UMWELTPROBENBANK
DES BUNDES

Guideline for Sampling and Sample Treatment

Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)

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

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1 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 present sampling guideline is in accordance with methods of the “emission-ecological forest vitality study" (KNABE 1981, 1983, 1984) and the VDI Guideline 3957 sheet 11 (VDI 2007). The guideline is an update of WAGNER et al. (1993) version and is applied to both, spruce and pine, with slight differences.

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

3 Function of the Specimen Type

Evergreen conifers are of particular importance as bioindicators, among other reasons, due to their:

- wide distribution,
- importance to forestry,
- great capacity to filter substances out of the air flow,
- year-round exposure of the assimilation organs.

The Norway spruce (*Picea abies L.*), as an important forest tree, shows a wide distribution and great abundance with economical and ecological relevance. In Germany the spruce is the most frequently occurring tree species with 28% of the forest area (SCHMIDT-VOGT 1977; BMVEL 2004)

With the increasing continental climate eastwards the Norway spruce is replaced by the Scots pine (*Pinus sylvestris L.).* Additionally the pine is the second most frequently occurring tree species in Germany (BMVEL 2004), with 23% of the forest area.

The selection of both species mentioned for the ESB is based on their prominent role as primary producers in numerous near-natural and anthropogenically influenced ecosystems throughout Central, Eastern and Northern Europe and far beyond their original natural range.

The following criteria underline its use as an accumulation indictor in the scope of the ESB:

- availability of comprehensive baseline and comparative data e.g. due to forest research programs, as well as the forest decline inventories (KNABE 1981, 1982; BMELF 1990; WEISS & TRIMBACHER 1998, EC-UN/ECE 2000; BMJ 2000, SCHRÖTER-KERMANI et al. 2006),
- 100 years and more, spruce and pine have been used both as sensitive effect indicators and as accumulation indicators (WAGNER & MÜLLER 1979; HÖPKER 1991; UMLAUF et al. 1992, 1994a,b; BAUR et al. 1998, TRIMBACHER & WEISS 1999; VISKARI 2000),

- wide distribution throughout Central, East and Northern Europe where they can also be found in areas with high pollution burden (SCHMITT-VOGT 1977).

4 Target Compartments

One-year-old shoots of spruce and pine reflect best the overall situation of an entire year, because they are physiologically most active.

Depending on pollutant type and age group, varying accumulation rates can arise, so that the predefinition of one age group is essential.

In several monitoring programs (i.e. VDI 3957 Bl. 11 (2007)) shoots of spruce and pine from different age groups are dried before the analysis. Thereafter, the needles are removed and analyzed apart. This approach is not feasible for the ESB. Due to the great amount of sample material (approx. 3 kg fresh weight) the separation of the needles would require a substantial effort. Likewise this procedure would cause the loss of highly volatile substances.

The inclusion of stems is essential because low volatile substances condense at particulate matter and adhere preferred to the bark of young shoots (WYTTENBACH el al. 1988; UMLAUF et al. 1994a,b).

5 Predefinitions for the Sampling

5.1 Selection and Definition of Sampling Sites

Representative sampling sites of a sampling area which normally covers several square kilometers, are selected using the stratified random sample principle after GREEN (1979).

Hence multiple (>10) sufficiently homogenous locations are chosen for a screening. Essential criteria for homogeneity are:

- distribution of the target tree species,
- age of the target tree species population,
- forest management,
- exposure.

The selected locations must contain a large number of suitable trees of various ages, to ensure long term sampling. On defining the sampling locations the minimum distance to major roads, railway lines, power lines, etc. should be at least 100 m. For elevated traffic routes the distance should be at least a tenfold of the height of the interfering object above the sampling location.

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

Additionally, provision should be taken from the outset to ensure that the selected locations, under the aspect of forest management, forest protection and ownership are suitable for long term sampling sites. This implies arrangements with the Forestry Authority and owners before starting. The sampling sites should be protected by contract and as far as possible, be excluded from usual forestry measures and other disturbing changes.

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 (one-year-old shoots) 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:

- more than 40 years old, predominant, dominant or co-dominant (Fig. 1),
- free from intense biological (e.g. bark beetle infestation) or mechanical damages (e.g. top rupture).

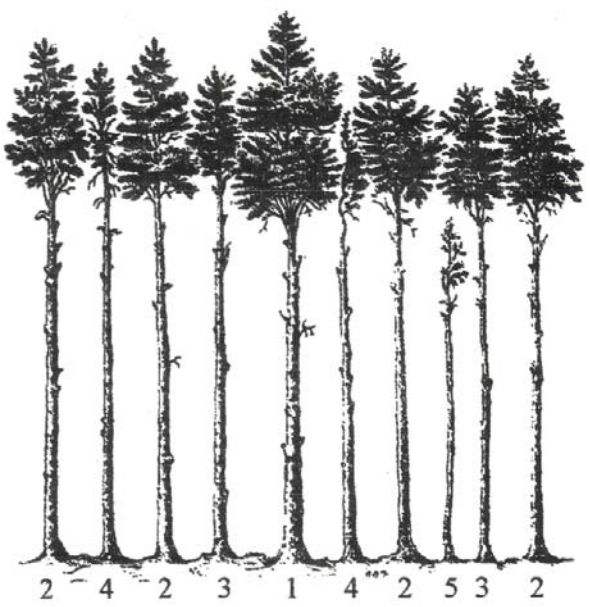

Fig. 1: Tree Categories by KRAFT (188 4) (1 = pr e-dominant, 2 = d ominant, 3 = co -dominant, 4 = domina ted, 5 = complete epigen ous) (from BMJ 2000))

5.3 Sampling Period and Frequency

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

The period chosen should be characterized by physiological stability of the trees. In accordance with the Environmental Specimen Bank a period at the end of the dormancy is chosen, to measure the emission situation of winter as well as that from the entire year.

Depending on the altitude and weather conditions sampling is carried out from March to May (between snowmelt and new budding).

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,
- useful addresses of tree climbers.

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 Implementation of the 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.

Collecting specimens from the crown area of standing trees is performed by and exclusively permitted to persons suitably qualified („cone pickers“) with respect to the safety regulations of the professional association. Their health condition also must be regularly checked. If special requirements exist, e.g. to protect the trees from which samples are taken against damage, appropriate tree climbing equipment is used. This equipment must also fulfill actual safety requirements.

6.1 Required Equipment and Cleaning Procedures

Field work:

- specimen data sheets for documentation during the sampling
- stainless steel scissors
- stainless steel trough to catch cut shoots

- 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°))
- ruler (reading accuracy 1 mm)
- scale paper (reading accuracy 1 mm)
- 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.

At least four branches are taken from the crown of each tree (approx. 7 -12 whorls with spruce, the outer crown with pines). When the branches are dropped and where they hit the ground care must be taken to avoid contamination. At least three branches of the spruce must have a complete number of the last seven needle years, at least the last five for pines.

From each branch the one-year-old shoots are cut using stainless steel scissors so that they fall directly into the stainless steel trough without being touched. Fig. 1 demonstrates the sampling procedure.

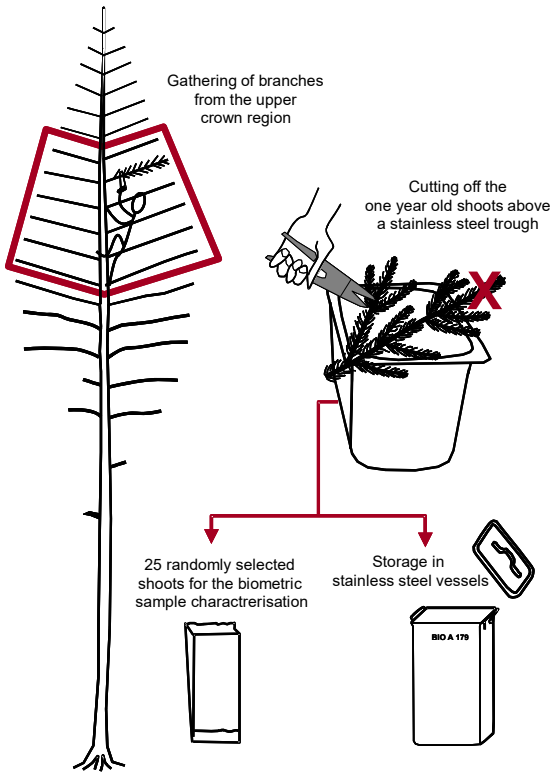

Fig. 2: Schematic represe ntation of the sampling (alter ed a ccording to WAGNER 1995)

For biometric sample characterization, 25 shoots are randomly selected out of the total sample

amount using stainless steel tweezers and collected in a paper bag labeled with the respective tree number.

The remaining shoots are transferred from the stainless steel trough to the storage containers with gloved hands after it 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).

7 Biometric Characterization

On site the parameters for the sample characterization are recorded pursuant of the respective specimen data sheets (description of the tree and description of the one-year-old shoots) prior to the removal of the shoots.

Using 25 randomly selected one-year-old shoots per tree, the following parameters are determined in the laboratory:

- shoot length including buds (1 mm reading accuracy),
- dry weight of the needles (0.01 g reading accuracy),
- dry weight of the stems with buds (0.01g reading accuracy),
- 1000-needle weight (0.001 g reading accuracy).

Paper bags containing the shoots are laid in a cabinet dryer (80°C (+/– 5°)) - directly on returning from sampling - for drying (not too densely packed to prevent overheating), and dried until weight stability is reached (approx. 2 days).

Needles and stems are separated from each other by shaking the paper bags. Remaining needles are removed by hand from the stem.

Shoot lengths (stem length including buds) are measured to a reading accuracy of 1 mm by using mm-paper. With curved stems, the length is estimated as precisely as possible e.g. by straitening the stems. The weight of the dried stems (this includes fallen off bud scales, bloom rests, etc.) as well as the weight of the dried needles is determined with a 0.01 g reading accuracy.

To determine the 1000-needle weight, exactly three times 100 whole, randomly selected needles are counted and weighed (N.B.: for pine needles, 100 intact double needles). From these individual weights, the 1000-needle weight is multiplied.

Coinciding, the weight relation of dried needles to dried stems is assessed.

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Checklist to Prepare and Conduct the Sampling

Specimen Type:	**Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)**
Target Compartments:	entire one-year-old shoots from at least four branches out of the upper, outer exposed crown region, of the spruce tree's 7.-12. whorl of the branches
Individual Specimens:	predominant, dominant or co-dominant trees (tree category 1, 2 or 3 by KRAFT) older than 40 years
Random Sample Number:	at least 15 trees
Sample Quantity for the ESB	150 g fresh weight (= one-year-old shoots) from 15 trees must be sampled to gain the needed quantity of 2.200 g
Sampling Period:	March until May (between snow thaw and prior to budding)
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 shoots, storage) • 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 select the leaves for the biometric characterization • disposable gloves • scales (effective range up to at least 3 kg, reading accuracy 1 g) • 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 (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 device (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°)) • ruler (reading accuracy 1 mm) • scale paper (reading accuracy 1mm) • 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 • needle coverage, yellowing (chlorosis) and necrosis, fructification one-year-old shoots (see specimen data sheets) • damage to needles and shoots (feeding on needles/shoots,

	chlorosis, necrosis), contamination for 25 shoots: • shoot length (reading accuracy 1 mm) • dry weight of the needles (reading accuracy 0.01 g) • dry weight of the stems including buds (reading accuracy 0.01 g) • 1000-needle weight (reading accuracy 0.001 g) • weight relation between needles and stems

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 1: Sampling Location

Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)

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

Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)

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

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 3: Sample Description (Tree)

Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)

Identification:

__ __ __ __ / X / __ __ __ __ / __ __ __ __ __ / __

Tree Number: __ __

Stand Type: ☐ Dense Stand ☐ Marginal Stand Zone

☐ Sparse Stand ☐ Forest Aisle

☐ Free Standing Solitary Trees

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

Needle Coverage: __ __ __ % (* automatic calculation in IS ESB)

(percentage estimate per needle age-group)

Needle Age-Group	1	2	3	4	5	Summation Pine Trees	6	7	Summation Spruce Trees
1st branch						__ __ __*			__ __ __*
2nd branch						__ __ __*			__ __ __*
3rd branch						__ __ __*			__ __ __*

All yellowish to whitish discolorations (Chlorosis): __ __ __ % (* automatic calculation in IS ESB)

(percentage estimate per needle age-group in 5%-steps)

Needle Age-Group	1	2	3	4	5	Summation Pine Trees	6	7	Summation Spruce Trees
1st branch						__ __ __*			__ __ __*
2nd branch						__ __ __*			__ __ __*
3rd branch						__ __ __*			__ __ __*

All brownish to reddish discolorations (Necrosis): __ __ __ % (* automatic calculation in IS ESB)

(percentage estimate per needle age-group in 5%-steps)

Needle Age-Group	1	2	3	4	5	Summation Pine Trees	6	7	Summation Spruce Trees
1st branch						__ __ __*			__ __ __*
2nd branch						__ __ __*			__ __ __*
3rd branch						__ __ __*			__ __ __*

Fructification: (related to cones and visible scars of current cone losses)

☐ None/Little ☐ Moderate ☐ Heavy

Water sprouts: ☐ None/Little ☐ Moderate ☐ Heavy

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 4: Sample Description (Shoots)

Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)

Identification:

__ __ __ __ / X / __ __ __ __ / __ __ __ __ __ / __

Tree Number: __ __

Damage to Needles

Biotic Damage: Percentage of the needles surface (Estimation at 5% intervals) __ __ __ %	**Damage Type** ☐ Nonexistent ☐ Feeding on Needles ☐ Sucking Spots of Insects ☐ Other:
Chlorosis: All yellowish to whitish discolorations (Estimation at 5% intervals) __ __ __ % * (* automatic calculation in IS ESB)	**Chlorosis Type** ☐ Nonexistent ☐ Blotchy, Skewbald ☐ At the Needle Basis ☐ At the Needle Top ☐ At the Entire Needles
Necrosis: (all brownish to reddish discolorations) (Estimation at 5% intervals) __ __ __ % * (* automatic calculation in IS ESB)	**Necrosis Type** ☐ Nonexistent ☐ Blotchy, Skewbald ☐ At the Needle Basis ☐ At the Needle Top ☐ At the Entire Needles
Damage to Shoots:	☐ Hollow Shoots ☐ Unopened Top Buds ☐ Galls, Pineapple Galls ☐ Other:
Description of Contaminations:	_____ _____ _____

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 4: Specimen Description and Storage

Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)

Identification:

__ __ __ __ / X / __ __ __ __ / __ __ __ __ __ / __

Tree Number: __ __

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

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

Specimen Description

Separation of needles and stems

Dry weight of the needles: __ __ , __ __ g

Dry weight stems with buds: __ __ , __ __ g

Weight relation of needles to stems: __ , __ __ * (* automatic calculation in IS ESB)

1000-needle weight:

Dry weight of 3 x 100 needles

__ , __ __ __ g	__ , __ __ __ g	__ , __ __ __ g

1000-needle weight: __ , __ __ __ g * (* automatic calculation in IS ESB)

Mean shoot length of 25 measured one-year-old shoots:

1 __ __ __ mm	11 __ __ __ mm	21 __ __ __ mm
2 __ __ __ mm	12 __ __ __ mm	22 __ __ __ mm
3 __ __ __ mm	13 __ __ __ mm	23 __ __ __ mm
4 __ __ __ mm	14 __ __ __ mm	24 __ __ __ mm
5 __ __ __ mm	15 __ __ __ mm	25 __ __ __ mm
6 __ __ __ mm	16 __ __ __ mm	
7 __ __ __ mm	17 __ __ __ mm	**Mean of 25 Measurements**
8 __ __ __ mm	18 __ __ __ mm	__ __ __ mm
9 __ __ __ mm	19 __ __ __ mm	(* automatic calculation in IS ESB)
10 __ __ __ mm	20 __ __ __ mm	

GERMAN ENVIRONMENTAL SPECIMEN BANK

Sampling Record

Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)

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