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Assessment and Monitoring of Air Pollution Effects on Forests

MANUAL

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

Part IV

Sampling and Analysis of Needles and Leaves

Updated 05/2005 [page 7: 2007]

CONTENTS

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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 Sampling

For each sample plot, relevant information with regard to fertilisation, liming, etc. should be indicated. Details should include the type of fertiliser used, the amount applied and the year of application.

2.1 Frequency and date

- *Level I:* Foliar analysis is optional on these 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.

- *Level II:* Foliar analysis is mandatory. Sampling and analysis must be performed at least every two years.

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.

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.

2.2 Number of trees to be sampled and analysed

- *Level I:* It is recommended to sample at least 3 trees of each main species. A composite sample should be made by mixing equal quantities of each sample after drying (if the three trees are analysed individually, the mean value is calculated for each element).

- *Level II:* At least 5 trees of each main species present in the plot have to be sampled but for statistical reasons it is recommended to sample more trees; the five samples are individually preserved in bags; for analysis, a composite sample is made by mixing equal quantities of each of the five samples (if the five trees are analysed individually, the mean value is calculated for each element).

2.3 Selection of the sample trees

The number of trees needed for the sampling (3 or 5) should be selected so that:

- the trees are spread over the total plot area, or around the plot, if the stand is homogeneous over a larger area (see below);
- 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 in the vicinity of the soil sampling plots. However, care must be taken that the main roots of the sample trees have not been damaged by soil sampling.
- 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 plot;
- the trees should be representative for the mean defoliation level of the plot (± 5% defoliation) at the time of selection;
- In case of loss 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 too few needles (or leaves) per year, it is allowed to alternate between two sets of 5 (level II), if necessary, to avoid damage of the sample trees. Each set must correspond to the above conditions.

2.4 Selection of leaves and needles

It is important that the leaves or needles sampled have developed in full light. 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.

For deciduous species and larch, sampling is done on current year leaves or needles.
For evergreen species, sampling of both the current year needles or leaves and the second year needles or leaves (current+1) is (see Annex 1):

- optional on Level I plots
- recommended on Level II plots

For 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.

If samples are rejected, reason for this should be noted.

2.5 Orientation

In general, sampling must be carried out in such a way that all the orientations are represented in the set of sample trees. 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 has to be the same. In such cases, it is necessary to document the orientation.

2.6 Quantity of material to be sampled

The recommended minimum quantities are:

- 10-20 grams of fresh needles or leaves 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.

It would be useful to record additional information about discoloration and symptoms of ozon and different diseases and insect attacks on the needle/leaf samples.

2.7 Methods of sampling

As trees must not be felled, any convenient way of sampling, taking into consideration type and size of stands etc., is acceptable, provided that it does not lead to contamination of the sample, to heavy tree damage, or to risks for the sampling team.

2.8 Pretreatment 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 polyethylene bags is recommended. If possible, samples are dried in a clean room and stored in a cool place in perforated polyethylene bags.

Great care must be taken to mark each sample clearly (forest, number of plot, 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.

Only trees of the 50 main species are to be sampled (see species code in the Annex 4).

3 Chemical analyses

3.1 Treatment before analysis

The determination of the dry mass of 100 leaves or 1,000 needles (by drying at 105ºC) is recommended for Level II (Table 1/A).

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 80ºC, for at least for 24 hours (Table 1/B1). 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, Al 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.

3.2 Elements to be determined

Levels I and II plots:

- mandatory: N, S, P, Ca, Mg and K
- optional: Zn, Mn, Fe, B, Pb, Cu, Cd and C

Only the total concentration of elements in needles or leaves must be given by reference to 105°C-dried material (Table 1/B2).

Table 1. Drying temperatures

Type of sample	Temperature of owen drying
A. Samples for the dry mass of 100 leaves or 1,000 needles	105 °C
B1. Leaves/needles drying before grinding	<= 80 °C
B2. Powder for moisture content determination (subsample of B1), not chemically analysed	105 °C

3.3 Digestion (or ashing) and analysis

The recommended methods for digestion or ashing and analysis and the validation of analytical results will be given in Annex 2. Other methods may be allowed, but each country should validate the national method (see Annex 2). It is necessary to compare the total element concentrations obtained by national methods with those certified on reference standard samples according to the results of the interlaboratory tests (EC & UN/ECE 1997, 1998).

4 Validation of the analytical results

The national laboratories have to participate in the needle/leaf inter-laboratory test of ICP Forests and EU at regular intervals of two years parallel to Level II foliar survey.
The results of the chemical analyses are evaluated and have to be within the following limits:

Nitrogen	10 %	Manganese	15 %
Sulphur	15 %	Iron	20 %
Phosphorus	10 %	Copper	20 %
Calcium	10 %	Lead	30 %
Magnesium	10 %	Cadmium	30 %
Potassium	10 %	Boron	20 %
Zinc	15 %	Carbon	5 %

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Annex

Annex 1: Choice of needles

Annex 2: Analytical methods

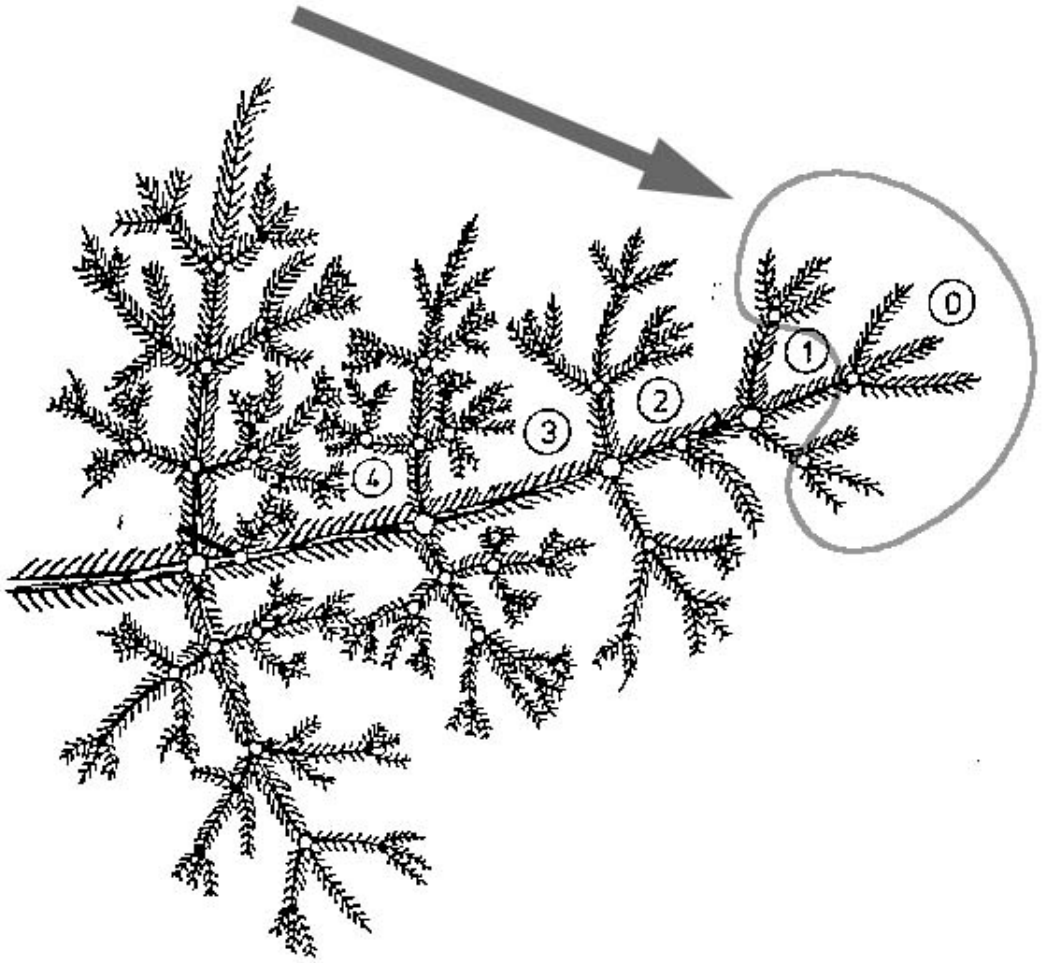
Annex 3: Forms

Annex 4: Explanatory items

Annex 1

Choice of needles

Choice of needles



Needle sets

0	current
1	current+1
2	current+2
3	current+3
4	current+4

Annex 2

Analytical methods

Contents

1 Methods of digestion (indicative)

As a guide, the preferred methods of digestion are given hereinafter.

1.1 Digestion by oxidants and/or hot acids at room pressure

- Kjeldahl method for N determination: concentrated H_2SO_4 with K_2SO_4 and Se as catalysts;
- methods derived from the Kjeldahl method for N determination: other catalysts than Se, which is toxic in the environment, such as Ti, Cu;
- $H_2SO_4 + H_2O_2$;
- $H_2SO_4 + HNO_3$;
- HNO_3;
- $H_2O_2 + HNO_3$;
- HNO_3 or H_2O_2 followed by $HClO_4$. Perchloric acid is very efficient but dangerous (risk of explosion by contact with organic material, or drying and heating perchlorates). Storage and manipulation therefore require great caution. $HClO_4$ digestion must be preceded by cold attack of the sample powder by H_2O_2 or concentrated HNO_3 during 24 hours in order to digest most of the organic tissues before adding $HClO_4$.
- HNO_3 + HF, teflon vessels; after digestion one has to fume away the HF with HNO_3.
- $HNO_3 + HClO_4$ + HF, teflon vessels. Perchloric acid is very efficient but dangerous (risk of explosion by contact with organic material, or drying and heating perchlorates). Storage and manipulation therefore require great caution. $HClO_4$ digestion must be preceded by cold attack of the sample powder by concentrated HNO_3 during 24 hours in order to digest most of the organic tissues before adding $HClO_4$. After digestion one has to fume away the HF.

1.2 Digestion in a microwave oven

- HNO_3
- $H_2O_2 + HNO_3$
- HNO_3 + HF, teflon vessels.

1.3 Pressurized digestion with HNO_3, or $HNO_3 + H_2O_2$ in quartz or teflon bombs

200 mg vegetal powder + 3 ml concentrated HNO_3

Remarks:
Of all digestion methods, the pressurized digestion of foliar products shows the most homogeneous results because of the low risk of element loss or contamination.

2 Determination

2.1 Titration

NH_4^+ after digestion by the Kjeldahl method and distillation of NH_3 in H_3BO_3

2.2 Colorimetry

- NH_4^+: indophenol blue; or FIA method (diffusion of NH_3 through a teflon membrane, and colorimetry in a solution of phenol + ethanol + NaCl + NaOH);
- P: phosphovanadomolybdate (yellow) or molybdene blue;
- B: carminic acid

2.3 Turbidimetry

S: turbidimetry of a suspension of insoluble $BaSO_4$ with a tensioactive agent (Tween 80). With dissolution by HCl and filtration before $BaSO_4$ precipitation is recommended;

2.4 X-Ray Fluorescence Analysis (RFA)

- energy dispersive (EDRFA)
- wavelength dispersive (WDRFA)

Remarks:
The determination of elements in plant material with X-Ray- or Röntgen-Fluorescence-Analysis-methods is normally only possible for elements with an atomic number higher than 10.
The quality of the RFA-method depends a high extent from:

- homogeneity of the plant material (texture if possible <80µm)
- the availability of qualified standards. The calibration is to be done with a high number of (certified) plant standardmaterials (NIST, BCR, samples from interlaboratory tests,...)
- the plant powder should be pressed to pellets with a pressure of about 15 tons (e.g. with a IR-press) without any addition of waxes or other additives.

2.5 Atomic Emission Spectroscopy (AES)

- Flame Atomic Emission Spectroscopy
- Flame Photometry for Na, K

2.6 Atomic Absorption Spectroscopy (AAS)

- Flame Atomic Absorption Spectroscopy (FAAS)
- Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)

2.7 Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)

- without ultrasonic nebulisation
- with ultrasonic nebulisation

2.8 Elemental-analyzer

More and more frequently special apparatus are used, performing automatically, in a closed circuit, oxidation, detection and quantification of gases coming from oxidation. Several firms supply such apparatus which are globally called CHN or NS apparatus.

3 Preferred methods for analysing mandatory and optional elements

3.1 Nitrogen (N)

3.1.1 Kjeldahl (N organic + NH_4^+)

a) Digestion: Kjeldahl method or derived methods
b) Determination: titration or colorimetry

Remarks:
Organic N is digested in concentrated H_2SO_4, in the presence of catalysts, and converted into NH_4^+. N may be present in NO_3^- or NO_2^- form is not transformed into NH_4^+ and therefore not determined by methods specific for NH_4^+, so there is a slight tendency to lower nitrogen results by this method.
Automatical or semi-automatical Kjeldahl-apparatus are commercially available.

3.1.2 Elemental-analyzer (Total N)

CHNS, NS, N-apparatus

3.2 Sulphur (S)

3.2.1 Elemental-analyzer

Direct determination by CNS, NS, S-apparatus.

Remarks:
A combustion temperature higher than 1200°C - in the sample, not in the oven - is necessary to digest $CaSO_4$ completely. Otherwise the sulphur content is too low.

3.2.2 X-ray fluorescence

Direct determination by X-ray fluorescence after pelleting.

3.2.3 Digestion/Turbidimetry

a) Digestion

Wet digestion at room pressure (with HNO_3, H_2O_2, $HClO_4$)
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: Turbidimetry

3.2.3 Digestion/ICP-AES

a) Digestion
Wet digestion at room pressure (with HNO_3, H_2O_2, $HClO_4$)
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: ICP-AES

Remarks:
There are two analytical wavelengths for sulphur. A vacuum monochromator is recommended for the determination, because both lines are in the lower UV range. In this range, oxygen absorbs the UV radiation and reduces the detection limit.

- At 182.036 nm, a Pb line directly overlaps the analytical line. Boron causes an elevated background at this s line. Both elements are in this matrix in a low concentration and should not interfere with the S line.
- At the analytical line of 180.734 nm, a Ca line directly overlaps.

3.3 Phosphorus (P)

3.3.1 X-ray fluorescence

Direct determination by X-ray fluorescence after pelleting.

3.3.2 Digestion/ICP-AES

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: ICP-AES

Remarks:
There are three important analytical wavelengths for phosphorus. A vacuum monochromator is recommended for two of them (lower 180 nm).

- 177.499 nm
- 178.287 nm
- 213.618 nm. At this line a vacuum monochromator is not required. A structured background is due to NO band emission. A well resolved Cu and Fe peak is close to the analytical wavelength.

3.3.3 Digestion/Colorimetry

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: Colorimetry
phosphovanadomolybdate (yellow)
molybdene blue

Remarks:
Check that all organic P compounds are in orthophosphate form (PO_4^{3-}); choose the best mineralization matrix for SiO_2 insolubilization.

3.4 Calcium (Ca)

3.4.1 X-ray fluorescence

Direct determination by X-ray fluorescence after pelleting.

3.4.2 Digestion/ICP-AES

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: ICP-AES

Remarks:
ICP-AES is a very sensitive Ca determination method. It is therefore better to use a lower sensitively wavelength for the determination (e.g. 317.933 nm). At this line only very high Cr and Fe concentrations interfer with the analyte line.

3.4.3 Digestion/FAAS

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: FAAS

Remarks:
If an Air/C_2H_2 - Flame is used, a spectral buffer (e.g. $La(NO_3)_2$) is required.

3.4.4 Digestion/AES

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: AES

Remarks:
If an Air/C_2H_2 - Flame is used, a spectral buffer (e.g. $La(NO_3)_2$) is required.

3.5 Magnesium (Mg)

3.5.1 X-ray fluorescence

Direct determination by X-ray fluorescence after pelleting.

3.5.2 Digestion/ICP-AES

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: ICP-AES

Remarks:
ICP-AES is a very sensitive Mg determination method. It is therefore better to use a lower sensitively wavelength for the determination (e.g. 285.213 nm). At this line only very high Cr and Fe concentrations interfer with the analyte line.

3.5.3 Digestion/FAAS

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: FAAS

3.5.4 Digestion/AES

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: AES

3.6 Potassium (K)

3.6.1 X-ray fluorescence

Direct determination by X-ray fluorescence after pelleting.

3.6.2 Digestion/ICP-AES

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: ICP-AES

Remarks:
There are two analytical lines for K determination with ICP-AES.

- 766.490 nm: At this line the detection limit is better, but there is a Mg line nearby, poorly resolved from the analytical line.
- 769.869 nm

3.6.3 Digestion/FAAS

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: FAAS

Remarks:
It is necessary to use a ionisation buffer (e.g. 1g/l $CsCl_2$) if an Air/C_2H_2-Flame is applied. The standard and the blank solutions should have the same acid concentration as the sample solution.

3.6.4 Digestion/AES

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: AES

Remarks:
It is necessary to use a ionisation buffer (e.g. 1g/l $CsCl_2$) if an Air/C_2H_2-Flame is applied. The standard and the blank solutions should have the same acid concentration as the sample solution.

3.7 Sodium (Na)

3.7.1 Digestion/ICP-AES

a) Digestion: Pressurized Digestion
b) Determination: ICP-AES

Remarks:
Because of the high contamination risk (dishwashers, detergents or sodium containing glasses) the use of pressurized digestion is recommanded.
There are two lines to determinate sodium with ICP-AES:

- At the first line (588.995 nm), the wing of nearby Ar line causes the background to slope. This can be minimized by using a higher viewing height. A good background correction is required.
- On the second line (589.592 nm), there is a direct overlap with a Fe line.

3.8 Zinc (Zn)

3.8.1 X-ray fluorescence

Direct determination by X-ray fluorescence after pelleting.

3.8.2 Digestion/ICP-AES

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: ICP-AES

Remarks:
The analytical line 213.856 nm has the best detection limit, but a structured background is due to NO band emission. Ni and Cu in higher concentration can interfere.

3.8.3 Digestion/FAAS

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: FAAS

3.9 Manganese (Mn)

3.9.1 X-ray fluorescence

Direct determination by X-ray fluorescence after pelleting.

3.9.2 Digestion/ICP-AES

a) Digestion
Wet digestion at room pressure

Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: ICP-AES

3.9.3 Digestion/FAAS

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: FAAS

3.10 Iron (Fe)

3.10.1 Digestion/ICP-AES

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: ICP-AES

Remarks:
Because of contamination problems the use of pressurized digestion is preferred.

3.10.2 Digestion/FAAS

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: FAAS

Remarks:
Because of contamination problems the use of pressurized digestion is preferred.

3.11 Copper (Cu)

3.11.1 X-ray fluorescence

Direct determination by X-ray fluorescence after pelleting.

3.11.2 Digestion/ICP-AES

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: ICP-AES

Remarks:
There are three Cu lines with low detection limits:

- 324.754 nm: At this line a structured background is due to OH band emission.
- 327.396 nm
- 224.700 nm: At this line a structured background is due to NO band emission. The nearby Fe and Pb lines cause wing overlap on the Cu line.

3.11.3 Digestion/FAAS

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: FAAS

3.12 Lead (Pb)

3.12.1 X-ray fluorescence

Direct determination by X-ray fluorescence after pelleting.

Remarks:
X-ray spectroscopy has sometimes too high detection limits.

3.12.2 Digestion/ICP-AES

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: ICP-AES

Remarks:
ICP-AES have sometimes too high detection limits. Possible Pb lines are 220.353 nm and 216.999 nm.

3.12.3 Digestion/GFAAS

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: GFAAS

Remarks:
GFAAS is a slow but sensitive method; it should be used for low Pb-content. It is necessary to use a matrix modifier (e.g. $NH_4H_2PO_4/Mg(NO_3)_2$ or $Pd/Mg(NO_3)_2$) because some lead compounds are volatile (e.g. $PbCl_2$).

3.13 Aluminium (Al)

3.13.1 X-ray fluorescence

Direct determination by X-ray fluorescence after pelleting, if Al-content is higher than 100 $\mu g.g^{-1}$.

3.13.2 Digestion/ICP-AES

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion in Teflon bombs with HF
b) Determination: ICP-AES

Remarks:
Due to incomplete sample dissolution all digestion methods not using HF normally lead to poor results. It is therefore urgently recommanded to use a digestion-method with HF addition. If there is a trace of HF in the sample it is necessary to use a HF resistent sample system (nebuliser and spray camber). If a Al_2O_3 torch (HF-resistent) is used, contamination problems are possible.
Some of the important lines are:

- 167.081 nm: A vacuum monochromator is recommended for the determination because this line is in the lower UV range. In this range, oxygen absorbs the UV radiation. Interfering elements in this matrix are Fe and P at higher concentrations.
- 396.152 nm: Ca causes an elevated background at this Al line. This can be treated by using a good background correction.

3.14 Boron (B)

3.14.1 Digestion/ICP-AES

a) Digestion
Wet digestion at room pressure
Wet digestion in a microwave oven
Pressurized Digestion
b) Determination: ICP-AES

Remarks:
HF should not be used for digestion, because of volantile BF_4^- compounds. There are contamination problems from dish washers, detergents, and boronsilicatglasses. Some of the important B lines are:

- 249.773 nm: A small B peak may be detected in subsequent derterminations, because of interaction of boron with the hot quartz of the plasma torch. A nearby Fe line is poorly resolved from the analytical line.
- 182.641 nm: A vacuum monochromator is recommended for the determination because this line is in the lower UV range. In this range, oxygen absorbs the UV radiation and reduces the detection limit. A small B peak may be detected in subsequent determinations because of interaction of boron with the hot quartz of the plasma torch. The nearby S line causes wing overlap on the B line.

3.14.2 Digestion/Colorimetry

a) Digestion
Wet digestion at room pressure (with HNO_3)

b) Determination: Colorimetry
Carminic acid

4 Data expression-Units

The total concentration of elements in needles or leaves must be given reference to dried at 105°C material. Because of possible element losses of nitrogen when drying at 105°C, it is recommended to dry the foliage powder for 8 hours at 80°C before the determination. The residual water content must be determined seperately by drying at 105°C for 8 hours and weighting, and the results given by the analysis performed on 80°C dried powder must be corrected.
For all other elements it is possible to dry the foliage powder before determination at 105°C for 8 hours.

- Major elements (N, P, S, K, Mg, Ca) must be expressed in $mg.g^{-1}$ oven dry powder (105°C) and C %.
- Trace elements (Fe, Mn, Zn, Cu, Pb, Na, Al, B) must be expressed in $\mu g.g^{-1}$ oven dry powder (105°C) and Cd $ng.g^{-1}$.

5 Validation of the analytical results

As has been indicated in 3.3, the total element concentrations obtained by the national methods have to be checked in order to be sure of the accuracy of these methods. Two steps of quality assurance should be foreseen:

Comparison of the results of the national methods with the concentrations of reference standard samples to validate the own analytical method. These reference standard samples, with certified total element concentrations, supplied e.g. by IRMM of the EC (Institute for Reference Materials and Measurement) or by ISO (International Standard Organization), or by the US group of foliar analysis, can be ordered by NFC for analysis, with the request to send the analysis results (with 3 repetitions) to the chairman of the working group,

within a delay of 6 months after receipt of the reference standard samples. The certified concentrations of the latter will be supplied when the results of all (or most of the) laboratories involved have been received.

The check the accuracy of the analysis, it should be a part of the validation of the analytical results. Each laboratory uses several own reference samples of different compositions (e.g. at least one high- and one low-concentration) to be included in each series of analysis and element concentrations which have been determined before by methods which give results in good agreement with the element concentrations of the reference standard samples. Laboratories should report the results of the validation to NFC.

5.1 Digestion, ashing and determination methods

Table 5.1-1: Summary of the digestion or ashing procedures

Chapter	N	S	P	K, Na, Ca, Mg, Fe, Zn, Mn	Al	Cu	Cd	Pb	Cl	B	F
1.1 Wet acidic oxydizing conditions											
1.1.1 Kjeldahl	X										
1.1.2 Oxidant and hot acids at room pressure											
H_2SO_4+H_2O_2	X		X								
H_2SO_4+HNO_3			X	X							
HNO_3			X	X		X					
H_2O_2+HNO_3			X	X		X					
$HClO_4$			X	X		X					
1.1.3 H_2O_2+HNO_3 in microwave oven			X	X		X					
1.1.4 HNO_3, or HNO_3 + H_2O_2 in teflon bombs at 180°			X	X	X	X	X	X	X	X	
1.1.5 HNO_3 digestion under backward column							X	X			
1.2 Dry ashing											
1.2.1 at room pressure 450-600°											
- without HF treatment			X	X							
- with HF treatment			X	X	X	X					
- with addition of stabilizing products							X (2)	X (1)	X (3)	X (4)	X (4,5)
1.2.2 Low temperatures in O_2											
1.2.3 Schöninger flask		X	X						X		
1.3 Integrated oxidation and detection (CHN, NS devices)	X	X									

(1) $Mg(NO_3)_2$; (2) NH_4NO_3 ; (3) Na_2CO_3 ; (4) CaO ; (5) NaOH

Table 5.1-2: Determination methods

	N	S	P	K	Na	Ca	Mg	Fe	Mn	Zn	Al	Cu	Cd	Pb	Cl	B	F
Tritration	X(1)														X(2)		
Colorimetry	X(3)	X(4)	X(5)												X(6)	X(7)	
Turbidimetry		X															
Ionic chromatography		X	X												X		X
Specific electrodes															X		X
Capillary electrophoresis		X													X		
Flame emission spectroph.				X	X												
Flame atomic abs. spectroph																	
- without graphite oven				X	X	X	X	X	X	X	X	X					
- with graphite oven												X	X	X			
- ICP-PN (Pneumatic Nebulization)		X	X	X	X	X	X	X	X	X	X	X			X	X	
- ICP-USN (UltraSonic Nebulization)												X	X	X			
Integrated oxidation and detection	X	X															
X-ray fluorescence		X	X	X	X	X	X	X	X	X	X				X	X	

(1) NH_3 distillation
(2) $AgNO_3$ in presence of CrO_4^{2-}
(3) Indophenol blue or FIA method (phenol + ethanol + NaCl + NaOH)
(4) Metorine
(5) Phosphovanadomolybdate or molybdene blue
(6) $Fe(SCN)_3$
(7) 1-1' dianthrimide