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International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests)

MANUAL

on

methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests

Part XII

Sampling and Analysis of Needles and Leaves

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1. Introduction

The aims of forest condition assessments are the monitoring of the health state of forests, and the detection of time trends and spatial patterns. Insight in the causes of changes can only be achieved if additional parameters from other ecosystem compartments are available. The nutritional state of trees is often indicative of processes at the ecosystem level. Inadequate nutrient supply may be a direct cause of low tree vitality or a factor which increases adverse air pollution effects. High concentrations of certain elements in the leaf or needle tissues may be the effect of intoxication or of high air-pollution levels. Unfavourable chemical conditions in the rooting zone of the soil may also lead to imbalances in the nutrient supply and subsequently to unbalanced nutrition of the trees. Thus, sampling and analysis of needles and leaves is essential. The analyses have to be performed at regular time intervals in order to establish potential relationships between changes in the stand condition and changes of the nutritional status. In the studies of intermediate or high intensity (see below), foliage sampling must be frequent enough to detect trends in the mineral nutrition of trees, not biased by interannual fluctuations in element concentrations.

2. Scope and application

The objective of this Part of the Manual is to provide harmonized and standard procedures for the sampling and analysis of leaves and needles at the monitoring plots of the UNECE ICP Forests. Harmonization is necessary to permit trans-national studies on the spatiotemporal trends of nutritional status and the impact of air-pollutants of forest trees. Procedures are intended for application at both large-scale (Level I) and intensive (Level II and core) monitoring plots. To have their data used in the international database and evaluations, National Focal Centers and their scientific partners participating to the ICP Forests programme should follow the methods described here and achieve the reported data quality requirements. Parameters measured within the foliage survey are listed in Table 1.

Table 1: Parameters measured within the foliage survey

Form	Parameter/variable	Level II	Level II core	Level I
FOM	mass of 100 leaves / 1000 needles (g)	m	m	o
FOM	N [mg/g]	m	m	o
FOM	S [mg/g]	m	m	o
FOM	P [mg/g]	m	m	o
FOM	Ca [mg/g]	m	m	o
FOM	Mg [mg/g]	m	m	o
FOM	K [mg/g]	m	m	o
FOM	C [g/100g]	m	m	o
FOO	Zn [µg/g]	o	o	o
FOO	Mn [µg/g]	o	o	o
FOO	Fe [µg/g]	o	o	o
FOO	Cu [µg/g]	o	o	o
FOO	Pb [µg/g]	o	o	o
FOO	Cd [ng/g]	o	o	o
FOO	B [µg/g]	o	o	o

3. Objectives

The objective of foliar analysis is to estimate the nutritional status of trees and the impact of air pollutants at the monitoring sites, the detection of time trends and spatial patterns and to contribute to the understanding and quantification of forest condition in Europe.

Specific objectives are:

Quantification of the mean element concentrations of N, S, P, Ca, Mg, K and C with an accuracy level of $\pm$20% and other nutrients and heavy metals with the accuracy level of $\pm$30% with a documented statistical precision.

Detection of temporal trends of mean element concentrations of nutrients and heavy metals on sample plots within 10 years with a documented statistical precision.

4. Location of measurements and sampling

4.1 Sampling design

4.1.1 Selection of sample trees

Sample trees (minimum of 5 trees) should be randomly selected to represent the population of trees fulfilling following qualifications

Only tree species listed in the species code in the Manual Part on data handling and the data submission forms are to be sampled. In case the plot represents a mixed stand the most common tree species (belonging to the dominant or predominant classes) is to be sampled

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

the trees belong to the predominant and dominant classes (forest with closed canopy) or to the trees with average height ± 20% (forest with open canopy);

the trees are different from those used for the crown assessment, so as to avoid crown damage due to successive samplings; if stand and site conditions are homogeneous on an area larger than the plot where the crown conditions have been assessed, it is recommended to choose the sample trees outside the crown assessment plot;

In case of loss, or significant damage (to the trunk or crown), of sample tree(s) the new tree(s) has to be selected according to the selection criterion mentioned above.

The same sample trees should be sampled over the years; the trees must be numbered. For species with small crowns and small foliage mass, it is allowed to alternate between two sets of sample trees (minimum of 5 trees per set), if necessary, to avoid damage of the sample trees. Each set must correspond to the above conditions.

4.1.2 Location and number of replicates

4.1.2.1 Large-scale Level I plots

At least 5 trees should be sampled from the main species within a stand. A composite sample by species can be made by mixing equal quantities of each sample per plot after drying. If the trees are analyzed individually, the mean value is calculated for each element for each plot based on the tree wise samples.

4.1.2.2 Standard Level II and core plots

In case there is no prior information about the representative sample size (i.e. number of sample trees) for the foliar parameter being measured (e.g. prior surveys or information in literature) sample size can be determined from a pilot study. Statistical analysis of the measurements should be made for each plot in order to ensure that the sampling procedure meets the specific objectives (see Chapter 3).

In every case, at least 5 trees of the main species present in the plot have to be sampled but for statistical reasons it is recommended to sample more trees. The samples are individually preserved in bags for analysis. A composite sample by species can be made by mixing equal quantities of each sample per plot after drying. But to get information about the variation of each element on the plot (see above) it is recommended to analyze the samples from the trees individually, the mean value is calculated then for each element for each plot. In case more than one species is sampled per plot above procedure is repeated for each species (i.e. different species are not mixed at any point).

4.1.3 Selection of leaves and needles

In general, sampling must be carried out in such a way that all the orientations are represented in the set of sample trees. However, it is important that the sampled leaves or needles have developed in light; hence south-facing branches are recommended and orientations in the canopy that are shaded should be avoided. If necessary, it is allowed to sample different orientations on each tree of the sample set. In special sites with evident influence of one orientation (e.g. steep slopes or strong dominant wind) only one orientation is sampled, which always have to be the same. In such cases, it is necessary to document the orientation.

In general, the current year needles or leaves of evergreen species are most convenient for judging the actual nutritional state, but for a number of elements the comparison of element contents in older needles with that in current year needles is also of interest.

The sampled leaves or needles must be taken from the upper third of the crown, but not from the very first whorls in the conifers; in stands where the different whorls can be clearly identified it is advisable to sample between the 7th and the 15th whorl.

4.1.3.1 All species

It is necessary to take care that leaves or needles which are sampled are mature ones, especially for species which have several flushes per year (e.g, *Pinus halepensis*, *Pseudotsuga menziesii*, *Eucalyptus* spp., *Quercus* spp.). For *Larix* spp. and *Cedrus* spp. samples are taken of the short twigs of the previous year.

4.1.3.2 Deciduous species (including larch)

Sampling is done on current year leaves or needles.

4.1.3.3 Evergreen species

Sampling of both the current year needles or leaves and the second year needles or leaves (current+1) is (see Annex I):

optional on Level I plots

recommended on Level II and core plots

In case separation of older foliage age classes is not possible (e.g. in *Quercus ilex* leaves can be separated only into current leaves and older than current leaves) it is allowed to sample and analyse the 1) current foliage (C-foliage) and 2) older than current foliage (containing C+1, C+2, C+3 etc. age classes, cf. Annex I).

In case total nutrient budget in a plot is to be determined also older foliage than current and current+1 foliage (if present) needs to be sampled and analysed (cf. chapter 6.4).

4.2 Sampling frequency

4.2.1 Survey status and sampling frequency

4.2.1.1 Large-scale Level I plots

Foliar analysis is optional in extensive monitoring (Level I) plots. If a country decides to perform such analyses, it is recommended that sampling and analyses are performed at least every ten years parallel to soil survey.

4.2.1.2 Intensive Level II and core plots

Foliar analysis is mandatory on intensive monitoring (Level II) plots. Sampling and analysis must be performed at least every two years, but many environmental changes might require annual sampling to be detected. In case sampling is performed biannually, the sampling should be performed in uneven years (2011, 2013, 2015 etc.) to obtain simultaneous data from different countries.

4.2.2 Date of sampling

Deciduous species (including larch): Sampling must be performed during the second half of the growing season and before the very beginning of the autumnal yellowing and senescence.

Evergreen species: Sampling must be performed during the dormancy period. The length of dormancy period depends on the region. Samples collected in the beginning of the year are considered to represent previous year's conditions (e.g. samples collected in February 2012 are considered to represent 2011 sample collection). Member states are requested to define for each region, treating the plains and mountains within each region separately, the most convenient period for sampling and analysis of the different species, and to stick to this period.

4.3 Sample collection, transport and storage

4.3.1 Methods of sampling

As trees must not be felled, any convenient way of sampling is acceptable (e.g. climbing, tree-pruner or hydraulic lift), provided that it does not lead to contamination of the sample, to heavy tree damage, or to risk for the sampling team. Contamination of the samples, e.g. through contact by hands or soil, should be avoided. In case climbing is needed to reach the upper third of the crown, this can be facilitated by installing a permanent pulley and rope to the sample trees. This can help to reach the same position in the canopy, reduce damage to the trunk and to enhance safety. However, the pulley and rope must be installed so that the sample trees are not damaged. Also in case crampons are used in climbing damage to the trunk must be avoided.

The sampled branches of each tree are stored in a bag for transport to laboratory (cf. 4.3.3). In case it is foreseen that transportation to laboratory takes long time, or takes place during warm weather, the samples should be stored in dark and cool during the transportation and during storage in laboratory before the pre-treatment.

4.3.2 Quantity of material to be sampled

The recommended minimum quantities are:

10-20 grams of fresh needles or leaves (resulting in 5-10 grams of dry material) for each sampled age class for mandatory analysis;

20-30 grams of fresh needles or leaves for both mandatory and optional analysis.

Each country may decide to sample a larger quantity of leaf material, according to the needs of its own analytical methods, or in order to conserve samples for future analysis. It is recommended to store dried, ground samples for the future use.

4.3.3 Pre-treatment before sending the samples to the laboratories for analysis

For broad-leaved trees, it may be advisable to detach the leaves from the twigs (and for some species, the small leaves from the axis) but this is not necessary for conifer needles. The shoots of the current year and those of the second year are separated and preserved in separate bags. The use of perforated resistant paper bags or polyethylene bags is recommended. If the samples cannot be send immediately to the laboratory they have to be dried in a clean ventilated room keeping the bags open, after which they should be stored in a cool place before sending. If samples are used also for visual assessment of possible discolourations and damages (see chapter 5.1.2.1.) the samples should be frozen after sampling or alternatively a sub-sample should be collected and freezed until the visual assessment. Great care must be taken to mark each sample clearly (forest, number of plot, number of tree, species, age of needles, etc.) before sending it to the laboratory for analysis. These identifications must be given on the outer side of the bag (directly on the bag by indelible ink, or by clasping a label on the bag). It is recommended to repeat these identifications on the inner side of the bag on a paper label written with indelible ink. The label should be folded in order to avoid leaves or needle contamination by contact with ink.

5. Measurements

5.1 Measurements and reporting units

5.1.1 Selected variables

Levels I and II and core plots:

mandatory: dry mass of 100 leaves or 1000 needles, N, S, P, Ca, Mg, K and C

optional: Zn, Mn, Fe, B, Pb, Cu and Cd

Only the total concentration of elements in needles or leaves must be given by reference to 105°C-dried material (Table 2).

Table 2: Drying temperatures

Type of sample	Drying temperature
A. Samples for the measurement of dry mass of 100 leaves or 1000 needles	105 °C
B1. Leaves / needles drying before grinding	≤ 70 °C
B2. Powder for moisture content determination (subsample of B1), not chemically analysed	105 °C

5.1.2 Analysis

5.1.2.1 Pre-treatment of the samples

Before drying and further processing the samples it is recommended to record additional information about colour, discolouration (e.g. symptoms of nutrient deficiency) and symptoms of ozone injury, different diseases and insect attacks and other damage on the needle/leaf samples. The method of this visual assessment should follow the procedures (methods and formats) used in the Crown Condition Assessment, the Damage Cause Assessment and Symptoms of Ozone Injury Assessment.

The determination of the dry mass of 100 leaves or 1,000 needles (by drying at 105°C) is recommended for Level II (Table 2). It is not necessary to cut the petioles of the leaves but in case of compound leaves it may be advisable to detach the small leaves from the axis if this has not been done in the forest. Use polyethylene gloves to avoid contamination. It is not necessary to wash the samples regularly, but it may be advisable in regions with a high level of air pollution or near the sea. The samples shall be washed with distilled water. Oven drying must be done at a maximum of 70°C for at least for 24 hours, and in any case till constant weight is achieved (Table 2). After drying the needles shall be removed from the twigs with the same precautions as for detaching the small leaves from their axis. Dry samples shall be ground in order to obtain a fine homogeneous powder. There will always remain some fibres, depending on the tree species; this is not a major inconvenience if they are small and if the powder is mixed carefully before taking samples for analysis. For Mn, Fe, Cu, Cd and Pb determination, it has to be assured that the grinder does not contaminate the samples. The grinder should be tested for the release of contaminating materials with dried fibrous cellulose. Before and after grinding the leaf/needle materials such test should be carried out.

5.1.2.2 Digestion (or ashing) and analysis

The recommended methods for digestion or ashing and analysis are given in Table 3a and 3b. Other methods may be allowed, but each country must validate the national method. To assure the homogeneity of the sample a minimum sample amount of 100 - 200 mg depending on the particle size for the analysis is recommended. It is necessary to compare the total element concentrations obtained by national methods with those of reference standard samples and with samples of the interlaboratory comparison tests (Fürst 2009, 2010).

Table 3a: Analytical pre-treatment procedures recommended for the analysis of foliage (ordered by element and frequency used in the ringtests).

Parameter	Pre-treatment	Comments
N	No pre-treatment	for element-analyzer
	Kjeldahl digestion method (sulphuric acid + catalyst)	Possible catalysts: Se, Cu, Ti/Cu, Hg
S	No pre-treatment	for element-analyzer, X-Ray method (pelleting)
	Microwave pressure digestion – closed system (nitric acid or nitric acid mixtures)	
	Pressure digestion – closed system (nitric acid or nitric acid mixtures)	
	Wet ashing at room pressure – open system (nitric acid or nitric acid mixtures)	S losses are possible
P, K, Ca, Mg, Fe, Mn, Zn, Cu	Microwave pressure digestion – closed system (nitric acid or nitric acid mixtures)	Contamination is possible from the dish washer (P, K, Zn), glass ware (Ca, Mg, K, Zn), water (Ca, Mg, Cu) and dust (Fe, Zn). Depending of the sample type Fe is not completely dissolved (fixed on the silica matrix)
	Wet ashing at room pressure – open system (nitric acid or nitric acid mixtures)	Contamination is possible from the dish washer (P, K, Zn), glass ware (Ca, Mg, K, Zn), water (Ca, Mg, Cu) and dust (Fe, Zn)
	Pressure digestion – closed system (nitric acid or nitric acid mixtures)	
	No pre-treatment	X-Ray method (pelleting)
Pb, Cd, B	Microwave pressure digestion – closed system (nitric acid or nitric acid mixtures)	Contamination is possible from the dish washer, water and dust Glass ware should not be used for B
	Pressure digestion – closed system (nitric acid or nitric acid mixtures)	
C	No pre-treatment	for element-analyzer

Table 3b: Analytical determination procedures recommended for the analysis of foliage (ordered by element and frequency used in the ringtests).

Parameter	Determination	Comments
N	element-analyzer	To avoid homogeneity problems a minimum sample weight of >100mg should be used.
	Titration after *Kjeldahl* digestion	
S	ICP-AES	e.g. 181.972nm
	element-analyzer	To avoid homogeneity problems a minimum sample weight of >100mg should be used.
	X-ray fluorescence	
P	ICP-AES	e.g. 213.618nm
	VIS-Photometry	All organic P compound must be digested to PO_4^{3-}.
	X-ray fluorescence	
Ca	ICP-AES	ICP-AES method is a very sensitive method, it is better to use a not so sensitive line (e.g. 317.933nm or 318.127nm)
	Flame-AAS	If you use an air/acetylene flame a spectral buffer (e.g. La^{2+} or EDTA) must be added and matrix adapted blank and standard solutions (same acid concentrations like the sample) are necessary.
	X-ray fluorescence	
Mg	ICP-AES	ICP-AES method is a very sensitive method, it is better to use a not so sensitive line (e.g. 285.213nm).
	Flame-AAS	Matrix adapted blank and standard solutions (same acid concentrations like the sample) are necessary.
K	ICP-AES	e.g. 766.491nm or 769.867nm
	Flame-AAS	If you use an air/acetylene flame a ionisation buffer (e.g. CsCl) must be added and matrix adapted blank and standard solutions (same acid concentrations like the sample) are necessary.
	Flame-AES	Matrix adapted blank and standard solutions (same acid concentrations like the sample) are necessary.
	X-ray fluorescence	
C	element-analyzer	
Zn	ICP-AES	e.g. 213.857nm
	X-ray fluorescence	
	Flame-AAS	Matrix adapted blank and standard solutions (same acid concentrations like the sample) are necessary.
Mn	ICP-AES	e.g. 257.610nm or 260.568nm
	Flame-AAS	Matrix adapted blank and standard solutions (same acid concentrations like the sample) are necessary.
	X-ray fluorescence	
Fe	ICP-AES	e.g. 238.204nm
	Flame-AAS	Matrix adapted blank and standard solutions (same acid concentrations like the sample) are necessary.
	X-ray fluorescence	
Cu	ICP-AES	e.g. 327.395nm or 324.754nm

Parameter	Determination	Comments
	Flameless-AAS	Recommend for low concentrations < 3µg/g a matrix modifier should be used (e.g. Pd)
	ICP-MS	Recommend for low concentrations < 3µg/g
	Flame-AAS	Matrix adapted blank and standard solutions (same acid concentrations like the sample) are necessary.
Pb	Flameless-AAS	Recommend for low concentrations < 1µg/g a matrix modifier should be used (e.g. Pd)
	ICP-MS	Recommend for low concentrations < 1µg/g
	ICP-AES	e.g. 220.253nm Only for higher concentrations and/or with special equipment (ultrasonic nebulizer)
Cd	ICP-MS	Recommend for low concentrations < 100ng/g
	Flameless-AAS	Recommend for low concentrations < 100ng/g a matrix modifier should be used (e.g. Pd)
	ICP-AES	e.g. 214.439nm Only for higher concentrations and/or with special equipment (ultrasonic nebulizer)
B	ICP-AES	e.g. 249.772nm Contamination problems (glass ware should not be used)
	ICP-MS	Contamination problems (glass ware should not be used)

5.2 Quality Assurance and Quality Control

5.2.1 Quality Assurance in the field

A national reference manual or standard operating procedures should be prepared, in which each step in the monitoring procedure is strictly defined and given to the personnel in connection with training in the field. Every step of the work, including special precautions needed to avoid contamination during sampling, coding system to identify the samples later on etc. should be well documented.

Communication between the field personnel and central project manager should be regular. All the operations and specific incidents or observations at the plot should be noted in the sampling logbook. When possible, visits should be made to the sampling plots and the sampling procedures checked by the project manager at least once a year.

5.2.2 Quality Assurance in the laboratory

The quality of the foliar analytical data is controlled by the regular organisation of Interlaboratory Comparisons (ring tests) by the Forest Foliar Co-ordinating Centre. Guidelines for QA/QC procedures in the laboratory are given in the Sub-manual Quality Assurance and Control in Laboratories. Documentary proof of the QA/QC adopted in each laboratory should be submitted, together with the annual results, to the European-level data centre (*FO.LQA File).

5.2.2.1 Plausibility limits

The plausibility limits (5-95 Percentile) for different tree species based on the Level II surveys are given in the Annex II.

5.2.2.2 Data completeness

All parameters reported in the *.plf, *.fom and *FO.lqa files are mandatory to report. When a country/federal state decides to report optional parameters (*.foo file), they should also fulfil the data quality requirements and submit respective obserations with the *FO.LQA file for these parameters. All reported values should be measured according to the methods described in chapter 5.1.

5.2.2.3 Data quality objectives or tolerable limits

See the manual part XVI "Quality Assurance and Control in Laboratories", and Table 4 for the tolerable limits of the measured parameters in the Interlaboratory Comparisons.

Table 4: Inter-laboratory tolerable limits for high and low concentrations of mandatory and optional foliage parameters.

Parameter	Conc. Range	Conc. Level	Inter-Laboratory Tolerable limit (% of mean)
N mg g^{-1}	Low	≤ 5.0	**± 15**
	High	> 5.0	**± 10**
S mg g^{-1}	Low	≤ 0.50	**± 20**
	High	> 0.50	**± 15**
P mg g^{-1}	Low	≤ 0.50	**± 15**
	High	> 0.50	**± 10**
Ca mg g^{-1}	Low	≤ 3.0	**± 15**
	High	> 3.0	**± 10**
Mg mg g^{-1}	Low	≤ 0.50	**± 15**
	High	> 0.50	**± 10**
K mg g^{-1}	Low	≤ 1.0	**±15**
	High	> 1.0	**±10**
Zn µg g^{-1}	Low	≤ 20	**± 20**
	High	> 20	**± 15**
Mn µg g^{-1}	Low	≤ 20	**± 20**
	High	> 20	**± 15**
Fe µg g^{-1}	Low	≤ 20	**±30**
	High	> 20	**±20**
Cu µg g^{-1}	Low	-	**±20**
	High		
Pb µg g^{-1}	Low	≤ 0.50	**± 40**
	High	> 0.50	**± 30**
Cd ng g^{-1}	Low	-	**± 30**
	High		
B µg g^{-1}	Low	≤ 5.0	**± 30**
	High	> 5.0	**± 20**
C g $100g^{-1}$	Low	-	**± 5**
	High		

5.2.2.4 Data quality limits

The national laboratories have to participate annually in the needle/leaf inter-laboratory test organized by Forest Foliar Coordinating Centre of ICP Forests. The laboratory results are

considered of sufficient quality when the laboratory received a qualification for the concerning parameter(s) after participation in the Interlaboratory Comparisons.

The foliar chemical Interlaboratory Comparisons should include at least four samples. When 50% or more of these samples in the ring test are within the tolerable limits, the laboratory is qualified to analyse the concerning parameter and the survey results can be reported to the central database. The results of the chemical analyses are evaluated and have to be within the limits listed in Table 4.

6. Data handling

6.1 Data submission

Forms for data submission (*.plf, *.fom, *.foo) and explanatory items are annually developed by the Programme Coordinating Centre of ICP Forests and are adopted by the Programme Task Force. The quality information from the labs has to be sent together with the relevant forms to the data centre using the submission form (*FO.LQA). The form and related explanatory items are available at the ICP-Forests webpage.

6.2 Data validation

Data checks should be done as soon as results from the analyses are available. Data validation and quality assurance should be applied in accordance with the guidelines for QA/QC procedures in the laboratory that are given in the manual part XVI "Quality Assurance and Control in Laboratories". The countries should use these methods (e.g. plausible ranges) to check data before submitting it. Excel files for analytical data validation, definition of a laboratory's methods including QA/QC procedures, and a control chart can be downloaded from the ICP Forests web page (http://www.icp-forests.org/WGqual_lab.htm)

6.3 Transmission to co-ordinating centres

All validated data should be sent yearly to the European central data storage facility at the ICP Forests Programme Coordinating Centre. Detailed time scheduled is provided by the relevant bodies.

6.4 Data processing guidelines

In case sample trees have been analysed separately a mean should be computed and submitted to the database. If trees are analysed separately the variation within plot should be examined in order to possibly reshape the sampling protocol. If total foliar element budgets of canopies are to be determined all the foliage age classes need to be sampled and analysed (cf. Rautio 2009: chapter 4). Further an estimation of total canopy foliage mass needs to be determined either by cutting down a new set of sample trees (outside the sample plots) and weighing the total foliage mass or by using existing biomass functions for different tree species (see e.g. Kittredge 1944, Zianis et al. 2005, Muukkonen & Mäkipää 2006, Repola 2009).

6.5 Data reporting

For details to the data submissions see Section 6.1. If values are below the *quantification* limit (not the detection limit), a value of -1 should be reported in the monitoring data submission forms (*.fom, *.foo). The quantification limit should be reported in the quality file (*FO.LQA). Definitions of the quantification and detection limits can be found in Section 2.3 of the manual part XVI "Quality Assurance and Control in Laboratories".

7. References

Fürst, A. 2009: 11th Needle/Leaf Interlaboratory Comparison Test 2008/2009. Techinal report Life+ QA-RFoliar09 in cooperation with ICP Forests. ISBN 978-3-901347-79-5. Forest Foliar Coordinating Centre / BFW, Vienna, Austria.

Fürst, A. 2010: 12th Needle/Leaf Interlaboratory Comparison Test 2009/2010. Techinal report Life+ QA-RFoliar10 in cooperation with ICP Forests. ISBN 978-3-901347-89-4. Forest Foliar Coordinating Centre / BFW, Vienna, Austria.

Kittredge, J. 1944. Estimation of thre amount of foliage of trees and stands. Journal of Forestry 42(12): 905-912.

Muukkonen, P. & Mäkipää, R. 2006. Biomass equations for European trees: addendum. Silva Fennica 40(4): 763-773.

Rautio, P. (ed.) 2009. FutMon (Life+) field protocol: Sampling procedure for evaluation of nutrient budgets in vegetation in FutMon intensive monitoring plots and more intensive foliage surveys (D2). 7 p.

Repola, J. 2009. Biomass equations for Scots pine and Norway spruce in Finland. Silva Fennica 43(4): 625-647.

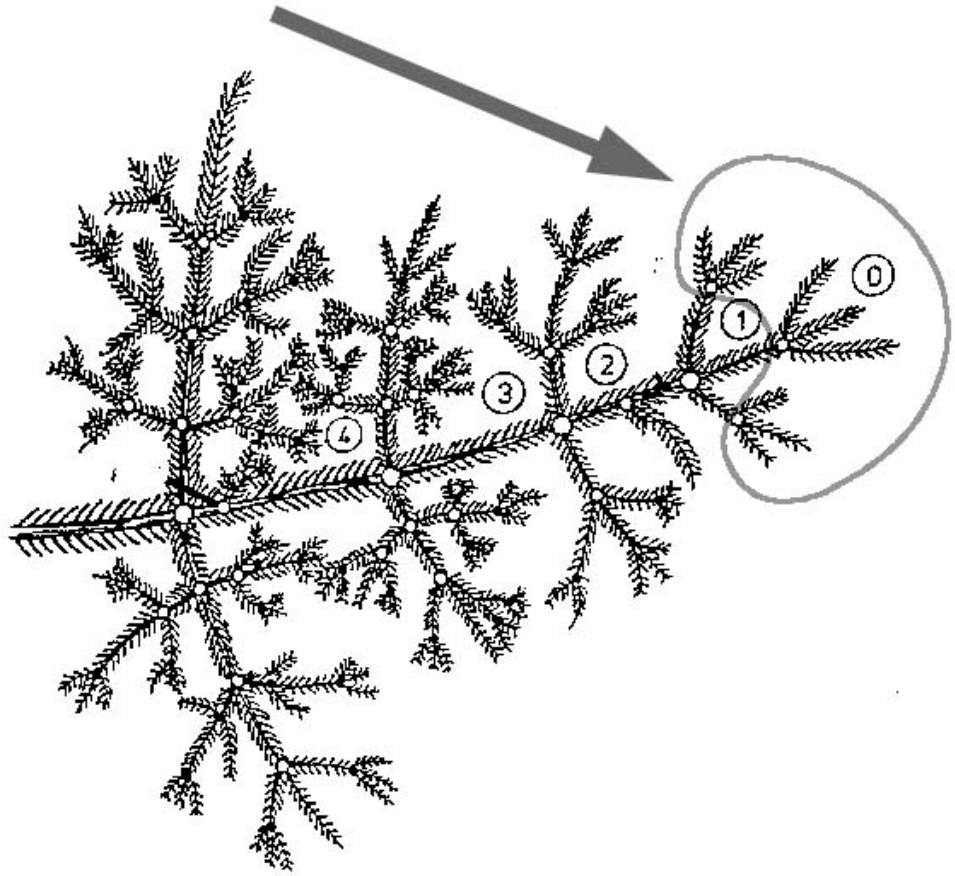
Zianis, D., Muukkonen, P., Mäkipää, R. & Mencuccini, M. 2005. Biomass and stem volume equations for tree species in Europe. Silva Fennica Monographs 4: 1-63.

8. Annexes

Annex I: Choice of needles

Annex II: Plausible ranges of element concentrations in the foliage of different tree species

Annex I - Choice of needles



Needle sets

0: current (C-needles)

1: current+1 (C+1-needles)

2: current+2 (C+2 needles)

3: current+3 (C+3-needles)

4: current+4 (C+4-needles)

etc.

Annex II - Plausible ranges

of element concentrations in the foliage of different tree species calculated from the Level II data sets as 5 - 95 Percentile (indicative values in grey)

Tree species	Leaf_type*)	Limit	N mg/g	S mg/g	P mg/g	Ca mg/g	Mg mg/g	K mg/g	C g/100g	Zn µg/g	Mn µg/g	Fe µg/g	Cu µg/g	Pb µg/g	Cd ng/g	B µg/g
Fagus sylvatica	0	low	20,41	1,26	0,89	3,44	0,65	4,81	*45*	17	127,2	62	5,67	-	50,3	9,09
	0	high	29,22	2,12	1,86	14,77	2,5	11,14	*55*	54,21	2902	177,9	12,18	6,79	461,5	40,04
Quercus cerris	0	low	12,86	0,91	0,63	4,81	0,98	1,19	*45*	13	509	83	6,89	-	63	15,9
	0	high	30,79	3,24	2,29	16,49	3,24	15,64	*55*	-	-	-	-	-	-	-
Quercus ilex	0	low	11,95	0,81	0,69	4	0,76	3,42	*45*	12,7	277,65	73,1	4	-	-	21,74
	0	high	17,24	1,41	1,22	10,32	2,62	8,46	*55*	41	5384,5	716,9	*7*	-	-	-
Quercus petraea	0	low	19,75	1,24	0,9	4,12	1,06	5,86	*45*	11	905	60,4	5,39	-	24	5,5
	0	high	29,84	2,01	1,85	10,46	2,26	11,16	*55*	25	4208,6	149,2	11,64	-	-	-
Quercus pyrenaica (Q.	0	low	17,85	1,18	1,48	4,6	1,4	3,52	*45*	18	434	81	8,07	-	-	-
	0	high	25,5	2,33	3,12	12,03	3	11,81	*55*	-	-	-	-	-	-	-
Quercus robur (Q.	0	low	20,31	1,36	0,97	3,33	1,09	5,8	*45*	14	218,75	63,8	5,5	0,138	40	23,41
	0	high	30,69	2,21	2,55	12,26	2,85	12,64	*55*	50	2819,7	232,8	14,1	17,993	183,2	54,79
Quercus suber	0	low	11,39	0,85	0,47	4,29	1,22	4,37	*45*	17	291,25	62,15	6,11	-	-	17,5
	0	high	23,09	1,61	1,53	11,02	2,55	9,85	*55*	47	2887,4	621	*20*	-	-	-
Abies alba	0	low	11,55	0,79	0,95	3,5	0,68	4,29	*47*	22	185	20,6	2,31	-	48	15,5
	0	high	16,16	1,69	2,23	11,71	1,9	8,48	*57*	45	2510	85,2	5,89	-	-	-
	1	low	11,67	0,95	0,86	4,19	0,37	3,97	*47*	20	250	32	2	-	56	14,4
	1	high	16,46	1,79	2,21	16,39	1,7	7,57	*57*	47,5	5240,5	120,95	6,45	-	-	-
Picea abies (P. excelsa)	0	low	10,39	0,7	1,01	1,83	0,66	3,65	*47*	16	164,65	22	1,41	-	-	7,2
	0	high	16,68	1,31	2,1	7,01	1,56	8,36	*57*	47	1739,4	91,2	5,94	2,92	226	29,39
	1	low	9,47	0,69	0,81	2,26	0,44	3,41	*47*	12	198,4	26,67	0,94	-	-	6,16
	1	high	15,97	1,34	1,82	9,77	1,51	7,05	*57*	51,83	2376,4	118,05	7,07	5,24	169,05	32,94
Picea sitchensis	0	low	12,67	0,98	1,04	1,21	0,78	5,56	*47*	8,4	147,36	30,54	*0,7*	-	-	6
	0	high	17,61	1,75	2,56	8,02	1,41	10,89	*57*	33,8	1489,1	232,29	5,91	-	-	*42*
	1	low	11,87	0,92	0,84	1,41	0,5	4,62	*47*	9,5	159,72	33,2	*0,7*	-	-	5
	1	high	18,19	1,94	2,43	8,23	1,18	10,05	*57*	29,25	1734	133,32	4,667	-	-	*52*
Pinus contorta	0	low	11,31	0,75	0,98	1,02	0,79	3,56	*47*	-	-	-	-	-	-	-
	0	high	21,51	1,66	1,73	2,7	1,31	6,06	*57*	-	-	-	-	-	-	-
	1	low	13,12	0,87	0,88	1,96	0,75	1,21	*47*	-	-	-	-	-	-	-
	1	high	20,22	*1,7*	1,55	4,41	1,5	6,02	*57*	-	-	-	-	-	-	-
Pinus halepensis	0	low	9,22	0,92	0,8	2,12	1,84	3,2	*47*	23	32	230	-	-	-	-
	0	high	14,28	1,68	1,79	8,04	2,89	8,67	*57*	-	-	-	-	-	-	-
Pinus nigra	0	low	8,42	0,51	0,81	0,97	0,56	3,88	*47*	18,8	60	29,25	1,81	0,56	399	8,9
	0	high	21,18	1,44	1,57	4,42	2,08	8,3	*57*	67,7	1072,4	131	18,08	-	-	-
	1	low	7,97	0,44	0,75	1,17	0,35	3,89	*47*	19	109	69	1,8	0,87	380	8,7
	1	high	23,49	1,93	1,71	6,9	2,06	7,34	*57*	*70*	*1000*	-	-	-	-	-
Pinus pinaster	0	low	6,85	0,61	0,55	0,8	1,01	3,26	*47*	15,6	41,4	22,9	1,697	-	-	15
	0	high	13,71	1,29	1,24	3,8	2,47	7,14	*57*	39	825	578,9	5,03	-	-	-
	1	low	6,25	0,55	0,4	1,09	0,94	2,4	*47*	12,3	35,4	23,3	1,13	-	-	20
	1	high	13,27	1,44	1,38	6,02	2,88	6,86	*57*	36,8	794,1	110,8	4,68	-	-	-
Pinus pinea	0	low	7,51	0,65	0,58	1,53	1,8	3,25	*47*	6	89	44	4,3	-	-	28,5
	0	high	*11,3*	*1,65*	*1,2*	*4,4*	*3*	*6,7*	*57*	-	-	-	-	-	-	-
Pinus sylvestris	0	low	11,4	0,75	1,11	1,61	0,64	3,77	*47*	32	172,05	18,25	2,28	-	50	9,17
	0	high	20,41	1,56	2,06	4,61	1,31	7,27	*57*	77,55	912	138,95	7,7	3,94	446,6	30,49
	1	low	10,94	0,77	1	2,57	0,5	3,51	*47*	31,5	222,05	28	1,96	0,14	60	7,38
	1	high	19,38	1,61	1,88	6,71	1,18	6,52	*57*	96	1331,95	170,5	6,88	5,59	507,2	33,9
Pseudotsuga menziesii	0	low	13,54	1	1	1,98	1,02	5,17	*47*	15	159,3	42,95	2,72	-	141	30,9
	0	high	22,71	1,8	1,7	5,91	2,1	8,96	*57*	45,3	1660,5	129,35	5,95	-	-	-
	1	low	13,55	0,99	0,71	3,09	1,14	2,97	*47*	14	443,6	57,9	2,91	-	-	-
	1	high	29,23	2,18	1,45	9,64	2,73	7,3	*57*	-	155,25	279,2	-	-	-	-