or lower.

As for indirect effects of acid rain, the deposition of air pollutants on forests will affect physiological activities that induce the depression of tree growth. That is, the addition of acid substance in soils will result in an increase of soil acidity to induce the dissolution of aluminum ions, which are very toxic to plant growth. Izuta et al. (2004) performed an experiment on the effects of soil acidification on dry-weight growth, net photosynthesis, and leaf nutrient status of Japanese beech (*Fagus crenata*) seedlings. In November 1997, brown forest soil originating from granite as the parent rock was collected, to which different amounts of hydrogen ions (H^+) were added as a H_2SO_4 solution at 20, 40, 60, and 100 mg L^{-1} on the basis of air-dried soil volume. These soil treatments were designated as S-20 (4.28 in soil pH), S-40 (4.20 in pH), S-60 (4.01 in pH), and S-100 (3.84 in pH), respectively. On the same day, four different amounts of H^+ were added as an HNO_3 solution to the other soil samples at 20, 40, 60, and 100 mg/L^{-1} on the basis of air-dried soil volume, and these soil treatments were designated as N-20 (4.79 in pH), N-40 (4.73 in pH), N-60 (4.00 in pH), and N-100 (3.64 in pH), respectively. The amounts of H^+ added to each soil sample in the soil treatment groups S-20 and N-20, S-40 and N-40, S-60 and N-60, and S-100 and N-100 were 40, 80, 120, and 200 mg H^+pot^{-1}, which correspond to 2,260, 4,520, 6,780, and 11,300 mg H^+ m^{-2} on the basis of potted soil surface area, respectively. Because the maximum deposition of H^+ on the soil surface in Japan between 1986 and 1993 was 47–113 mg m^{-2}, the amounts of H^+ added to the potted soil in each treatment corresponded roughly to the cumulative H^+ loads for 20–48, 40–96, 60–144, and 100–240 years, respectively. Control soil (5.14 in soil pH) and soil treated with lime at 1 g L^{-1} (5.60 in soil pH) on the basis of air-dried soil volume were not supplemented with H^+ as a H_2SO_4 or HNO_3 solution. On 27 April 1998, three-year-old Japanese beech seedlings were transplanted to 2-L plastic pots (soil surface areas of 177 cm^2) that were filled with the treated soils mentioned before. For 526 days, from April 1998 to November 1999, the seedlings were grown in a naturally lit phytotron (day time air temperature at 20°C /13°C at night and relative air humidity of 70%). On 27 April, 8 June, and 16 September 1998, and on 7 June and 5 October 1999, the eight seedlings per soil treatment were harvested to determine leaf number, leaf area, stem diameter, and the dry mass of plant organs.

Table 1 indicates the results of the experiments conducted on 5 October 1999, including studies of stem diameter, leaf area per plant, and dry mass and ratio of shoot dry mass to root dry mass (S/R) of the Japanese beech tree seedlings.

Figure 1 shows the relationship between the pH of soil solution and relative whole-plant dry mass per plant, indicating the clear reduction of whole-plant dry mass below 4.3 in soil solution pH. Figure 2 indicates the relationship between the molar ratio of total concentration of calcium (Ca), magnesium (Mg) and potassium (K) to (aluminum [Al] + manganese [Mn]) concentration (BC/(Al+Mn) ratio) in soil solution and relative whole-plant dry mass per plant, indicating growth depression below the BC/(Al+Mn) ratio of ca. 2. This suggests that the molar ratio of BC/(Al+Mn) in soil solution is a suitable soil parameter for evaluating critical loads of acid deposition in efforts to protect Japan's beech forests (Izuta et al. 2004).

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Table 1. Stem diameter, leaf area per plant, dry mass, and ratio of shoot dry mass to root dry mass (S/R) of *F. crenata* seedlings on 5 October 1999

Soil treatment	Stem diameter (cm)	Leaf area (cm^2)	Leaf number	Dry mass (g)				S/R ($g\ g^{-1}$)
				Leaf	Stem + Branch	Root	Whole-plant	
Lime	0.968 a	463.2 abc	74 ab	2.026 bc	7.354 a	8.465 a	17.545 a	1.16 b
Control	0.892 ab	448.5 abc	77 ab	2.165 ab	7.671 a	7.407 a	17.242 a	1.33 ab
S-20	0.892 ab	454.0 abc	84 ab	2.085 bc	8.219 a	8.422 a	18.726 a	1.28 ab
S-40	0.903 ab	355.2 bcd	72 ab	1.730 bcd	7.255 ab	6.654 ab	15.639 ab	1.44 ab
S-60	0.869 ab	331.6 bcd	57 bc	1.216 de	6.031 ab	5.252 bc	12.499 bc	1.36 ab
S-100	0.661 c	244.4 d	38 c	0.983 e	3.187 c	3.698 c	7.868 d	1.07 b
N-20	0.885 ab	485.0 ab	91 a	2.345 ab	8.325 a	7.900 a	18.570 a	1.28 ab
N-40	0.868 ab	586.8 a	85 ab	2.725 a	8.274 a	8.190 a	19.189 a	1.45 ab
N-60	0.881 b	335.8 bcd	58 bc	1.523 cde	6.328 ab	5.091 bc	12.942 bc	1.56 a
N-100	0.806 b	295.7 cd	58 bc	1.333 de	4.842 bc	4.196 c	10.371 cd	1.59 a

Note: Each value is the mean of six determinations. The values followed by different letters within a column are significantly different according to Duncan's multiple-range test ($P<0.05$).

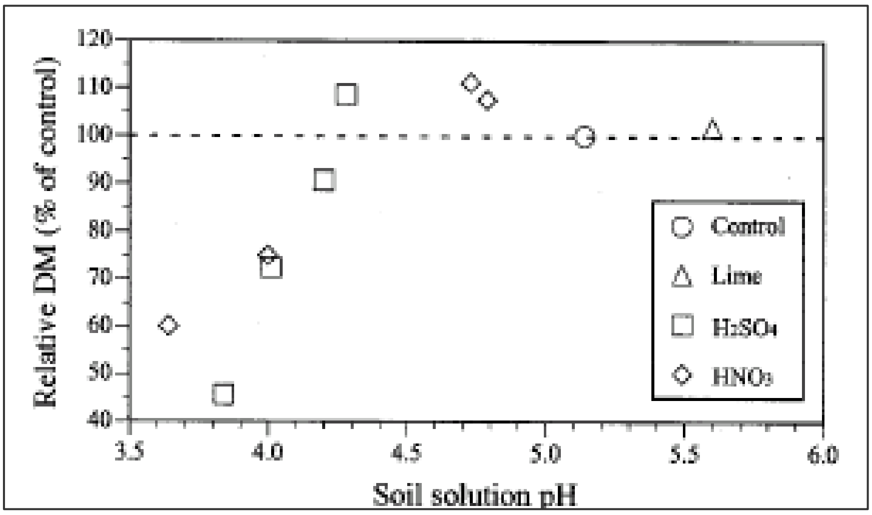

Figure 1. The relationship between the pH of soil solution and relative whole-plant dry mass per plant of *F. crenata* seedlings grown in acidified soil to that of the seedlings grown in the control soil (relative dry matter [DM]). The seedlings were grown in brown forest soil acidified by adding H^+ as H_2SO_4 (square) or HNO_3 solution (diamond). Control soil (circle) and that treated with lime (triangle) were not supplemented with H^+ as H_2SO_4 or HNO_3 solution (Izuta et al. 2004).

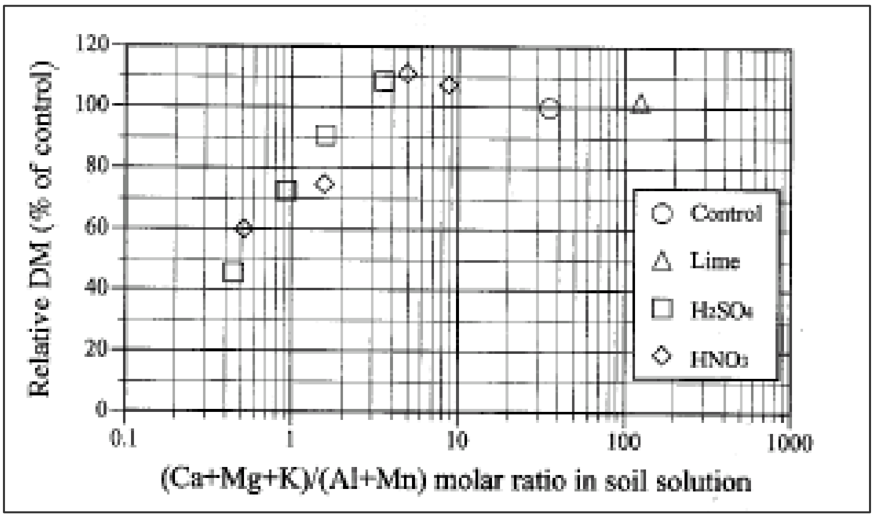

Figure 2. The relationship between the molar ratio of total concentration of Ca, Mg, and K to Al concentration in soil solution ((Ca+Mg+K)/(Al+Mn)) and relative whole-plant dry mass per plant of *F. crenata* seedlings grown in acidified soil to that of the seedlings grown in the control soil (relative DM). The seedlings were grown in brown forest soil acidified by adding H^+ as H_2SO_4 (square) or HNO_3 solution (diamond). Control soil (circle) and that treated with lime (triangle) were not supplemented with H^+ as H_2SO_4 or a HNO_3 solution (Izuta et al. 2004).

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Article

Visible Foliar Injuries Induced by Simulated Acid Rain in Several Japanese Forest Tree Species*[1]

Takeshi IZUTA*[2], Takuya KOBAYASHI*[3], Hideyuki MATSUMURA*[3], Yoshihisa KOHNO*[3] and Takayoshi KOIKE*[4]

The effects of simulated acid rain on the appearance of visible foliar injuries were investigated in 2-year-old seedlings of Japanese cedar, 8-year-old seedlings of Nikko fir, 2-year-old seedlings of Japanese white birch and 3-year-old seedlings of Japanese beech. The seedlings were exposed to simulated acid rain (SAR, SO_4^{2-}:NO_3^-:Cl^-=5:2:3, equivalent ratio) of pH 4.0, 3.0, 2.5 or 2.0, and deionized water of pH 5.6 (control rain), three times per week, for 20 weeks from May to September. The exposure to SAR or deionized water was conducted at a rate of 20mm per day during the nighttime (8-10 h per day), and the total amount of precipitation during the exposure period of 20 weeks was ca. 1100mm.

When the Japanese cedar seedlings were exposed to SAR of pH 2.0 for 20 weeks, visible necrotic injuries appeared at the tip of the needles. Chlorotic and necrotic injuries were observed at the tip of the needles in Nikko fir seedlings exposed to SAR of pH 2.5 and 2.0 for 20 weeks. In Japanese white birch and beech seedlings, exposure to SAR of pH 2.5 or 2.0 for 20 weeks caused visible foliar injuries such as marginal necrosis and necrotic spot. Therefore, the threshold pH of SAR for the appearance of visible foliar injuries was considered to be 2.5 or below, which suggests that visible foliar injury is a useful plant indicator for evaluating the direct effects of natural acid fog of pH 2.5 or below on Japanese forest tree species.

Keywords: simulated acid rain, visible foliar injury, *Cryptomeria japonica* D.Don, *Abies homolepis* Sieb. et Zucc., *Betula platyphylla* Sukatchev var. *japonica* (Miq.) Hara, *Fagus crenata* Blume

1. INTRODUCTION

Dry or wet deposition of acidic substances in forest ecosystems is one of the most serious environmental problems worldwide[1]. There have been many experimental studies on the effects of simulated acid rain on dry weight growth and physiological functions, such as photosynthesis and dark respiration, of forest tree species native to Europe and North America[2]. However, only few studies have been reported on the effects of simulated acid rain on Japanese forest tree species[3-7]. To protect Japanese forest ecosystem from the adverse effects of acidic substances not only now, but also in the

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near future, it is necessary to clarify the effects of acid rain or fog on Japanese forest tree species.

To evaluate the direct and adverse effects of natural acid rain or fog on Japanese forest tree species, we need to select some plant indicators sensitive to acid rain or fog. In general, the extent of visible foliar injury is considered to be a useful plant indicator to evaluate the adverse effects of environmental stresses, such as gaseous air pollutants, on crop and woody plants. However, very limited information on acid rain- or fog-induced visible foliar injuries is available for Japanese forest tree species[8].

In the present study, we investigated the effects of simulated acid rain on the appearance of visible foliar injuries in seedlings of Japanese cedar, Nikko fir, Japanese white birch and Japanese beech to clarify whether visible foliar injury is useful as a plant indicator for detecting the direct effects of natural acid rain or fog on Japanese forest tree species.

2. MATERIALS AND METHODS

Two-year-old seedlings of Japanese cedar (*Cryptomeria japonica* D.Don), 8-year-old seedlings of Nikko fir (*Abies homolepis* Siebold et Zuccarini), 2-year-old seedlings of Japanese white birch (*Betula platyphylla* Sukatchev var. *japonica* (Miq.) Hara) and 3-year-old seedlings of Japanese beech (*Fagus crenata* Blume) were used as plant materials in this study. Seedling sizes of Japanese cedar, Nikko fir, Japanese white birch and Japanese beech at the beginning of the experiment were ca. 9cm, 17 cm, 59cm and 16cm in height, respectively. The seedlings were grown in 1.8 L (Japanese cedar, Nikko fir and Japanese beech) or 5.0 L (Japanese white birch) pot filled with brown forest soil originating from granite mother rock. The soil was collected by the authors from the B horizon under a coniferous forest at the Kusaki University Forest of Tokyo University of Agriculture and Technology (TUAT) in Gunma Prefecture, central Japan.

In April 1995, the seedlings were placed in a greenhouse at the Central Research Institute of Electric Power Industry (CRIEPI, Abiko, Chiba Prefecture, Japan). The seedlings were then exposed to simulated acid rain (SAR) of pH 4.0, 3.0, 2.5 or 2.0, and deionized water of pH 5.6 (control rain), three times per week, for 20 weeks from May to September. The exposure to SAR or deionized water was conducted at a rate of 20mm per day during the nighttime (8-10 h per day). The SAR was a mixture of deionized water, sulfate, nitrate and chloride (SO_4^{2-}:NO_3^-:Cl^-=5:2:3, equivalent ratio). The total amount of precipitation during the exposure period of 20 weeks was ca. 1100 mm. Average air temperature and average daily cumulative solar radiation in the greenhouse during the exposure period of 20 weeks were 20.5℃ (max. 29.3℃, min. 12.9℃) and 7.3 MJ · m^{-2}, respectively. The evaluation of visible foliar injuries was conducted immediately after the exposure period of 20 weeks.

3. RESULTS AND DISCUSSION

No visible injury developed on the needles of Japanese cedar seedlings exposed to control rain or SAR of pH 4.0, 3.0 and 2.5. However, when the seedlings were exposed to SAR of pH 2.0 for 20 weeks, visible necrotic injuries appeared at the tip of the needles (Fig. 1). The color of the needles tended to deepen when the seedlings were exposed

Fig.1 Visible foliar injury of Japanese cedar seedlings exposed to simulated acid rain of pH 2.0 for 20 weeks.

to SAR of pH 3.0 for 20 weeks (Fig. 2).

The 20-week exposure to the control rain or SAR of pH 4.0 and 3.0 did not cause any visible injuries on the needles of Nikko fir seedlings. However, chlorotic and necrotic injuries were observed at the tip of the needles in the seedlings exposed to SAR of pH 2.5 and 2.0 for 20 weeks (Fig. 3). Defoliation was induced during the exposure period in the seedlings exposed to SAR of pH 2.5 and 2.0. When the seedlings were exposed to SAR of relatively low pH for 20 weeks, the green color of the needles tended to deepen (Fig. 4).

Japanese white birch seedlings exposed to control rain or SAR of pH 4.0 and 3.0 for 20 weeks did not show any visible foliar injuries. As shown in Fig. 5, marginal necrosis and necrotic spots appeared on the leaves of seedlings exposed to SAR of pH 2.5 and 2.0 for 20 weeks. Furthermore, defoliation was observed in the seedlings exposed to SAR of pH 2.0 during the exposure period (Fig. 6).

In Japanese beech seedlings, no visible foliar injury was induced by 20-week exposure to control rain or SAR of pH 4.0 and 3.0. However, exposure to SAR of pH 2.5 for 20 weeks caused visible foliar injuries such as marginal necrosis and necrotic spots. Fig. 7 shows the developmental process of visible foliar injury in the seedlings exposed to SAR of pH 2.5. As the frequency of exposure to SAR of

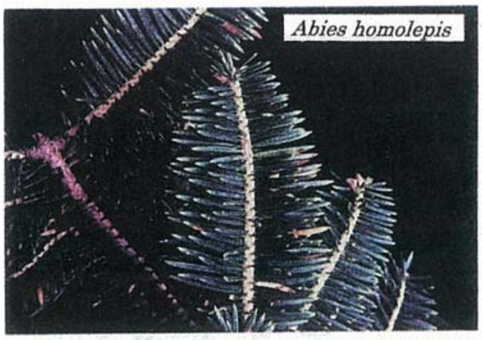

Fig.3 Visible foliar injury of Nikko fir seedlings exposed to simulated acid rain of pH 2.5 for 20 weeks.

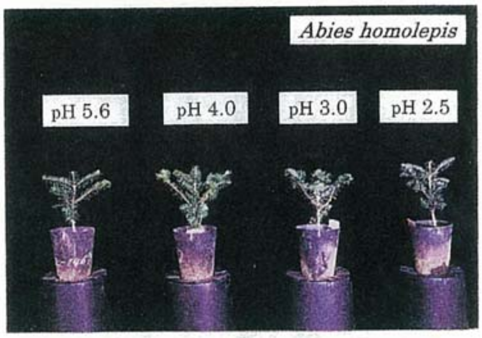

Fig.4 Nikko fir seedlings exposed to control rain of pH 5.6 and simulated acid rain of pH 4.0, 3.0 or 2.5 for 20 weeks.

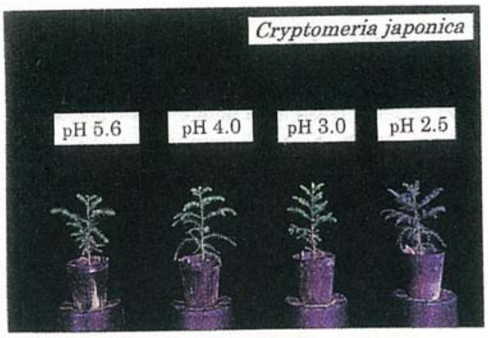

Fig.2 Japanese cedar seedlings exposed to control rain of pH 5.6 and simulated acid rain of pH 4.0, 3.0 or 2.5 for 20 weeks.

Fig.5 Visible foliar injury of Japanese white birch seedlings exposed to simulated acid rain of pH 2.5 for 20 weeks.

Fig.6 Japanese white birch seedlings exposed to simulated acid rain of pH 3.0 (left) and 2.0 (right) for 20 weeks.

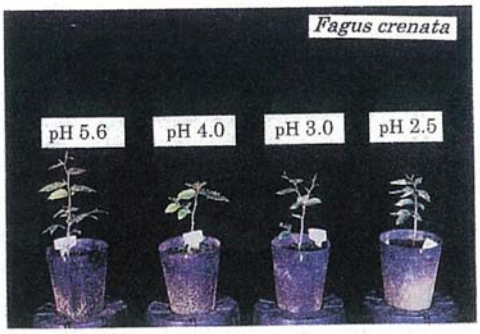

Fig.8 Japanese beech seedlings exposed to control rain of pH 5.6 and simulated acid rain of pH 4.0, 3.0 or 2.5 for 20 weeks.

Fig.7 The developmental process of visible foliar injury in Japanese beech seedlings exposed to simulated acid rain of pH 2.5.

Table 1. Threshold pH of simulated acid rain for appearance of visible foliar injuries in seedlings of Japanese cedar, Nikko fir, Japanese white birch and Japanese beech.

Species	pH of simulated rain				
	5.6	4.0	3.0	2.5	2.0
Japanese cedar	−	−	−	−	+
Nikko fir	−	−	−	+	+
Japanese white birch	−	−	−	+	+
Japanese beech	−	−	−	+	ND

− No visible injury developed.
\+ Visible foliar injury developed. ND No data.

pH 2.5 increased, the degree of marginal necrosis also increased. As a result, early fall of the leaves with visible injuries was induced by exposure to SAR of pH 2.5 (Fig. 8).

Table 1 shows the threshold pH of SAR for the appearance of visible foliar injuries in the seedlings of Japanese cedar, Nikko fir, Japanese white birch and Japanese beech. Among the 4 species tested in the present study, Japanese cedar was relatively insensitive to SAR of low pH compared with the other 3 species. As a whole, the threshold pH of SAR for the appearance of visible foliar injuries was considered to be 2.5 or below. Kohno *et al.*[8] reported that deciduous broad-leaved trees are relatively sensitive to SAR compared with coniferous trees, and most of the tree species tested in their study did not show any visible foliar injuries when seedlings were exposed to SAR of pH 4.0 or above. In Japan, the pH value of natural rain is generally 4.0 and above[9]. Therefore, it is very difficult to evaluate the direct effects of natural acid rain on the 4 tree species tested in the present study, based on the appearance and/or extent of visible foliar injuries. In Japan, however, fog with relatively low pH was often observed in the field. For example, acid fog of pH 1.95 was observed in Mt. Ohyama in the Tanzawa Mountains (Kanagawa Prefecture, central Japan)[10]. Therefore, visible foliar injury is considered to be a useful plant indicator for detecting the direct effects of acid fog of pH 2.5 or below on Japanese forest tree species.

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