

**UNITED NATIONS ECONOMIC COMMISSION FOR EUROPE
CONVENTION ON LONG-RANGE TRANSBOUNDARY AIR POLLUTION**

International Co-operative Programme on
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 IIIa

Sampling and Analysis of Soil

updated 06/2006
to be applied from 2007 on
(as well as within the BioSoil project)

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Elaborated by:

Expert Panel on Soil

Forest Soil Co-ordinating Centre, Research Institute for Nature and Forest, Belgium

With the financial support of the Regulation (EC) N° 2152/2003 of the European Parliament and of the Council of 17 November 2003 concerning monitoring of Forests and Environmental Interactions in the Community (Forest Focus)

1. Introduction

The present part of the manual outlines the sampling, analysis and reporting procedures for a set of soil parameters.

The purpose of the large-scale representative soil survey (Level I) is first of all the assessment of basic information on the chemical soil status and its changes over time, and secondly the assessment of soil properties which determine the forest soil's sensitivity to air pollution. Besides providing soil data for the study of atmospheric deposition effects at the broader scale, the soil survey will serve other purposes, as studies related to climate change (e.g. inventory of carbon storage) and sustainable forest management (e.g. in addition to acidification status, also nitrogen studies and nutrient imbalances).

The intensive soil studies are conducted in selected areas on permanent plots (Level II) where other measurements for the analysis of the forest ecosystem are concentrated. The objectives of the special forest ecosystem analysis are the verification of hypotheses and in-depth analyses of damage mechanisms and the derivation of fundamental knowledge of forecasting future developments.

A third major objective of the large-scale representative soil survey (Level I) is to allow the evaluation of the (quality of) forest soils on a European scale. For the sake of data comparability between countries, a prime prerequisite is that the same methods for soil sampling and analysis are used throughout the network. As such, analytical results obtained by national methods, different from those described in this manual, cannot directly be compared with analytical results obtained by the international reference methods in this manual. Notwithstanding, the participating countries are encouraged to make efforts (where necessary and possible) to allow the comparison of the data obtained in the first survey with those of future surveys.

2. Sampling

2.1. Georeferencing

- One common datum, the World Geodetic System 1984 (WGS84) will be applied and no projection system (geographical lat/long coordinates in degrees, minutes, seconds). A GPS measurement (if technically possible) should be taken in the centre of the plot.
- Information on the precision should be provided in the Data Accompanying Report Questionnaire (DAR-Q).
- For Level I, the coordinates of crown condition survey plots should coincide.

2.2. Pedological Characterization of the plots

The pedological characterization:

- Is mandatory for Level I and Level II plots;
- Should be a general characterization, including a detailed site description with information on soil parent material (See Annex 3 “Explanatory Items N° 16”) and at least one profile description according to the FAO guidelines (FAO, 1990a). The soils should then be classified according to the most recent official version of the World Reference Base of Soil Resources (WRB)-classification system. All qualifiers need to be reported. The DAR-Q will mention which official version of WRB (FAO et al. 1998, IUSS Working Group WRB 2006) has been used.
- Has to be carried out only once before the start of the first sampling activities, given all necessary information to allow for soil classification according to WRB is available.
- The described soil profile(s) should be located at a location which is representative for the dominant soil type in the actual sampling area. For Level II this should be in the buffer zone of the plot.

- The analytical data for soil classification should be reported.
- Includes the identification of the **dominant humus form** on the observation plot (See Annex 3 “Explanatory Items” N° 6) according to the adopted description and classification guidelines.

Note: In the framework of the EU Forest Focus Demonstration Project. BIOSOIL, FSCC developed Guidelines for Forest Soil Profile Description, adapted for optimal field observations which are partly based on the 4th edition of the Guidelines for Soil Profile Description and Classification (FAO, In Press). In the BioSoil project it is recommended to use these guidelines in stead of the above mentioned FAO guidelines of 1990.

2.3. Soil sampling

2.3.1. Allocation of Soil Sampling Sites

Level I:

- judgmental design;
- sampling sites may be located within the plot area, but only if samples are collected from bores. Sites that should be avoided are areas around tree stems (1m) and animal holes, disturbances like wind-thrown trees and trails. A record of the places sampled should be kept, so that they will not be resampled at a later date.

Level II:

- random design or systematic design with a random component;
- sampling sites have to be located in the buffer zone of the plot; sites to be avoided: see Level I.

2.3.2. Sampling Time

In order to reduce temporal variations, especially in the organic layer, sampling activities should be confined to periods with low biological activity, e.g. winter or dry season, based on expert judgement. However, the countries that participated in the first survey, have to carry out the sampling activities in the same period (season) as for the first survey. The sampling dates have to be reported in the reduced plot file (form 4a).

2.3.3. Sampled Layers

2.3.3.1. General

The organic layer at the soil surface, which may consist of one or more of the following organic horizons: litter (OL), fermentation horizon (OF) and/or humus (OH) in aerated organic layers and Hf, Hfs or Hs in water saturated organic layers, is sampled separately from the underlying mineral soil. Buried organic layers are sampled in the same way as mineral layers. Material discarded for the representative sample can be used to refill bore holes or pits.

2.3.3.2. Organic Layer Sampling

A distinction has to be made between an organic layer that is saturated (H) or not saturated (O) with water according to the FAO-definition (FAO 1990a). The thickness of the different horizons OL, OF and OH or Hf, Hfs and Hs, constituting the organic layer and as defined in Box 1, has to be measured and reported.

Box 1: Definitions of the organic layersDistinction between saturated (H) and aerated (O) organic layers

A distinction is made between the water saturated organic layers, designated as ‘H’, and the aerated organic materials indicated as ‘O’ (FAO, 1990a):

- **Organic O-layers or horizons** are dominated by organic material, consisting of undecomposed or partially decomposed litter, such as leaves, needles, twigs, mosses and lichens, which has accumulated on the soil surface; they may be on top of either mineral or organic soils. O horizons are not saturated with water for prolonged periods. The mineral fraction of such material is only a small percentage of the volume of the material and generally is much less than half of the weight.
An O layer may be at the surface of a mineral soil or at any depth beneath the surface if it is buried. A horizon formed by illuviation of organic material into a mineral soil is not an O horizon, though some horizons formed in this manner contain much organic matter.
- **Organic H-layers or horizons** are dominated by organic material, formed from accumulations of undecomposed or partially decomposed organic material at the soil surface which may be under water. All H horizons are saturated with water for prolonged periods or were once saturated but are now artificially drained. An H horizon may be on top of mineral soils or at any depth beneath the surface if it is buried.

Distinction of fresh, partly -, and well decomposed horizons in the organic O-layers

A subdivision of the organic O-layers is made according to the following definitions (partly based on Jabiol et al., 2004):

OL-horizon (Litter, Förna): this organic horizon is characterised by an accumulation of mainly leaves/needles, twigs and woody materials (including bark), fruits etc. This sublayer is generally indicated as litter. It must be recognized that, while the litter is essentially unaltered, it is in some stage of decomposition from the moment it hits the floor and therefore it should be considered as part of the humus layer. There may be some fragmentation, but the plant species can still be identified. So most of the original biomass structures are easily discernible. Leaves and/or needles may be discoloured and slightly fragmented. Organic fine substance (in which the original organs are not recognisable with naked eye) amounts to less than 10 % by volume.

*Note: this horizon is generally called the **litter layer** (Klinka et al., 1981, Green et al., 1993, Jabiol et al., 1995, Delecour, 1980).*

OF-horizon (fragmented and/or altered) is a zone immediately below the litter layer. This organic horizon is characterised by an accumulation of partly decomposed (i.e. fragmented, bleached, spotted) organic matter derived mainly from leaves/needles, twigs and woody materials. The material is sufficiently well preserved to permit identification as being of plant origin (no identification of plant species). The proportion of organic fine substance is 10 % to 70 % by volume. Depending on humus form, decomposition is mainly accomplished by soil fauna (mull, moder) or cellulose-decomposing fungi. Slow decomposition is characterised by a partly decomposed matted layer, permeated by hyphae.

*Note: this is the **fragmented layer** in non-saturated soils (Klinka et al., 1981, Green et al., 1993, Jabiol et al., 1995, Delecour, 1980)*

OH-horizon (humus, humification): characterised by an accumulation of well-decomposed, amorphous organic matter. It is partially coprogenic, whereas the F horizon has not yet passed through the bodies of soil fauna. The humified H horizon is often not recognized as such because it can have friable crumb structure and may contain considerable amounts of mineral materials. It is therefore often misinterpreted and designated as the Ah horizon of the mineral soil and not as part of the forest floor as such. To qualify as organic horizon, it should fulfil the FAO requirement, as

described above. The original structures and materials are not discernible. Organic fine substance amounts to more than 70 % by volume. The OH is either sharply delineated from the mineral soil where humification is dependent on fungal activity (mor) or partly incorporated into the mineral soil (moder).

*Note: This horizon coincides with what is called the **humus layer** (Klinka et al., 1981, Green et al., 1993, Jabiol et al., 1995, Delecour, 1980)*

Distinction of subhorizons in the organic H-layers (Englisch et al., 2005):

Hf horizon (fibric): consists largely of poorly decomposed plant residues (tissues recognizable at naked eye)

*Note: this horizon coincides with what is classified as **fibric** (Klinka et al., 1981, Green et al., 1993) or **fibrist** (Delecour, 1980).*

Hs horizon (sapric): consists largely of well decomposed plant residues

*Note: This horizon coincides with what is classified as **humic** (Klinka et al., 1981, Green et al., 1993) or **saprist** (Delecour, 1980).*

Hfs horizon: decomposition of plant residues is intermediate between Hf and Hs, consists of fragmentized and partly oxidized peat

*Note: This horizon coincides with what is classified as **mesic** (Klinka et al., 1981, Green et al., 1993) or **hemist layer** in saturated soils;*

The following two horizons can be seen as special cases of the Hs horizon:

Hz horizon (zoogenic): consists of largely well decomposed plant residues with high and well recognisable earthworm activity (casts)

Hsl horizon (limnic): consists of largely well decomposed plant residues with high mineral content under aquatic conditions.

The OL-horizon has to be sampled separately. The OH-horizon has to be sampled separately only if it is thicker than 1 cm; otherwise, it may be sampled together with the OF-horizon. Optionally, the individual horizons (OL, OF, OH) may be sampled and analysed separately.

Note: For the BioSoil project the OF and OH layer can be sampled together (OF -layer).

For the submission of data, these horizons are designated as OL, OF, OFH and OH for the aerated organic (O) layers and as Hf, Hs, Hfs for the saturated H-layers.

Separation of the mineral and the organic layer

Care should be taken to correctly separate the organic layer from the mineral soil material. Separation will be done in the field, but will be checked in the laboratory, following the internationally accepted criteria (WRB definitions, see Box 2) to make a distinction between both layers.

Box 2: Definition of organic soil materials

Organic soil material (FAO et al., 1998) consists of organic debris which accumulates at the surface under either wet or dry conditions and in which the mineral component does not significantly influence the soil properties.

Diagnostic criteria. Organic soil material must have one of the two following:

1. if saturated with water for long periods (unless artificially drained), and excluding live roots, *either*:
 - a. 18 percent organic carbon (30 percent organic matter) or more if the mineral fraction comprises 60 percent or more clay; *or*
 - b. 12 percent organic carbon (20 percent organic matter) or more if the mineral fraction has no clay; *or*
 - c. a proportional lower limit of organic carbon content between 12 and 18 percent if the clay content of the mineral fraction is between 0 and 60 percent; *or*
2. if never saturated with water for more than a few days, 20 percent or more organic carbon.

According to these criteria, organic carbon determination (which is mandatory for both Levels of the survey) has to be used to check whether the separation has been done correctly. If necessary, clay analysis is required to guarantee a proper separation. If the separation was not done correctly, a new sample has to be taken.

Sampling method

A frame of 25 by 25 cm is recommended, but alternatives with a minimum total surface of 500 cm² are acceptable; for mor humus, an auger with a diameter of 8 cm can be used.

Determination of the organic layer weight

In the field, the total fresh weight of each layer (OL, OF, OH or H) has to be determined, preferably together with the thickness of each organic layer (OL, OF, OH or H) (see also § 3.1.1.). Of each layer a subsample is collected for determination of moisture content (weight %) in the lab. Based on the result of the moisture content, the total dry weight (kg/m²) of each layer can be calculated.

Note: For the BioSoil project the OF and OH layer can be sampled together (OFH layer).

2.3.3.3. Mineral Layer Sampling

Location

Where possible, the organic and mineral soil should be sampled at exactly the same locations, i.e. sample the mineral soil underneath the organic layer that has already been removed for sampling.

Procedure

Sampling should be done by fixed depth. The top of the mineral soil corresponds with the zero level for depth measurements.

Mineral soil layers are designated as 'Mij', where i is the first number of the upper depth limit and j is the first number of the lower depth limit (e.g. M01 corresponds to the 0-10 cm layer). Table 1 shows the layers that should be sampled.

Table 1: Status of layers to be sampled in both levels

Level I ⁽¹⁾		Level II ⁽¹⁾	
Mandatory	Optional	Mandatory	Optional
0-10 cm	0-5 cm	0-10 cm	0-5 cm
10-20 cm	5-10 cm	10-20 cm	5-10 cm
	20-40 cm ⁽²⁾	20-40 cm ⁽³⁾	
	40-80 cm ⁽²⁾	40-80 cm ⁽³⁾	

¹ Note that the entire thickness of the predetermined depth should be sampled and not the central part of the layer only.

² Optional, but recommended if big changes between topsoil and subsoil are to be expected

³ Only mandatory for a first assessment, not to be repeated (optional) for a second survey if all mandatory parameters were determined with the reference method, see also par. 3.3.1., key soil parameters

If the upper surface of an indurated horizon (e.g. parent rock) is above the lower limit of sampled soil (20 cm for Level I; 80 cm for Level II), the soil is to be sampled till the depth of the limiting horizon. For example, a M48 layer subsample taken at a location where the rock surface reaches up to 65 cm below the soil surface is composed of material from the mineral soil between 40 and 65 cm depth. The depth range of the upper limit of the indurated horizon is reported under ‘Observations’ in form 4b.

Sampling method

Augering preferred but pits are allowed, especially in case of stony soils where augerings are impossible.

2.3.3.4. Sampling of peatlands

The sampling design is based on the WRB definition of Histosols (= peat soils) which is based on the 40 cm boundary. As long as the peatlayer is less than 40 cm the existing sampling design for mineral forest soils shall be applied (separate sampling of the organic layers and mineral soil according to the fixed depth layers). From the moment the peat is ≥ 40 cm, the peatlayer shall be sampled according to the PEATLAND SAMPLING DESIGN.

This means that the peatlayer is sampled at fixed depths, mandatory 0 – 10 and 10 – 20 cm and optionally at 20 – 40 and 40 – 80 cm. In the reporting forms a separate name for the peatlayers shall be used, namely H01, H12, H24 and H48 in the records for the organic layers. The list of parameters (mandatory and optional) follow the rules for the OF, OH or OFH layer.

If the conditions allow (lower water table), the mineral soil below the peat soil (> 40 cm) can be further sampled according to the standard depths (M01, M12, M24, M48).

2.3.3.5. Sampling for Bulk Density

For the determination of bulk density, the sampling scheme deviates from the one for the mineral layer sampling that is needed for the analyses of all other parameters. Per plot, five samples with a minimal volume of 100 cm³ have to be taken from the mineral topsoil (0-10 cm) of non-stony soils using the core or excavation method (Soil Analysis Method 4 in Annex 1). Determination of bulk density by measurement is mandatory for level II, but if this measurement has been done according to the reference methods for the first survey, it has not to be repeated. For Level I, bulk density is a mandatory parameter too, but it can be estimated using pedo-transfer functions (see also § 3.2.2). A typical example of a pedotransfer function is the Adams (1973) equation:

$$BD = \frac{100}{\frac{1.72 * \%OC}{0.244} + \frac{100 - 1.72 * \%OC}{MBD}}$$

Where %OC is the percentage total organic carbon and MBD is the mineral bulk density (usually estimated at 1.33 kg/m³ or determined based on the ‘Mineral Bulk Density Chart’ developed by Rawls and Brakensiek, 1985 – See Table 2).

Table 2: Mineral Bulk Density Chart (Rawls and Brakensiek, 1985)

clay	sand										
	%	10	20	30	40	50	60	70	80	90	100
10	1.4	1.2	1.25	1.27	1.4	1.52	1.58	1.69	1.65	1.53	
20	1.4	1.25	1.35	1.45	1.53	1.6	1.67	1.72			
30	1.4	1.3	1.4	1.5	1.57	1.63	1.68				
40	1.4	1.35	1.44	1.55	1.61	1.68					
50	1.4	1.35	1.44	1.53	1.62						

If pedotransfer functions are used, regional calibration and validation are necessary. Information on how to determine the usefulness and predictive quality of bulk density PTFs for forest soils can be found in De Vos et al (2005).

2.3.4. Number of Samples and Sample Size

2.3.4.1. Number of Samples in Composite

Level I: For every layer, mandatory 5 subsamples have to be taken (a composite of 5 is allowed) (e.g. if taken with an auger ≥ 8 cm diameter), but more subsamples are required according to the variability of the site. Mandatory 1 composite sample has to be analysed and reported, more can be analysed optionally to determine the variability of the site. In case of very stony soils where sampling by auger is not possible, 1 composite of at least 3 subsamples can be accepted for the optional depth layers (M24 and M48) only.

Level II: For every layer, mandatory a MINIMUM of 24 subsamples has to be taken, to be combined in at least three composite samples (i.e. at least 3 composites of each 8 subsamples or 4 composite samples of each 6 subsamples). Mandatory at least 3 values have to be reported (1 from each composite), to obtain information on the sampling variability. The samples should be representative for the whole plot area. The distance between 2 sampling points should be at least 5 meter in order to avoid autocorrelation.

The subsamples have to be of equal weight, except for situations with a variable lower depth limit. In such a case (e.g. an indurated horizon within the depth range of the sampled layer), the weight of each subsample is function of the thickness of the actually sampled layer. In the above example (section 2.2.3.3 last part), the weight of the subsample taken should be a fraction equal to $(65-40)/(80-40)$ of the normal weight.

2.3.4.2. Sample Size

The minimum weight of each representative sample should be large enough for all analyses (mandatory and optional parameters) and possible repetitions or reanalyses in time. It is also advisable to keep the sample in a storeroom. The ISO 11464 method (Soil quality – Pretreatment of samples for physico-chemical analysis) recommends a sample size of at least 500 g of fresh soil for each sample.

2.4. Conservation and Preparation of Samples

Preparation of soil samples is based on the ISO 11464 method (Soil quality – pretreatment of samples for physico-chemical analysis). Collected samples should be transported to the laboratory as soon as possible and be air dried or dried at a temperature of 40 °C (ISO 11464, 1994). They can then be stored until analysis. To recalculate the analysis results on weight basis, the moisture content of the sample has to be determined by oven-drying the sample once at 105°C (ISO 11465, 1993).

Living macroscopic roots and all particles, mineral and organic, with a diameter larger than 2 mm, should be removed from the samples by dry sieving as a preparation for analysis. The particles not passing the 2-mm sieve are weighed separately for the determination of the coarse fragments content (required for bulk density). To guarantee a harmonised approach, samples should not be further milled or ground. For those analyses for which finely ground material is required [this is Carbonate Content (SA07), Total Organic Carbon (SA08), Total Nitrogen (SA09) and Total Elements (SA12)] further milling or grinding is allowed.

The sample materials for storage should be kept without preservative under normal room conditions with minimal temperature and humidity fluctuations, shielded from incident light.

3. Physical and Chemical Characterization

3.1. Physical Characterization of the Organic Layer

3.1.1. Amount of Organic Layer

Parameter

Determination of the weight of the organic layer (volume-dry weight, kg/m²). For method of soil moisture content, see Annex I SA02).

Method

In the field, the total fresh weight of each layer (OL, OF and OH or Hf, Hsf, and Hs) has to be determined, preferably together with the thickness of each organic layer (OL, OF and OH or Hf, Hsf, and Hs) (see also § 2.2.3.2.). Of each layer a subsample is collected for determination of moisture content (weight %) in the lab. Based on the result of the moisture content, the total dry weight (kg/m²) of each layer can be calculated.

Note: For the BioSoil project the OF and OH layer can be sampled together (OFH layer).

3.2. Physical Characterization of the Mineral Layer

3.2.1. Particle Size Distribution

Parameters to be determined

The determination of the soil granulometry and classification according to the USDA-FAO textural classes (Figure 1) is mandatory for the mineral layers for Level II, only if not already determined for the first survey (no repetition required if this parameter was already measured).

For Level I, information on textural class for the mineral layers is mandatory too (though again only if not done in the first survey). However, for Level I an estimate based on the finger test in the field on 1 composite of each layer can be accepted for classifying the soil texture according to the USDA-FAO textural classes. In addition an estimate of the clay content is mandatory as well. Practical guidelines can be consulted in the Guidelines for Forest Soil Profile Description (Mikkelsen et al., 2006).

Relevance

Texture is needed for the profile description and WRB classification (mandatory for both Levels). In addition texture, and in particular the clay content, is required for the determination of nutrient exchange ability of the soil (interpretation of other - mandatory - parameters).

Box 3: Definition of particle size classes

The particle size classes of the fine earth fraction (< 2 mm) are defined as follows (FAO, 1990a):

Clay < 2 µm
Silt 2 – 63 µm
Sand 63 – 2000 µm

Method

Level I: finger test for estimation of soil texture classified according to USDA-FAO texture triangle (FAO, 1990a), and for estimation of the clay content (%). Optional: reference method as described for Level II.

Level II: reference method as described in Soil Manual of ICP forests (Annex 1: SA03)

Practicability

Repetition of the determination of the granulometry is not required. For Level I, extra time and costs are minimised if estimated by finger test.

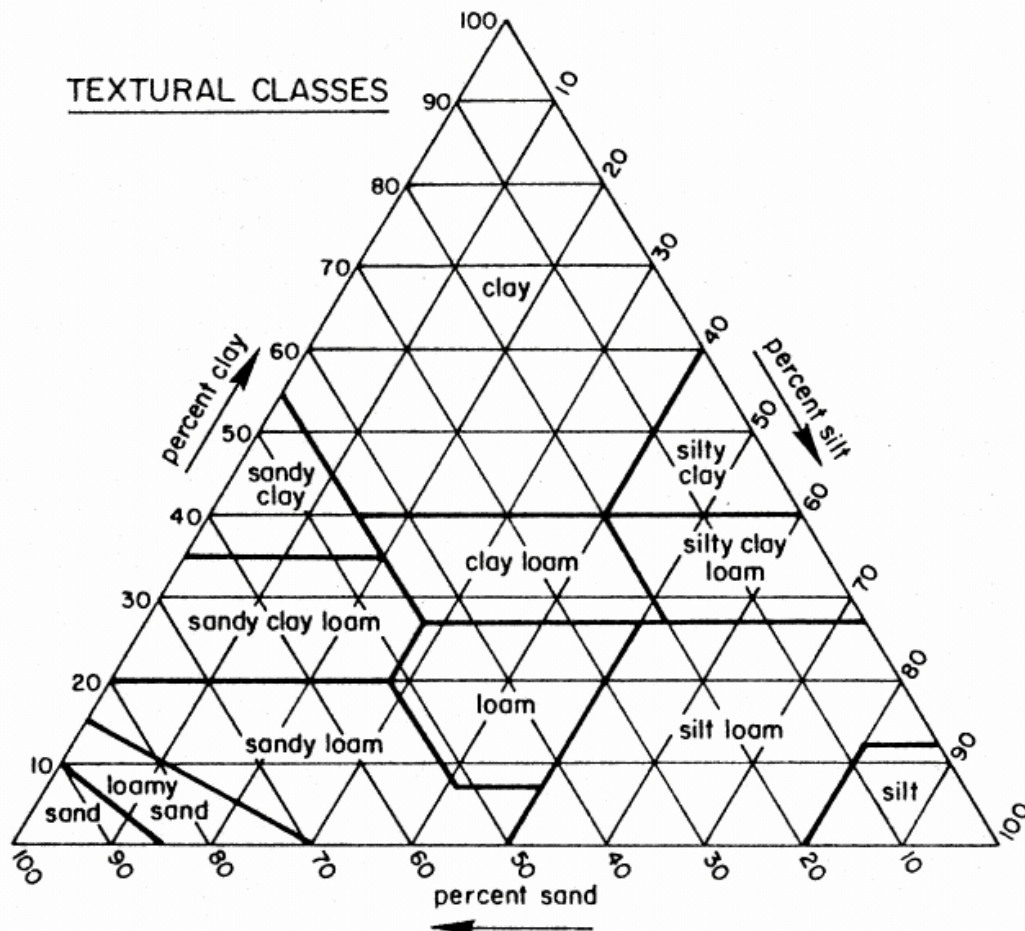


Figure 1: Textural classes according to USDA (1951) and adopted by FAO (FAO, 1990a)

3.2.2. Bulk Density of the total mineral soil

Optional and mandatory parameters

One value of bulk density has to be reported mandatory for the mineral topsoil (0-10 cm) of non-stony soils. For Level I, this value may be obtained either by estimation, pedotransfer functions or measurement. For Level II, the bulk density has to be measured. Determination of the bulk density of the deeper layers is optional for both Levels. No re-measurement is required if this parameter was determined according to the reference method for the first survey.

Methodology

For measurement: five samples have to be taken with a minimal volume of 100 cm³ per plot and per layer. In addition, the determination of bulk density requires estimation of coarse fragments according to the USDA-FAO classes (FAO, 1990a). This can be measured or estimated in the soil profile. This estimation according to the fixed depths shall be done in addition to the normal profile description which follows the genetic layers.

Definition

Bulk density is defined as the mass (weight) of a unit volume of oven dry soil. The volume includes both solids and pores. In mineral soils with no coarse fragment content the bulk density of the total mineral soil is equal to the bulk density of the fine earth.

Relevance

The physical arrangement of the soil components is important for determining the nutrient supply to plants and in calculation of stocks. In addition, bulk density has an indirect influence on the concentrations of air pollutants in the soil (Vanmechelen *et al.*, 1997).

3.2.3. Coarse Fragments

Report the amount of coarse fragments (boulders, stones and gravel with a diameter > 2mm) of the individual mineral layers in volume %. The abundance of coarse fragments can be measured in the laboratory, but is usually estimated during routine soil profile observations. In the case that very coarse materials are present (stones and boulders), the quantity of these materials has to be estimated in the field (e.g. method established in Finland, as described in Annex 1: SA05 or in Germany, see Annex1: SA04).

The determination of coarse fragments is mandatory for the 0-10 cm mineral layer and optional for 10-80 cm mineral layer in both Level I and Level II. In case of re-assessment (if this parameter was already measured according to the reference method in first survey) the parameter is optional. For Level I the parameter may be estimated, for Level II it must be measured using the methods described in Annex 1: SA05.

3.2.4. Combined approach to estimate bulk density, coarse fragments and fine earth stock in stony soils

Relevance

Recent investigations (Riek and Wolff, 2006) have revealed that the soil physical parameters (in this case bulk density and fine earth stock) can only be recorded with field methods at specific locations in an inadequate or scarcely reproducible manner. This applies to soils with a high content of coarse gravel (2 – 6 cm) and/or the presence of stones (6 – 20 cm) and boulders (> 20 cm). Because of their low volume, the core samplers normally used in forest monitoring are not able to representatively collect stones or large portions of coarse fragments in the field. In these cases, the excavation method may produce good results but it is probably too expensive, time-consuming and destructive in the framework of large-scale monitoring.

Methodology

The combined approach can improve the determination of these parameters at locations with a high content of coarse gravel and/or the presence of stones and boulders and lead to a better approximation of the real coarse fragments content.

In the case of a high content of coarse gravel and/or the presence of stones and boulders, the quantity of bulk density of both fine earth and coarse fragments has to be estimated / sampled in the field. Methods should be selected according to the prevailing conditions (i.e. coarse fragment content and size) at each individual sampling site.

In the analysis each method or each combined method leads to the determination of (partially) different parameters which means that different calculation formulas are needed. A description of the different methods and/or combined methods, the related parameters and calculation methods are described in Annex SA04.

If the mineral soil contains no coarse fragments or the (estimated) coarse fragment portion is less than 5 % (**case 1**), then the bulk density of the fine earth (BD_{fe}) is approximately equivalent to the bulk density of the total mineral soil (BD_s) (see paragraph 3.2.2).

In case of mineral soils with a coarse fragment content of more than 5% which can be sampled with a core sampler or any other (representative) sampler with coarse fragments < 20 mm (**case 2**), a representative volume sampling with core sampler, root auger, AMS core sampler with liner or hollow stem auger is done. When calculating the bulk density of the fine earth, the volume of the coarse fragment content is subtracted from the total volume of the sampler and the mass of the coarse fragments subtracted from the mass of the fine earth referring to this volume.

In case the mineral soil cannot be representatively sampled with a core sampler or any other samples (coarse fragments > 20 mm) (**case 3**), there are two possibilities of taking the coarse fragments into

account. The amount of coarse fragments has to be estimated in the field or be determined by additional sampling with a shovel or a spade (representative volume sampling).

When representative volume sampling is not possible, sampling with mini-core samplers and estimation at the profile (coarse fragments > 60 mm) is required (**case 4**). The bulk density of the fine earth in the spaces between the coarse material [soil skeleton] is determined with a mini-core sampler. In addition, a disturbed spade /shovel sample is taken in order to determine factor f (correction factor for a possible coarse fragment portion in the mini-core sampler). Furthermore, the coarse fraction portion > 60 mm is estimated at the profile.

3.3. Chemical Characterization of Collected Samples

3.3.1. Selected Key Soil Parameters for the Level I and II Survey

An overview of the key parameters to be measured is presented in Table 3. The key parameters CEC and Base Saturation will be calculated from the data reported, and as such do not have to be submitted and are not included in the table. Note that the minimum requirement for a number of the mandatory parameters, indicate that in the mineral layers below 20 cm the parameters should be measured once and not necessarily be re-measured a second time. Temporal changes are in the first place expected in the upper layers. Mandatory Level II parameters of the deeper layers (20-40 and 40-80 cm) that were already measured with the reference method for the first survey, do not have to be determined again. This means that if *all* the mandatory parameters of these deeper layers were assessed with the reference method, re-sampling of these layers is not needed.

Table 3: Chemical and physical key soil parameters ⁽¹⁾

Parameter	Unit	Decimals	Level I					Level II				
			Organic Layer		Mineral Layer			Organic Layer		Mineral Layer		
			OL	OF+OH, H-layers ⁽²⁾	0-10 cm	10-20 cm	20-40 cm 40-80 cm	OL	OF+OH, H-layers ⁽²⁾	0-10 cm	10-20 cm	20-40 cm ⁽³⁾ 40-80 cm ⁽³⁾
<i>Physical soil parameter</i>												
Organic layer weight	kg/m ²	2	O	M	-	-	-	O	M	-	-	-
Coarse fragments	%	0	-	-	M ^{(3), (4)}	O ^{(3), (4)}	O	-	-	M	O ^{(3), (4)}	O ^{(3), (4)}
Bulk density of the fine earth	kg/m ³	0	-	-	M ^{(3), (5), (6)}	O ⁽⁴⁾	O	-	-	M ^{(3), (5)}	O	O
Particle size distribution (FAO, 1990a)	-	-	-	-	M ^{(3), (7)}	M ^{(3), (7)}	O	-	-	M ⁽³⁾	M ⁽³⁾	M ⁽³⁾
Clay content	%	0	-	-	M ⁽⁷⁾	M ⁽⁷⁾	O	-	-	M	M	M
Silt Content	%	0	-	-	O	O	O	-	-	M	M	M
Sand Content	%	0	-	-	O	O	O	-	-	M	M	M
<i>Chemical soil parameter</i>												
pH(CaCl ₂)	-	1	-	M	M	M	O	-	M	M	M	M
Organic carbon	g/kg	1	-	M	M	M	O	-	M	M	M	O
Total nitrogen	g/kg	1	-	M	M	M	O	-	M	M	M	O
Carbonates	g/kg	0	-	M ⁽⁸⁾	M ⁽⁹⁾	M ⁽⁹⁾	O	-	M ⁽⁸⁾	M ⁽⁹⁾	M ⁽⁹⁾	O
Aqua Regia extracted P, Ca, K, Mg, Mn	mg/kg	1	O	M	O	O	O	O	M	O	O	-
Aqua Regia extracted Cu, Pb, Cd, Zn	mg/kg	1	O	M	M	-	-	O	M	M	-	-
Aqua Regia extracted Al, Fe, Cr, Ni, S, Hg, Na	mg/kg	1	O	O	O	-	-	O	O	O	-	-
Exchangeable Acidity	cmol(+)/ kg	2	-	M ⁽¹⁰⁾	M	M	O	-	M ⁽¹⁰⁾	M	M	M
Exchangeable Cations: Ca, Mg, K, Na, Al, Fe, Mn, H	cmol(+)/ kg	2	-	M ⁽¹⁰⁾	M	M	O	-	M ⁽¹⁰⁾	M	M	M
pH(H ₂ O)	-	2	-	O	O	O	O	-	O	O	O	O
Total Elements: Ca, Mg, Na, K, Al, Fe, Mn	mg/kg	1	-	-	-	-	-	-	-	O	O	O
Oxalate extractable Fe, Al	mg/kg	1	-	O	O	O	O	-	O	M	M	M

¹ Abbreviations : M = mandatory parameter, O = optional parameter

² If the OH - horizon > 1 cm, the OF - and the OH - horizons should be analysed separately and each value has to be reported

³ In case of a re-assessment (if the parameter was already measured according to the reference method for the first survey) , the measurement is optional

⁴ May be obtained by estimation or measurement

⁵ Mandatory only in non-stony soils

⁶ May be obtained by estimation, pedo-transfer function or measurement

⁷ May be obtained by finger test, consists of texture classified according to USDA-FAO texture triangle

⁸ Only mandatory if pH(CaCl₂) > 5.5

⁹ Only mandatory if pH(CaCl₂) > 6

¹⁰ In calcareous soil, the measurement of this parameter is optional

Relevance

The relevance of the key parameters is given in Table 4.

With regard to the nutrients, the amount extracted by aqua regia has been made mandatory for the OF+OH horizons and H layers of the organic layer and optional for the mineral topsoil. While from this extraction not the real total content is obtained, it is useful as an estimate of the nutrient stock. Extra costs and work are minimal as it can be measured from the same extraction to be made for the heavy metals (mandatory for both the OF+OH horizons, H-layers and the mineral topsoil). For the determination of the 'real' total amounts, more specialised material and skill are required. As these 'real' total contents are important for the calculation of weathering rates and critical loads, they have been made optional for Level II.

Note that the measurement of Carbonates is required also for the correction of the organic carbon content if the $\text{pH}(\text{CaCl}_2) > 5.5$ in the organic and > 6 in the mineral layer.

For the determination of the pH, measurement on a CaCl_2 -extract is recommended. $\text{pH}(\text{H}_2\text{O})$ has been made an optional parameter for reasons of comparability, as this is mostly used in literature.

Table 4: Relevance of the key parameters

Type of parameter	Key parameters	Layer	Relevance
Carbon and nitrogen	C_{tot} , N_{tot} , (Carbonates)	Organic	Forest nutrition, atmospheric N deposition, climate change
		Mineral	Forest nutrition (0-20 cm), C- & N sinks
Nutrients	Total P, Ca, Mg, K, Mn	Organic	Atmospheric deposition of basic cations, stock of main nutrients
		Mineral	Weathering rates, critical loads of acidity, stock of main nutrients
Acidity, Exchange characteristics	pH, Carbonates, CEC, BS, Exchangeable cations, Exchangeable Acidity	Organic	Buffering acid input
	pH, Carbonates, CEC, BS, Exchangeable cations, Exchangeable Acidity, Al_{ox} , Fe_{ox}	Mineral	
Heavy metals	Pb, Cu, Zn, Cd	Organic	Atmospheric metal deposition
		Mineral	Atmospheric metal deposition, calculation critical loads (0-20 cm), deficiency of oligo elements
Physical soil parameters	Organic layer weight	Organic	Calculation of stocks
	Bulk density of the fine earth (BD_{fe}) and the coarse fragment content	Mineral	

Practicability

Elements that require special equipment or particular skill to determine correctly have been made optional or were skipped altogether.

3.3.2. Reference analytical methods

The analytical methods proposed as the reference methods for the 1st survey are proposed again for the second survey, though adapted to ISO methods. The full description of the reference methods is given in Annex 1.

Table 5 gives an overview of the reference methods. Note that the parameters are grouped according to the analysis method. As such it is made clear which elements can be measured in the same run, without additional costs and hardly extra work involved.

Table 5: Overview of reference methods for the chemical parameters

Parameter	Reference Analysis Method ¹		Unit ²	
	Extractant	Measurement method(s) ³		
pH(CaCl ₂)	0.01 M CaCl ₂	pH-electrode		
pH(H ₂ O)	H ₂ O	pH-electrode		
Total nitrogen	-	Dry Combustion or Modified Kjeldahl	g/kg	
Organic carbon ⁴	-	Dry Combustion at ≥ 900 °C		
Carbonates	HCl	Calcimeter		
P	Aqua Regia	ICP	Colorimetry	mg/kg
K, Ca			AAS	
Mg				
Mn				
Heavy metals: Cu, Cd, Pb, Zn				
Other: Al, Fe, Cr, Ni, Na				
Hg		Cold vapour AAS		
S		ICP		
	CNS - analyser			
Free Acidity (or sum of AC ⁵) and free H ⁺	0.1 M BaCl ₂	titration to pH 7.8 or 'German' method (difference in pH before and after extraction and model)	cmol+/kg	
Exchangeable Cations	Al, Fe, Mn,	0.1 M BaCl ₂	ICP, AAS	-
	K, Ca, Mg, Na			FES
Reactive Fe and Al	Acid oxalate	AAS, ICP	mg/kg	
Total Elements: Ca, Mg, Na, K, Al, Fe, Mn	Method using HF or Lithium metaborate, that brings all elements into solution		mg/kg	

¹ Full descriptions are given in Annex

² Results have to be expressed on an oven dry basis

³ For the measurement of a number of parameters there are several alternatives for the equipment that can be used

⁴ Note that for organic carbon a correction has to be made for carbonates

⁵ Alternative for the titration of the exchangeable acidity is the sum of the exchangeable Al, Fe, Mn and H (=Acid Cations)

4. Quality assurance, quality control

4.1. Objectives

The guidelines below are based on the guidelines for quality assurance and control in the field and in the laboratory, as dealt with in detail in the submanual on the Measurement of Deposition (Annex VI.4 Quality Assurance and Quality Control for Atmospheric Deposition Monitoring by E. Ulrich and R. Mosello). The guidelines are intended to be used by the laboratories working on the analysis of the soil samples of the various assessments of the Pan European Monitoring Programme. In this paragraph, the focus is put on the analysis of the soil samples. These guidelines should allow the laboratories to develop their own methods to control the quality of soil analysis and should improve comparability of analysed data over the Programme.

4.2. Starting points

Quality assurance and Quality Control (QA/QC) are national issues. The National Focal centre (NFC) is responsible to select an adequate laboratory, the laboratories are responsible for the QA/QC. Laboratories use the proper equipment and the reference methods for the digestion and chemical analysis of samples. The laboratories set up good methods to control the quality of the analysis carried out. Proof of the obtained quality is documented and submitted to European level. Laboratories apply local, national and international reference material to ensure the quality over time and comparability on (inter-)national level.

4.3. Principles

Repetitive analysis of reference materials is the basis of a good QA/QC programme. The repeated digestion/analysis over time of the same sample will provide insight in the random and systematic deviation from the average over time.

When the mean stays the same over a longer period of time, no trend is taking place. The measured values allow the calculation of the standard deviation around this mean.

Mapping of the values of the repeated analysed samples will show trends in time. The standard deviation can be used to define the limits (e.g $2 \times SD$) outside where the encountered value is highly improbable. Values outside this range function therefore as a warning signal. It indicates that something different than 'normal' has taken place. There are many possible reasons and such a warning signal has to be followed by proper laboratory actions.

4.4. Reference materials

Reference materials come in various sorts and prices. International Reference Materials (IRM) are expensive and should be used only when really needed. In many cases the concentrations are not in the ranges encountered in the daily practices.

National Reference Materials (NRM) are in many cases easier to get and often not so expensive as IRM. They are in most cases issued by national laboratories and very useful to ensure the quality over the laboratories within a country.

Local Reference Materials (LRM) are (to be) prepared by the laboratory itself and can be easily prepared in large quantities, very cheaply. It can also be made in the correct concentration ranges for the more important parameters.

Especially these LRM have a high importance for the QA/QC activities.

4.4.1. Preparation of Local Reference Material

Due to the nature of the soil samples and its two-step analysis LRM samples of both the solid phase (to control the quality of digestion) and the liquid phase (to control the quality of the chemical analysis) is needed.

Solid phase LRM: Take several larger samples from a site (e.g L/H horizons, mineral soil: 0-10 cm and 20-40 cm). Dry all sampled material and homogenise the sample material to ensure a uniform mixed sample. Split or riffle the sample in several parts and store in cool and dry place. It may be useful to prepare several LRM-sets for the different parts and concentration regimes in the country (e.g. a LRM for the samples of a clay soil in the coastal area with high concentration of sea salt versus a LRM on sandy soil in and inland situation).

Liquid phases LRM: After digestion of larger part of the solid phase LRM, store the liquid LRM in a cool and dark place.

In general, no control of high concentrations is done, because the errors are higher the lower the concentration is. Often, higher concentrated solutions are diluted in order to reduce the concentration so that they fit into the ranges for which the analysers were calibrated.

The quantity of the LRM has to be large enough to be used for a longer period of time (preferably up to one year). The needed annual quantity will depend on the type of analytical equipment and method used by the laboratory. The sample should be stored in such conditions that no (or minimal) changes take place over time.

Note: a small standard deviation around the mean is very nice and an indicator of very accurate and precise work, but not the first objective of this QA/QC.

4.4.2. Initiation of Local Reference Material

When the LRM-sample has been prepared, a test run has to be made. For this initiation the equipment has to be calibrated as good as possible. A number of replicates (e.g.5 repetition of the solid phase and 30 of the liquid phase) of the LRM is to be analysed plus at least one (but preferably more) samples of a NRM or even an IRM. All relevant parameters are analysed.

From the results of the NRM and IRM the accuracy in absolute sense is determined for each parameter. The spread of the results of the LRM gives an indication on the SD. Naturally the smaller the SD, the better the results. The results of this first test-run should be treated according to the ISO standard 8258 (1991) (Shewhart control charts). The mean value of the parameter in the LRM is of less importance, but should be in the same range as the values of the real samples.

From this point each parameter has a SD. This could result in an evaluation of the included parameters and the relevance of the analysis by the methods applied. When the SD is significant larger then the expected values the relevance to analyse the parameter is small. Other methods/equipment may have to be used to analyse the parameter within an acceptable range.

This procedure is to be repeated whenever equipment is changed, important parts are replaced or when trends seem to have taken place over time. In the latter case the absolute values obtained from the NRM and IRM are of high importance.

4.4.3. Implementation of the Local Reference Material

After the successful initiation of the LRM a systematic re-sampling of the LRM (liquid phase) takes place in every batch or series. Depending on the number of samples to be analysed and the methods and equipment used, this could be in the range of one LRM per 10 analysed real samples to 1:20. For the solid phase (digestion and analysis) this could be reduced to 1:100.

The results of the repeated analysis of the LRM allow the evaluation of the stability of the method/equipment over time. It is therefore important that no changes take place in the LRM sample over time. It is therefore strongly recommended that every analysis of the LRM is mapped in a graph over time (see ISO 8258). The example in Figure 4 shows lines with the mean (10), the 1*SD (9 and 11) and 2*SD (8 and 12) are indicated as a result of the initiation of the LRM.

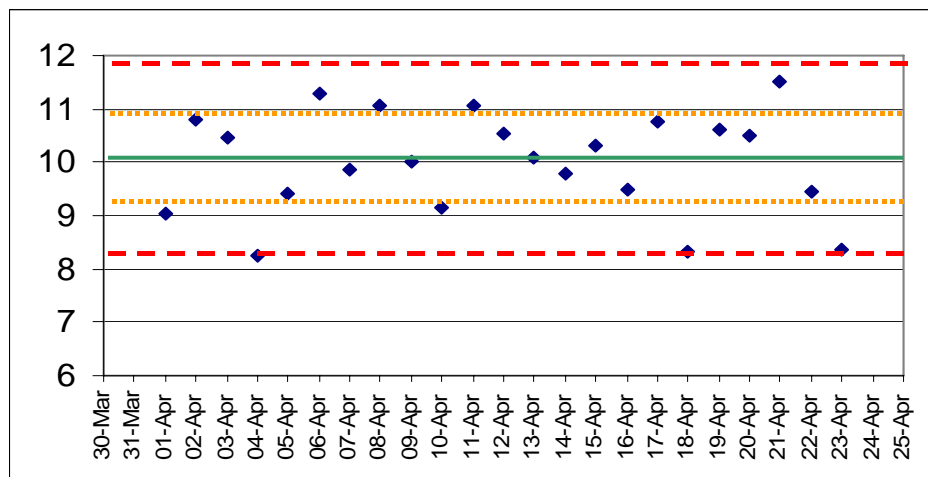


Figure 4: Example of a control graph for medium chloride concentrations in countries near the sea (in mg/l) with the mean around 10 and a SD on 0.9. The dots are the daily means of the measured values of the LRM over time.

4.4.4. Basic principles for use of the Shewhart control chart

Assuming that the concentrations in the LRM do not change and the methods/equipment remain the same, the spread of the measured values should be within the range of $1*SD$ (68%) and randomly distributed around the mean. These two requirements need to be tested every time a set of samples is processed. The graph visualises these things easily, but statistical calculation will be needed to calculate the probability of the occasion.

This means that when measured values fall (far) outside the $1*SD$ lines a problem has occurred. Similarly when the mean value (of e.g. the last 10 values) is drifting away from the original mean, systematic changes are taking place.

A check of methods/equipment has to be carried out. If the values fall out of the $2*SD$ range, the equipment must be checked entirely and re-calibrated. Further on, the whole series of samples having been analysed during the time when the $2*SD$ range was exceeded, has to be reanalysed.

4.4.5. Translating the results of the repeated sampling of Local Reference Material into QA/QC

The mapping of the values of the repeated sampling of the LRM over time is a proof of consistent analysis. Evaluation of the results per parameter leads now to a quality statement per parameter. This statement is only valid for the concentration range of the concerned LRM. It is therefore recommended to use in very different concentration situations more LRM's. In general, it is sufficient to use two sample concentrations, one for low concentrations and one for medium concentrations (e.g. for nitrogen 1 and 5 g/kg). Samples with higher concentrations are regularly diluted by the lab technicians in order to fit in the calibrated concentration range. Moreover, higher concentrations have a much smaller percentage of error/variation (reproducibility: 1-5%) than low concentrations (reproducibility: 5-10 %) or very low concentrations (reproducibility: 10-20%).

Box 4: Definition of reproducibility

The analytical results are obtained by the same method, using the same LRM, but not at the same moment (sometimes with days or weeks of delay between two subsequent analyses) and sometimes by different persons in the laboratory. The percentage of reproducibility is the variation coefficient of the annual mean of one LRM.

4.5. Co-operation with neighbouring laboratories

As mentioned before, the NRM and IRM may not have concentrations in the right ranges. This makes the tests less valuable. On the other hand when well initiated LRM are available, these could be used in neighbouring labs as an additional NRM or IRM. The cost is of course minimal and at the same time regional coverage with good samples is achieved. In a further step of cooperation, the analysis of parameters for which special equipment is needed (e.g. low concentrations of heavy metals), could be concentrated in a limited number of labs. In many cases pooled samples can be used to further reduce costs.

4.6. Submission of information on quality to European Level

Besides the actual measured values of the samples, also information on the QA/QC will be requested at European Level. This information consists of:

- Complete description of the methods applied in view of the QA/QC, using a fixed coding system, in the form of a Data Accompanying Report Questionnaire (DAR-Q)
- Description of the complete procedures of handling of the sample, including information on storage and delay time; produce a flow chart of the way the samples go from the field till the end of the different determinations; this chart should show the flow in time and space
- Shewhart control charts of the LRM for the various parameters (including the statistical analysis such as initial Mean and SD, differences of the mean over time, points of action/re-measurement/calibration and or re-initiation)
- Calculation of the coefficient of variation (reproducibility, definition see above) for all LRM, depending on the concentration (for each concentration a CV has to be calculated).

5. Data reporting

The following rules apply:

- Data will be reported separately for the H- and O-horizons and for the mineral soil.
- For the organic layers reporting is done according to the OL-, OF-, OH-, OFH-, Hf, Hs, Hfs horizons or as described in Box 1 of this manual.
- For the mineral soil reporting is done according to the defined mandatory depth layers.
- For the peat layers, reporting is done according to the defined depth layers (Mandatory: H01, H12 and Optional: H24 or H48) and following the parameter list for the OF, OH and H-layers of the organic horizons.

Data shall be submitted using the forms in Annex 2.

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