UMWELTPROBENBANK
DES BUNDES

Guideline for Sampling and Sample Treatment

Lombardy poplar (*Populus nigra 'Italica'*)

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Für Mensch und Umwelt

Contents

Guidelines for Sampling, Transport, Storage and Chemical Characterization of Environmental and Human Samples

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German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an instrument of environmental monitoring for the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) underlying specialized and administrative co-ordination of the Federal Environmental Agency (Umweltbundesamt, UBA). The ESB collects ecologically representative environmental specimen in addition to human samples, maintains and examines them concerning relevant environmental substances (BMU 2008).

Long term storage is accomplished under conditions, which exclude condition change or loss of chemical characteristics, over a period of numerous decades. The archive stores samples for retrospective examination of such substances whose danger potential for the environment or for human health is today unknown.

Comprehensive information of the ESB is available at www.umweltprobenbank.de.

2 Guideline Objective

Sampling is the first and most important step to safeguard the quality of samples and data. It is the result of science-based and standardized methods, to avoid contamination and inhibit loss of chemical information. The exceptionally high demand of true quality results derives from the extraordinary value of the samples as archive material. Representativeness and reproducibility of the samples are the basis for spatial and temporal comparison.

The current guideline is an update of Wagner et al. (1996) version. It is conform to the VDI guideline 3792 sheet 11 (VDI 2007).so that the research results are comparable with other monitoring programs.

Transport, further sample treatment and storage as well as chemical analysis have to be done following the actual guidelines of the ESB.

Function of the Specimen Type

In contrast to evergreen conifers, the leaves of the summer green deciduous trees are only exposed during the vegetation period to environmental influences. Thus they are indicators for characterizing effects of air-borne pollution, during the vegetation period. They are used complementary to evergreen conifers.

Due to its frequency in densely populated areas and in agrarian landscapes, the Lombardy poplar *(Populus nigra* 'Italica') is a suitable specimen for urban regions. It is a male clone of the European black poplar *P. nigra* L. which developed into existence through unique mutation. It is vegetatively reproduced as a clone and spread by man (TOEPFER 1926, cited acc. to HEGI 1980). The following criteria underline its use as an accumulation indicator in the scope of the ESB:

- worldwide it is spread in almost all developed countries as ornamental tree and wind shelter planting in urban areas and agrarian landscapes (WEISCHET 1963; FAO 1979),
- great genetic analogy (clone),
- great ecological valance and high resistance to environmental influences (e.g. SAUER 1967; EMSCHERMANN 1972; DIMITRI 1973),
- physiologically and eco-physiologically highly investigated species (e.g. JOACHIM 1953; SEBALD 1959; CANNON et al. 1972; SEVERIN & KÖSTER 1982; KSIAZEK et al. 1984; OMASA et al. 2000),
- continuous exposure of the leaves; the pollution burden of the leaves represents the integrated effective dose of the main vegetation period, even if only one sampling is performed per annum,
- existence of comprehensive knowledge as an accumulation indicator in field-tests (e.g. HALLEZ et al. 1979; CLAUSSEN & Bartels 1982; DITTMANN et al. 1984; GRIMMER et al. 1985; WAGNER 1987; CAPELLI et al. 1989; TERHORST & WITTIG 1988/89; DJINGOVA et al. 1993, 1995, 1996, 1999, 2001; SAWIDIS et al. 1995; MARTH et al. 1999).

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4 Target Compartments

In accordance with the ESB, leaves without stalks are collected as target compartments. The dense foliage and the very prominent reticular venation are the reason why leaves of the poplar accumulate and bind a considerable quantity of particles (SAWIDIS et al. 1995).

As long as pollutant accumulation is related to exposure, the age plays a crucial part in the evaluation of environmental samples. The trees continue to grow new leaves during the whole summer, therefore the youngest leaves on top of the long shoots must not be sampled.

5 Predefinitions for the Sampling

5.1 Selection and Definition of Sampling Sites

In urban-industrial and densely populated areas it is necessary to define sampling sites containing several locations within a sampling region, to sufficiently detect the spectrum of the regionally differentiated burden.

Preceding the first sampling a screening has to be conducted to define the sampling site(s) and the random sample number. Objective of this screening is to determine the availability and spatial distribution of the specimen type, the diversification of the characteristics, the material composition, and the spatial schemes of the pollution load.

First step of the screening is a mapping of all plots where the Lombardy poplar occurs and which are suited as locations within the respective sampling site. In doing so, the criteria listed in chap. 5.2 have to be met.

From the selected plots trees are sampled and tested for homogeneity by biometrical and analytical characterization. In case of adequate homogeneity the location is determined as part of the sampling site.

Subsequently, number and position of the sampling sites within the area under investigation are defined.

5.2 Selection of Individuals and Sample Size

The sample size for the annual routine sampling is obtained by interpretation of the screening results.

For routine sampling of the ESB, samples should be taken from at least 15 trees per sampling site. By a given sample collective of 15 trees a minimum of 150 g fresh weight leaves without stalks per tree should be collected to represent the respective tree to a sufficient extent.

The trees are randomly selected within the locations and should comply with the following criteria:

- 20 years old or older, to exclude the juvenile stage,
- with branches from the base on upwards,
- free from intense biological (e.g. droughty crowns) or mechanical damages.

Specimens with early yellowing leaves, rust infestation (over 10% of the leaves),serious aphid infestation, and those with chlorosis, necrosis, or damage through feeding on leaves, are not selected unless this feature characterizes the average condition at the sampling location.

Each time, the individual trees must be randomly chosen. The trees selected for sampling must be free-standing without being considerably shielded by buildings or vegetation etc. and without being located near local sources of emission.

5.3 Sampling Period and Frequency

In long term programs as that of the ESB sampling should be carried out annually.

Sampling should take place in the late summer before leaf discoloration. Since this period varies both annually and in dependence with the climate zone, varying sampling periods for different sampling areas result which need to be substantiated in the area related sampling schemes. The sampling should be completed in the lower areas by the end of August and in higher areas by mid September.

With repeated sampling on the same site, sampling should be carried out depending on

weather condition and phenology in the same determined period.

5.4 Area Related Sampling Scheme

Based on the sampling guidelines, specific definitions for the individual sampling areas and sites must be made and documented in an area related sampling scheme. This includes amongst others:

- location and demarcation of the sampling sites,
- required sample size,
- time frame for sampling,
- addresses of the appropriate authorities.

Describing the characteristic elements of the sampling sites within the area related sampling scheme secures long-term continuous sampling. In the case of changes within the sampling site or the sampled population the document has to be updated.

In case of major changes, so that comparability of the samples could not be guaranteed anymore, a new site has to be selected.

6 Sampling Procedure

All data collected in the course of sampling and through the biometric sample description are documented in the respective specimen data sheets (see appendix). A record is kept for each sampling with the following contents:

- all persons involved in the sampling,
- chronological procedure of the sampling,
- the underlying version of the sampling guideline and the area related sampling scheme for the current sampling,
- alterations to the sampling guideline and the area related sampling scheme.

6.1 Required Equipment and Cleaning Procedures

Field work:

- specimen data sheets for documentation during the sampling
- shears with telescopic handles extendable to a length of 5 m
- several stainless steel scissors
- stainless steel trough to catch the cut leaves
- stainless steel containers (3.5 l or 5.5 l) with lids and fasteners
- waterproof pen for inscribing the paper bags and stainless steel containers
- stainless steel tweezers
- paper bags (1 bag per tree)
- disposable gloves
- scales (effective range up to at least 3 kg, reading accuracy 1 g)
- measuring tape for measuring the trunk circumference
- tree height measuring instrument
- air-thermometer (reading accuracy 1°C)
- soil-thermometer (reading accuracy 1°C)
- camera for documentation
- liquid nitrogen
- tools and protective clothing for liquid nitrogen handling
- cooling device (dewar vessel) for the rapid deep-freezing and storage of the samples in the gas phase above liquid nitrogen (LIN), corresponding to the number of required stainless steel containers

Laboratory:

- specimen data sheets for the biometric sample description
- cabinet dryer (80°C (+/– 5°))
- precision scales (reading accuracy 1 mg)
- weighing pans
- stainless steel tweezers

Cleaning procedures:

Sample containers and all equipment is cleaned in a laboratory washer using a chlorine-free powerful washing agent in a first step. After cold and hot (90-95°C) rinsing, neutralization using 30 % phosphorus acid in warm water is performed, followed by hot and cold rinsing with deionized water. After this procedure the containers are dried in a cabinet dryer at 130°C (+/– 5°) for a minimum of an hour (sterilization). The containers remain closed while they are left to cool. Sterilization is not applied to synthetic materials.

6.2 Sampling Technique

Sampling is only carried out in dry weather and stopped if it starts raining. Morning dew must have evaporated on the leaves in the tree crown prior to starting or continuing the collection. Inevitable deviations must be precisely noted in the sampling record.

Samples are taken at a height of 5 to 7 m above ground by evenly including all directions. The youngest leaves on top of the long shoots are not used.

The branches are cut off using a pair of shears with telescopic handles, extendable to about 5 m. At least four branches are cut from all around the crown periphery of each tree (Fig. 1). In the dropping procedure and where the branches hit the ground care must be taken that there is no contamination. The cut branches are placed in the shadow until they are processed.

After biometric specimen description (chap. 7), the leaves without their stalks are cut using stainless steel scissors so that they fall directly into the stainless steel trough without being touched. The youngest leaves at the top of the long shoots remain unconsidered. Fig. 1 demonstrates the sampling procedure.

For biometric sample characterization, 25 leaves are randomly taken from each tree out of the total sample amount using stainless steel tweezers and collected in a paper bag labeled with the respective tree number.

The remaining leaves are transferred from the stainless steel trough to the storage vessel with gloved hands after it is has been weighed empty. After filling the container, the gross weight is determined and noted in the corresponding data sheet.

The samples are immediately rapid-frozen on-site in a dewar vessel for the further storage and transportation of the samples in the gas phase above liquid nitrogen (LIN).

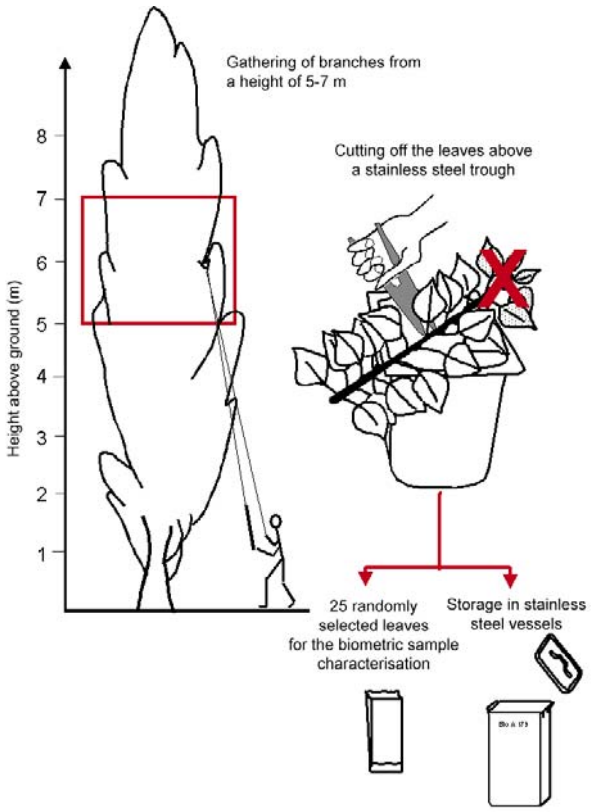

Fig. 1: Schematic representation of the sampling (altered according to WAGNER 1995)

7 Biometric Characterization

On site the parameters for the sample description are recorded persistent with the respective specimen data sheets (description of the tree and description of the leaves) prior to the removal of the leaves.

With the help of the position of a leaf on a long shoot, the age of a leaf (~ duration of exposure) can be estimated pretty well.

Using 25 randomly selected leaves per tree, the dry weight is determined (reading accuracy 0.01 g) in the laboratory. The paper bags containing the 25 randomly selected leaves per tree are placed in the drying cabinet (80°C (+/– 5°)) for drying (not too densely packed to prevent overheating), and left to dry for about 2 days (until weight stability is reached).

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Checklist to Prepare and Conduct the Sampling

Specimen Type:	**Lombardy poplar (*Populus nigra* 'Italica')**
Target Compartments:	leaves without leaf stalks
Individual Specimens:	poplars > 20 years (to exclude the juvenile stage)
Random Sample Number:	at least 15 trees per sampling site
Sample Quantity for the ESB	150 g fresh weight (= leaves without stalks) from 15 trees must be sampled to gain the needed quantity of 2.200 g
Sampling Period:	Early August until middle of September (prior to leaf discoloration)
Sampling Frequency:	1 sampling per annum
Equipment Required for Field Work:	• specimen data sheets for documentation during the sampling (sampling location, atmospheric condition, description of the tree and the leaves, storage) • shears with a telescopic handle extendable to about 5 m • several stainless steel scissors • stainless steel container (trough) to catch the cut leaves • waterproof pen to inscribe the paper bags and stainless steel containers • stainless steel tweezers, to collect the leaves for the biometric characterization • disposable gloves • scales (effective range up to at least 3 kg, reading accuracy 1g) • measuring tape for measuring the trunk circumference (reading accuracy 1 cm) • tree height measuring instrument • air-thermometer, soil-thermometer (reading accuracy 1°C) • camera for documentation
Sample Packing until Further Processing:	• stainless steel containers (vessels, 3.5 or 5.5 l) with lids and fasteners (1 container per tree), • paper bags (1 bag per tree)
Transport and Interim Storage:	cooling unit (dewar) for the rapid deep-freezing and storage of the samples in the gas phase above liquid nitrogen (LIN)
Required Equipment for Laboratory Work:	• specimen data sheets for the biometric sample description • cabinet dryer (80°C (+/- 5°) • precision scales (reading accuracy 1 mg) • weighing pans • stainless steel tweezers
Biometric Sample Characterization:	tree (see specimen data sheets): • stand type, trunk circumference and tree height leaves: • damage (feeding on leaves, chlorosis, necrosis), contamination of 25 leaves: • petiole gall infestation • dry weight of the leaves (reading accuracy 0.01 g)

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 1: Sampling Location

Lombardy poplar (*Populus nigra* 'Italica')

Identification:

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ **/** __

	Specimen Type
	Specimen Condition
	Collection Date (MM/YY)
	Sampling area (SA)
	Sampling Region (SR)
	Sampling Site (SS)
	Additional information

Tree Numbers: from __ __ to __ __

Gauß-Krüger-Coordinates:

Easting: __ __ __ __ __ __ __ Northing: __ __ __ __ __ __ __

Datum: _____ Ellipsoid: _____

Altitude: __ __ __ __ m (above sea level)

Slope Gradient: __ __ __ %

Exposure: _____

Size of the Sampling Location: __ __ km² __ __ ha __ __ a __ __ m²

Land Use: _____

Sampling Location: _____

Remarks: _____

_____

Person(s) in Charge:

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 2: Weather Conditions

Lombardy poplar (*Populus nigra* 'Italica')

Identification:

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

Tree Numbers: from __ __ to __ __

Last Precipitation Date Preceding the Sampling: __ __ . __ __ . __ __ __ __

Type of Precipitation: ___

(See Table Below)

Start of the Sampling:		**End of the Sampling:**
__ __ . __ __ . __ __	Sampling Date	__ __ . __ __ . __ __
__ __ : __ __	Time	__ __ : __ __
__ __	Air Temperature in 1,5 m Height (°C)	__ __
__ __	Soil Temperature in 10 cm Depth (°C)	__ __
__ / 8	Cloud Covering	__ / 8
__	Type of Clouds	__
__ __ __	Wind Direction	__ __ __
__	Wind Force in Degree Beaufort (see table below)	__
__	Type of Precipitation (see table below)	__

Type of Clouds:
0 = unclouded
1 = Cirrus
2 = Stratus
3 = Cumulus
4 = Fog
5 = High Fog
6. = Stratocumulus




Cirrus | Stratus | Cumulus | Stratocumulus




Type of Precipitation:
0 = No Precipitation
1 = Rain
2 = Drizzle
3 = Snow
4 = Dew
5 = Rime
6 = Torrential Rain
7 = Hail

Wind Force (according to Beaufort):
0 = Calm
1 = Very Slight Breeze
2 = Slight Breeze, moves leaves
3 = Light Breeze, moves twigs
4 = Moderate Breeze, moves thin branches
5 = Bright Breeze, moves medium sized branches
6 = Strong Wind, moves thick branches
7 = Stiff Wind, shakes trees

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Specimen Data Sheet 3: Sample Description

Lombardy poplar (*Populus nigra* 'Italica')

Identification:

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __ __

Tree Number: __ __

Stand Type:

☐ Grove ☐ Marginal Stand Zone

☐ Tree Row ☐ Forest Aisle

☐ Free Standing Solitary Trees

Trunk Circumference (in 1,3 m height): __ __ __ cm **Tree Height:** __ __ m

Location of Sampled Branches in Crown:

☐ Upper Outer Crown
☐ Upper Inner Crown
☐ Lower Inner Crown
☐ Lower Outer Crown (Normal Case)

Damages at Leaves

Feeding on Leaves:

__ __ __ %
(Percentage of the leaf surface, estimation at 5% intervals)

Damage Type

☐ Nonexistent ☐ Leaf Skeletonizing
☐ Pitting ☐ Sucking Spots of Insects
☐ Mining ☐ Other:

Chlorosis:

__ __ __ %
(all yellowish to whitish discolorations, estimation at 5% intervals)

Chlorosis Type

☐ Nonexistent
☐ As Stippling
☐ Blotchy, Skewbald

Chlorosis Dissemination on Leaf

☐ Nonexistent
☐ In the Middle
☐ Tip Burn
☐ Marginal Scorch
☐ Interveinal
☐ At Whole Leaves

Necrosis:

__ __ __ %
(all brownish to reddish discolorations, estimation at 5% intervals)

Necrosis Type

☐ Nonexistent
☐ As Stippling
☐ Blotchy, Skewbald

Necrosis Dissemination on Leaf

☐ Nonexistent
☐ In the Middle
☐ Tip Burn
☐ Marginal Scorch
☐ Interveinal
☐ At Whole Leaves

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 4: Specimen Description and Storage

Lombardy poplar (*Populus nigra* 'Italica')

Identification:

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

Tree Number: __ __

Modification of or Overlay on Leaf Surface:	**Type of Overlay**
(Estimation at 5% intervals)	☐ Nonexistent
Overall Top Side	☐ Honeydew
__ __ __ %	☐ Sooty Mould
Overall Bottom Side	☐ Rust Fungus Infection
__ __ __ %	☐ Other Fungal Diseases on Leaves
	☐ Galls on Leaves
	☐ Other: _____
	_____

Petiole Gall Infestation: __ __ __ % of the leaves

Dry Weight of the Leaves: __ __ , __ __ g, related to 25 randomly selected leaves

Storage

Storage condition: ☐ Dry Samples (standard) ☐ Humid Samples

Number of Stainless Steel Vessel	Weight Empty [g]	Weight Filled [g]	Weighted Sample [g]	Remarks
__ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	
__ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	

Remarks: _____

_____

GERMAN ENVIRONMENTAL SPECIMEN BANK

Sampling Record

Lombardy poplar (*Populus nigra* 'Italica')

Sampling Area: _____ Identification: __ __ __ __ __

Underlying Version of the Sampling Guideline: __ __ . __ __ . __ __

Underlying Version of the Sampling Scheme: __ __ . __ __ . __ __

1. Objective of the Sampling: _____

_____

2. Actual Timeframe of the Sampling:

Date	Time		Sample No.		Remarks
	from	to	from	to	

3. Participants: Conductor/Recorder: _____

Other: _____

_____

4. Checklist referring to Sampling Scheme and Sampling Guideline: ☒ as prescribed

- ☐ 4.1 Sampling Period
- ☐ 4.2 Sampling Site and Sampling Location (Selection/Definition)
- ☐ 4.3 Selection of the Individual Specimens
- ☐ 4.4 Technical Preparations
- ☐ 4.5 Cleaning Procedure for the Packages
- ☐ 4.6 Sampling Technique/Method of Capture
- ☐ 4.7 Sample Amount
- ☐ 4.8 Data Collection
- ☐ 4.9. Transport and Interim Storage

Number, Kind of, and Reason for Possible Variations (Clear Text):

_____

_____

_____

_____

Remarks:: _____

_____

_____ __ __ . __ __ . __ __ _____

Recorder Date Signature