## Environmental Specimen Banking Program (EBS)

# Biomonitoring and Environmental Specimen Banking

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Abstract

The environmental specimen banking program (ESB) of the Federal Republic of Germany implies a collection of specimens from representative areas of Germany, stored under stable conditions for deferred analysis. It is an important part of the ecological assessment program. The ecological concept is presented as the frame of the entire ESB program. It is based on the selection of representative sampling areas and specimens. Standard operating procedures (SOP's) for sampling and characterization of specimens, as well as sampling designs specific to the areas, are presented as further important parts of the quality assurance system in relation to the correct sampling of specimens.

**Keywords:** Environmental specimen banking; biological monitoring; effect monitoring; accumulation indicators; sampling; standard operating procedures; quality assurance; sample characterization; area selection

## 1 Introduction

The environmental specimen banking program (ESB) of the Federal Republic of Germany is a permanent institution of the Federation under the general responsibility of the Federal Ministry of Environment, Nature Conservation and Nuclear Safety, Bonn, and the administrative coordination of the Federal Environmental Agency, Berlin.

Its formation began in 1985 after a five-year pilot project promoted by the Federal Ministry of Research and Technology during which it was tested for technical practicability and declared to be successful (BMFT 1988). The ESB is defined as a collection and long-term storage of representative biotic and abiotic environmental samples held under chemically stable conditions over a period of at least several decades for future retrospective analysis.

Tasks and objectives (Umweltbundesamt 1993):

- Determination of the concentration of substances which at the time of storage had not yet been recognized as hazardous chemicals or could not be analyzed with sufficient accuracy (retrospective monitoring);
- success control of current and future measures of prohibition and limitation in environmental issues;
- permanent control of the concentration of already determined hazardous substances through systematic characterization of the samples before their archiving;
- trends on the local, regional and global development of pollution;
- standardized recording of methods for sampling, analysis, characterization and storage as a precondition for the comparability of the results;
- revision of former monitoring results;
- use of samples as reference for the documentation of improvements of the analytic efficiency.

In order to achieve these objectives it was necessary to elaborate an ecological framework for the collection of representative environmental samples from representative areas of the Federal Republic of Germany and their storing in accordance with high quality standards. After their biometrical and ecological-biogeographical characterization the samples are therefore frozen in the gaseous phase over liquid nitrogen, directly at the site of sampling.

The deep freezing started there is not interrupted until the final storage. After the transport the frozen samples are cryo-homogenized and divided in portions of 10 grams each. About 250 subsamples are produced per sampling. Most of them are stored in the gaseous phase on liquid nitrogen at temperatures below -150 °C. In the process of chemical characterization in each case, 6 of these subsamples are analyzed for anorganic substances, chlorinated hydrocarbons and polycyclic aromatic hydrocarbons.

The course of the samples from the sampling area to their storage – now described only briefly – is during all phases a great demand on quality assurance, a process which is becoming more and more important in economics and science and is a precondition for the reliability and the comparability of results in environmental research and assessment. While quality management and quality assurance systems are the object of measures of accreditation and certification for chemical analysis and effect research in the laboratory (e.g. GLP, ISO 9000f.), this is not the case for field research which includes sampling (Klein & Paulus 1995, Wagner 1995). This fact, however, jeopardizes the success of all subsequent processes, i.e. sample treatment, storage and analysis, since failing representativeness, inaccuracies or errors committed in the preparation and the sampling itself cannot be corrected later.

For these reasons, the ecological contributions to the methodology and the conception of the ESB-Program of the Federation initially provided a method to develop and carry out constituents of a quality assurance system *(→ Table 1)* for the whole range of tasks connected with sampling in the field. The steps of the procedure, which are described in the following, can be applied in an adapted form as well to further areas of environmental research, monitoring and management (see KLEIN & PAULUS 1993; PAULUS & SPRENGART 1993; SPRENGART & KLEIN 1993; WAGNER 1993; PAULUS et al. 1996).

Table 1: Characteristics of the quality assurance system of the German ESB-Program

- ⇒ Detailled description and exact demarcation of representative sampling areas and sites
- ⇒ Selection of specimen sets based on purpose and ecosystem type
- ⇒ Observance of standard operating procedures
- ⇒ Sampling in accordance with the principles of area related, stratified random sampling
- ⇒ Extensive characterization of samples
- ⇒ Avoidance of all contamination during the sampling and storing
- ⇒ Immediate conservation of samples in the field at low temperatures under the guarantee of constant deep frozen conditions until the storage
- ⇒ Long-term storage of homogeneous subsamples under stable conditions
- ⇒ Recording of all steps of the procedure and of deviations from the instructions and aims

Representativeness and precision are the central concepts of quality assurance concerning all steps from the planning of the sampling to the analysis. **Representativeness** reflects the trueness of information from environmental samples for the representation of the actual pollution of an ecosystem.

It is defined by:

- **ecosystemical representativeness** in the sense that the selected indicators or parts of them, which can be used for the assessment of hazardous substances, function sufficiently as surrogates;
- **statistical representativeness** in the sense that, considering naturally and anthropogeneously caused variability, adjusted spot checks can be guaranteed in order to accurately represent the collected population, defined according to fixed standards;
- **chemical integrity** of the samples until the chemical analysis, which can be distorted through contamination or improper treatment of the samples (conservation of samples);
- **accuracy of the analysis** method in the sense that the results of the analytical method used come as close as possible to the true value of an environmental sample.

**Precision** reflects the degree of exactness with which the methods used for sampling, sample treatment and analysis are repeatedly able to represent equal environmental conditions in the same manner, and not dependent on how close the results come to the correct value (KLEIN & PAULUS 1995; see RAMSEY 1994, BOENKE 1995, GERTZ 1995), *(→ Fig. 1)*.

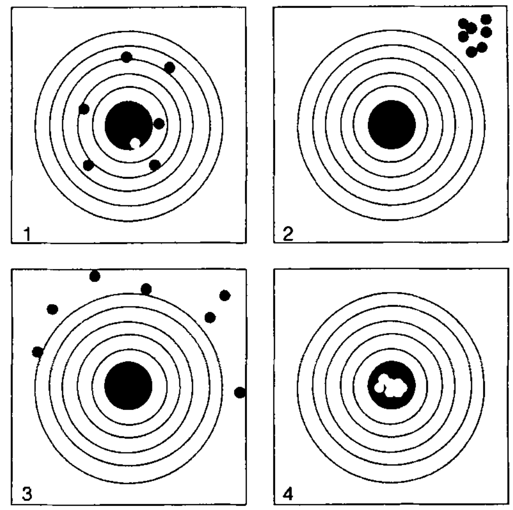


Fig.1: Precision and trueness in biomonitoring (after KLEIN & PAULUS 1995)
1: True but imprecise result, 2: Precise but wrong result,
3: Imprecise and wrong result, 4: True and precise result

## 2 Sampling Areas

The sampling areas of the ESB have been selected in such a way that the main ecosystem types of the Federal Republic of Germany are represented, taking into account the different intensity of anthropogenic influence and land use. Therefore, the areas as a whole can be regarded as being representative for the environmental situation of the Federal Republic of Germany.

Representativeness is defined as the synthesis of regional and national representativeness:

- **Regional representativeness:** On the basis of existing ecological knowledge, an area is regionally representative if it shares the greatest possible number of biotopes and biocenosis with the larger specific region in which it occurs
- **National representativeness:** A set or network of areas are nationally representative to the extent that they are a cross-section of the main types of ecosystems in the Federal Republic of Germany. To be representative in this sense, the set should contain the most information possible that relates to the condition and state of development of the environment of the Federal Republic of Germany.

The method of selection that has led to the sampling areas as shown in **Figure 2** is described in detail in LEWIS et al. (1989), PAULUS et al. (1992) and PAULUS & KLEIN (1994). Pragmatical aspects have been taken into account for the realization of such an extensive program as well.

Compared to a nationwide surveillance approach, where only a few widespread specimens are sampled from many sampling areas, the development of these study areas provides the advantage of being able to use many different accumulation indicators together in one and the same area. Thus, a broader spectrum of hazardous substances can be examined, and systemic level phenomena (such as food chain effects and substance transfers) can be considered. Furthermore it is possible to register information adequately for the interpretation of data from residue analysis (see e.g. GREEN 1979; MÜLLER 1980; LEWIS 1985; BIGNERT et al. 1993).

In this context, subareas have been selected according to the watershed concept (see e.g. NATIONAL BOARD OF WATERS AND ENVIRONMENT 1989; BRÅKENHIELM 1990) in which the sampling of the individual specimen sets could be performed randomly.

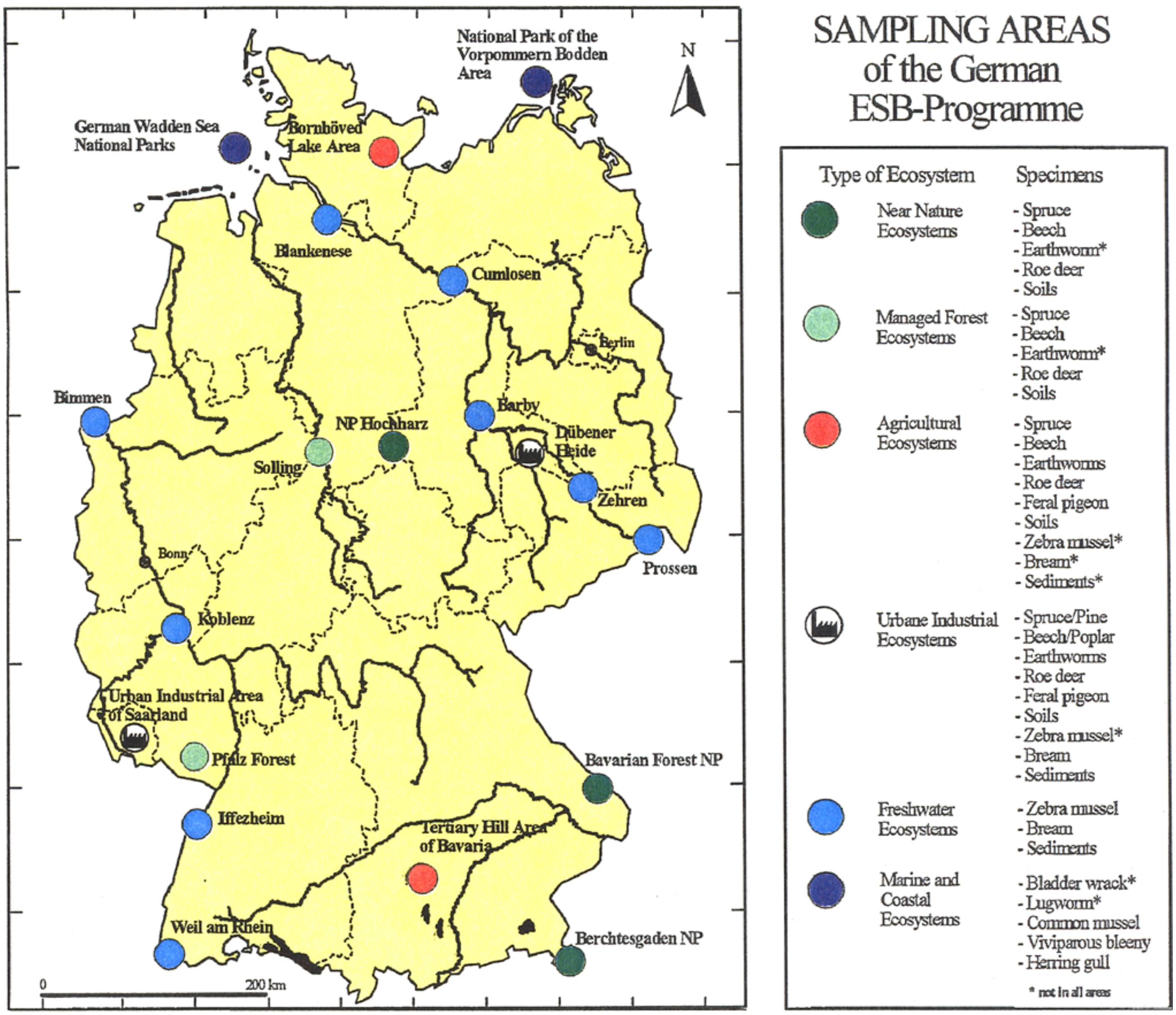


**Fig. 2:** Sampling areas and specimen sets of the German ESB-Program (in adaptation to PAULUS & KLEIN 1994)

## 3 Specimen Sets

Representative biological specimen sets are of significant importance for the ESB because of their integrative accumulation of potentially hazardous substances in time and space. At the same time they represent integral models for assessment of the bioavailability and hazardous potential of xenobiotics.

The chance to use the best possible indicators for the documentation of still unknown hazardous substances, as well as the possibility of making comparisons between different areas, increase with the number, the diversity and especially the representativeness of the selected specimen sets. The different species complement one another with the content of information because they don't inevitably demonstrate an equal accumulation of all hazardous substances. For that reason ecologically and biogeographically representative sets have been selected for each sampling area.

**Ecosystemic representativeness** (see above) means that the different trophic levels and the important functions of an ecosystem are represented through appropriate specimen types. For pragmatical reasons, e.g. nature conservation, the top consumers of a food chain which frequently have an especially high accumulation potential can only be considered insufficiently (see KLEIN 1993).

**Biogeographical representativeness** is defined as the representation of the typical present-day flora and fauna elements of the area of the FRG through few species. Although these two criteria are essential to the concept, they will have to be complemented and be relativized by further demands. Detailed lists of criteria are available in e.g. LUEPKE (1979), LEWIS et al. (1984), MÜLLER & WAGNER (1986, 1988), PAULUS & KLEIN (1994) and KLEIN & PAULUS (1995).

For ecological, biogeographical and practical reasons it was not always possible to place exactly identical sets into all comparable ecosystem types. Therefore, additional species with complementary information content had to be integrated, i.e. species which are comparable with regard to their function in the ecosystem (see below).

Under restricted conditions concerning the available capacity, the sets shown in **Figure 2** were selected for the ESB-Program of the Federation; as a rule, samplings of each set are performed once a year.

## 4 Standard Operating Procedures (SOPs)

Within the context of the ESB-Program of the Federation, standard operating procedures were developed for each specimen type to guarantee a high degree of standardization concerning the selection and delimitation of sampling areas, the number and selection of individuals for sampling, the time, rhythms and frequency of sampling, the technical equipment, the packing of samples and the cleaning of equipment, the methods of sampling and catching, the treatment and the characterization of samples and the transport of samples.

Important natural variations can lead to a misrepresentation of the content of chemicals and therefore had to be taken into special consideration, for example with regard to standardization.

**Temporal variation:**

Can be caused by daily, seasonal or other rhythms, as well as by irregular or temporary events.

In this context, KLEIN et al. (1995) – working on the question of a dependency of the information potential of Zebra mussels *(Dreissena polymorpha)*, used as accumulation indicator, on their phenology in Lake Constance – could for instance illustrate the modification of PCB contents through the influence of different seasonally specific forms of variation. On the one hand, they detected a positive correlation between the concentration of the lipophilic PCBs in the soft body and fat content of the animals that were subject to seasonal variations *(→ Fig. 3)*. On the other hand, the circulation type of Lake Constance, classed as a warm-monomictic lake, is mirrored during the winter period in an increased PCB concentration available for the mussels, i.e. in a higher PCB content of the mussels.

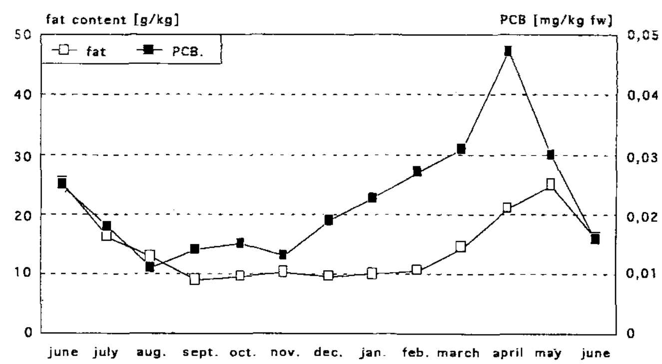


Fig. 3: Seasonal variation of total PCB and the mean fat content in the soft body tissue of the zebra mussel *(Dreissena polymorpha)* in Lake Constance (see KLEIN et al. 1995)

**Spatial variation:**

Occurring in all scales of resolution and is determined by the geographical structure of abiotic environment compartments, by the distribution of xenobiotics and the distribution, specific to populations, of the different species. As a rule, this dispersion is stationary for plants, for animals it depends on mobility (geographic mobility, migration). ELLIOT (1977) emphasizes that the geographic distribution of individuals of a population can be random, homogeneous or patchy. As a rule, he regards the patchy distribution which mathematically leads to a very big variation compared to the arithmetical mean.

**Genetic variation:**

The genetic variation can already be well-developed within a population. The accumulation of chemical substances, as well, can vary from individual to individual. HALBWACHS & ARNDT (1991) point to the fact that every organism has a genetically determined, phylogenetically acquired, specific susceptibility towards affecting factors. Within certain limits, stress factors can be tolerated. This physiological susceptibility can vary extensively during the evolution of an organism and is not identical for all individuals of a population.

**Sex and age-dependent variation:**

Chemicals and their effects can vary as a function of age in all organisms. In the human organism and in that of heteroecious animals, variation can additionally occur as a function of the different sexes.

**Innerorganism variation:**

Is of importance when parts of the body (compartments) – instead of the whole body – are analyzed for residues as accumulation indicators. These variations can be caused either by an different exposure to hazardous substances – such as is visible in spruce shoots in accordance with their position in the crown – or by the temporally induced accumulation during the exposition of parts of an organism, such as in conifer shoots of different age *(→ Fig. 4)*. Or they can be caused by biochemically determined differences in the transport and deposition of hazardous substances in the organism.

| Class | Two-year old shoot µg F/g dw | One-year old shoot µg F/g dw |
|---|---|---|
| 1 | < 8 | < 6 |
| 2 | 8 - 12,9 | 6 - 9,9 |
| 3 | 13 - 19,9 | 10 - 15,9 |
| 4 | 20 - 28,9 | 16 - 23,9 |
| 5 | ≥ 29 | ≥ 24 |

Fig. 4: Different fluorine contents in one- and two-year old spruce shoots with reference to immission effects.
Class 1: none; Class 2: possible; Class 3: starting; Class 4: remarkable; Class 5: very high (from KNABE 1984)

## 5 The Principle of Stratified Random Sampling

### 5.1 Stratified sampling

Stratification describes the division of a very heterogeneous population into a determined number of subunities or strata which are characterized by a comparably small variability of naturally and anthropogeneously provoked parameters (see GREEN 1979; LEWIS 1985; KLEIN et al. 1994; PAULUS et al. 1994). In passive biomonitoring, sampling will be confined to that stratum which, in a defined sampling period, is characterized by a maximum information potential representing the pollution of the area under examination and simultaneously provides a good reproducibility of sampling. However, even after a stratification, there is no „typical fish" or „typical beech" in a stratum. Thus, a certain number of spot checks in a stratum will always be necessary in order to adequately represent the remaining variability and to obtain a representative and reproducible sample. Through a stratification, it is also possible to pool the single samples within each defined stratum. BIGNERT et al. (1993) state that a pooled sample always represents a weighted mean value of the original samples. Only through a stratification, i.e. the reduction of the variability of samples, can an acceptable parameter be the result. Pooled samples, in particular, are the aim of biomonitoring and of the ESB of the Federation in order to get a mean value of pollution in the area under examination, using the smallest possible number of samples and a minimum of analysis.

### 5.2 Production of spatially representative samples

In order to obtain a sample of the environment which allows a correct assessment of the pollution of an examined area, the precondition of the sampling – as already stated above – is a certain number of sampling sites and spot checks, in accordance with the specific heterogeneity of the area. The number of spot checks, however, must be adapted to the variations remaining after standardization. Methods for calculating the specific amount of spot checks can be found, for example, in MACE (1964), PIELOU (1984), KEITH (1988), FISCHER (1991) and KÖHL (1991). In contrast to specific variations of the specimen types used themselves (such as temporal, genetic, age dependent, innerorganism) – which can be reduced through general standardization, providing better applications of the results of different monitoring studies – geographical variations of an area in examination will have to deal with special strategies in order to guarantee a spatially representative sampling. If extensive geographical heterogeneity is provided through large-scale environmental patterns, GREEN (1979) recommends breaking up the examination area into relatively homogeneous subareas and allocating samples in proportion to the size of the subareas. On the basis of a stratified spot check it would be possible to obtain a spatially representative mean value for the whole area through a pooled sample.

### 5.3 Random sampling

A fundamental principle to increase the information potential of environmental samples is to leave the selection of the individuals for sampling from the defined collection of samples to chance (random sampling). Only a random sampling guarantees a representative statement about the area in examination and, at the same time, an unrestricted applicability of statistical evaluation methods which are indispensable for a logically correct exhaustion of the information potential of environmental samples. The principle of random sampling – where each single individual of the population selected for sampling has exactly the same chance of being chosen – minimizes the loss of information always provoked by subjective selection. Examples for errors in this context are seen when avoiding subareas with complicated sampling conditions or through the selection of „typical" individuals (GREEN 1979; HARTUNG & ELPELT 1989; FISCHER 1991; PAULUS et al. 1994).

Methods for developing stratified random sampling strategies are described in PAULUS et al. (1994). The authors, using deep burrowing earthworms of the species *Aporrectodea longa* as accumulation indicators, were able to show that it is possible, even in a heterogeneous sampling area – with a spot check amount of 400 g per sampling collected from 10 sampling sites – to obtain spatially representative samples on the basis of random sampling.

## 6 Sample Characterization

An essential part of the general program is the extensive characterization of the samples stored in Environmental Specimen Banking. Especially the knowledge about ecological parameters is of great importance for a sufficient comprehension of the data from residue analysis (see MÜLLER 1980; BIGNERT et al. 1993). GIEGE et al. (1993) stress the following statement: „Ecotoxicological research is not all chemical analysis. An analysis result must always be put in its biological perspective ...".

Sample characterization in the ESB-Program of the Federation is differentiated into three areas:

- Documentation of sample treatment
- Biometrical characterization
- Ecological-biogeographical characterization, completed by an analytical sample characterization.

**The documentation of sample treatment** covers each step from the sampling to the final storage of the samples. EDP storing ensures a complete control of the entire process. Thus, the demand for transparency, which is essential to the quality assurance of samples, is complied with.

**The biometrical sample characterization** is done through investigations during the sampling with the aid of standardized data sheets, suitable as well for EDP purposes. Biometrical data are the basis for the decision which samples can be compared with regard to which substance content and about the degree of biological variability of the samples. Thus, PAULUS et al. (1995) were able to demonstrate that the element contents in spruce needles of the same branch and from the same year can already be changed through a different infestation of *Sacchipantes viridis* (→ *Fig. 5*). Therefore, it is important to record the degree of infestation of the shoots when sampling in order to be able to correctly interpret the possible differences between spruce needles of different spatial and different temporal origin. Otherwise the risk might be to attribute different concentrations of contaminants, caused by insect infestations, to different environmental pollution rates.

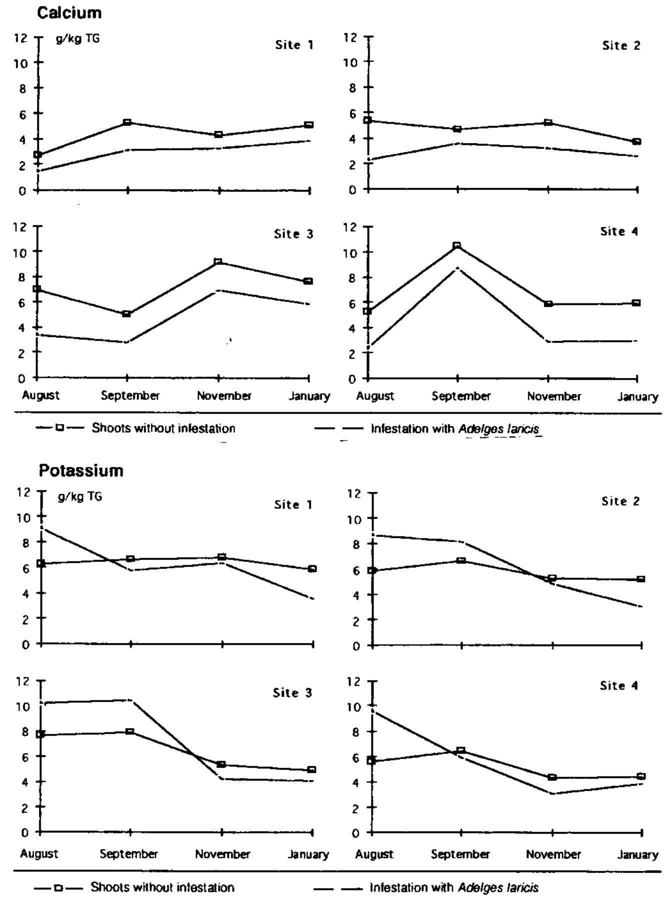


Fig. 5: Influence of *Sacchipantes viridis-infestation* on element contents in spruce shoots

A further problem – stated by PAULUS et al. (1995) – refers to the influence of changing needle size of spruces from year to year on the concentration of airborn hazardous substances. The authors explain that the different needle sizes of the trees being sampled – caused by different climatic conditions in the individual sampling years – result in different concentrations of airborne contaminants, although the contents of the analyzed substances in the atmosphere probably did not change.

The main reason is the fact that smaller needles have a larger relative surface area and therefore a bigger adsorption surface compared with the total mass of the needles as a reducing factor. When relating the content of substances to the mass (dry weight) of the material, a much higher absorption surface is considered if the needles are small or thin. This proves that, without regarding the needle size, – as is normally performed when only the needle weight is used as a basis – an incorrect interpretation of pollution trends may be the consequence (→ *Fig. 6*).

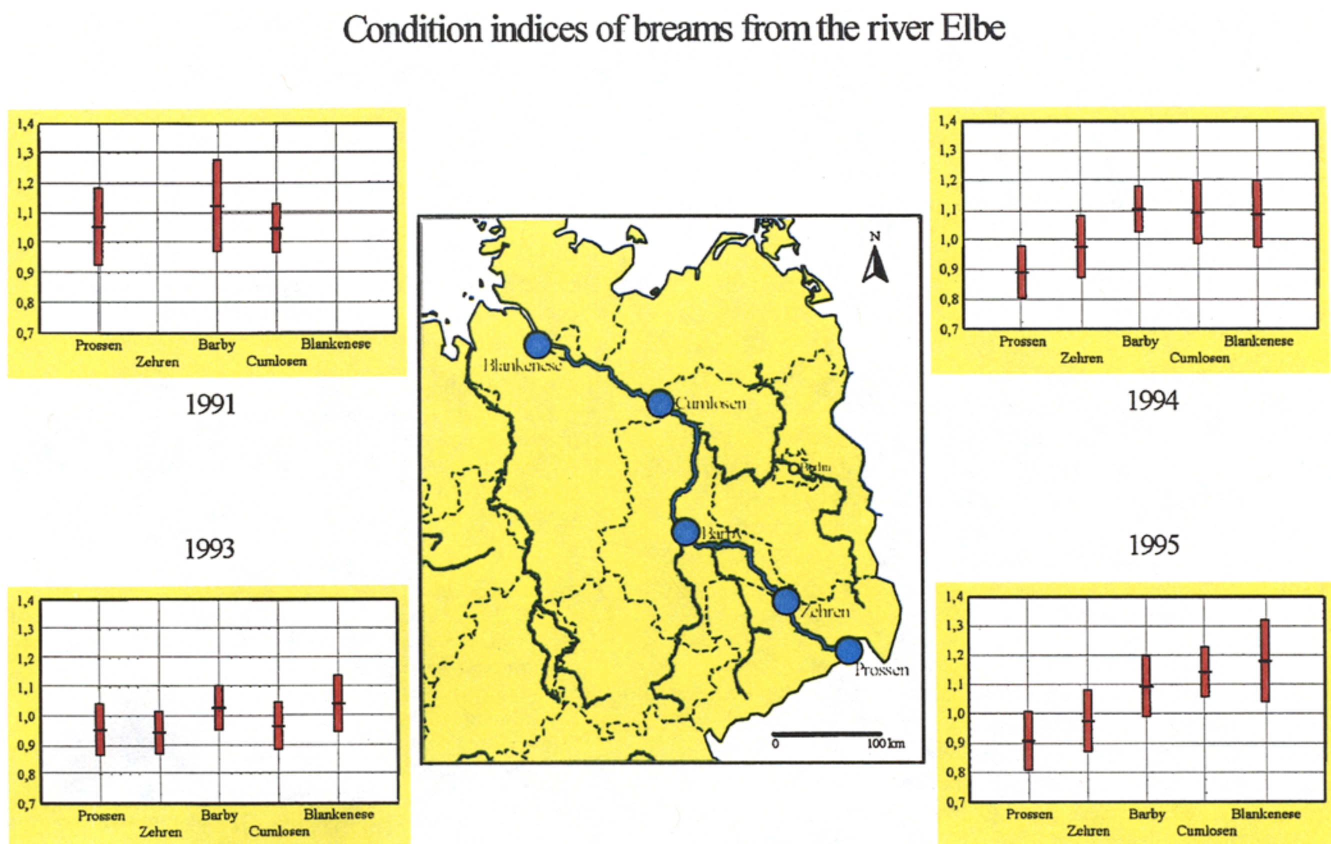


**Fig. 7:** Condition indices of breams (Abramis brama) sampled in 1995 in the Elbe river (from WAGNER et al. 1996).
The indices resulting from body size and weight point to a continual improvement in the living conditions of breams from the Czech border in the direction of the estuary. Grömping et al. (1996) presume a connection with the total content of chlorinated hydrocarbons in liver and muscular tissue of the fish which correlates negatively with the condition indices

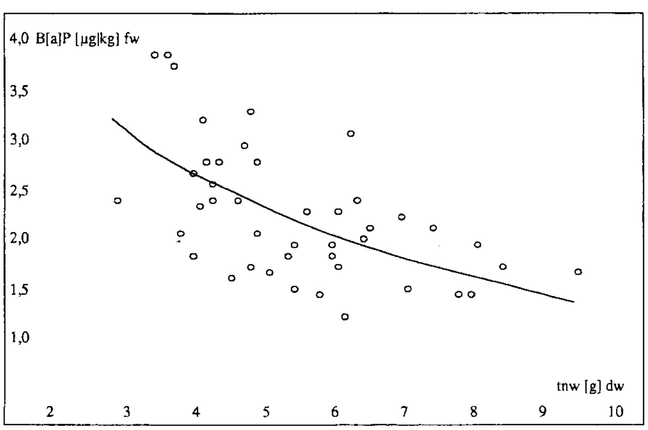


**Fig. 6:** Relation between the B[a]P-concentrations and the thousand needle weight (tnw) of spruces from the Bornhoeved Lake Area

Biometrical investigations furthermore provide irretrievable data about the effects of hazardous substances on environmental samples, such as the documentation of the proportion of necrotic needle cells in the total needle surface of a spruce, the resistance to breakage of pigeon eggs, or the determination of the condition index for fish *(→ Fig. 7)*. The specimen types used as accumulation indicators for ESB can thus also be used as indicators for specific effects of contaminants.

These biometrical data have to be completed by parameters which allow us to document the physiological condition and the genetic structure of environmental samples at the time of sampling.

**Ecological-biogeographical characterization** means that, in addition to the mentioned direct data about environmental samples, "indirect" data are recorded in order to characterize the origin of the samples as well. On the one hand, it covers the geographical, ecological and biogeographical characterization of the sampling area from which the samples are taken; on the other hand, it covers studying, filtering and evaluation of so-called external, area-related data, i.e. data recorded by other research programs such as ecosystem research. An important aid in this context are Geographical Information Systems (GIS) which relate the external and internal data to yield an extensive ecological-biogeographical characterization of the investigated area. A simple example may demonstrate the significance of this characterization. TREMP (1992) showed that the aluminium content in the shoots of *Scapania undulata,* caused by precipitation of aluminium hydroxides on the surface of the moss leaves, increases with an increasing pH-value of the water as well. This is a condition which is quite different from the accumulation in moss itself. Without knowing about the influence of the pH-value, therefore, no correct statements can be made about the ecological effects of aluminum contamination.

## 7 Conclusions

The ESB-Program of the Federation at the moment is one of the most sophisticated biomonitoring programs worldwide which includes the option for retrospective environmental analysis. It distinguishes itself by its high quality standards from sampling to storage and analysis, and because of its temporal continuity and ability of giving reliable long-term trends on pollution. Because of its conception, it is qualified as a basic constituent for ecological environmental assessment as well as a reference system for other biomonitoring programs.

Lacking standards for assessment, gaps in the determination of sample quality, changed pollution types and new scientific findings are challenges which the ESB still has to face in order to be able to meet its functions as well in the future. The elaboration of concepts for the objective control of the accuracy of the sampling in the field are indispensable preconditions for an extensive quality assurance system. Furthermore, we will have to develop strategies which, through extensive analysis of the information potential of environmental samples, enable us to point to possibilities and limits of data from residue analysis for the assessment of the environmental conditions and to ensure the functionality of the ESB as an extensive reference system. Finally, the aspect of the effects of chemicals as well as that of other stressful factors such as viruses, bacteria and parasites, and to provide samples for retrospective analyses of such factors must be considered more extensively. Then it will be possible to make important contributions to the recording of the vitality of ecosystems and thus to an extensive environmental assessment.

The few examples shall be sufficient to illustrate that, with the ESB-Program of the Federal Republic of Germany, a system for environmental assessment has been built up which not only can react flexibly to new demands but, especially because of its retrospective character, its high demands on quality and its bioindicative approach, plays an important part – beyond mere residue analysis as well – within the scope of ecological environmental monitoring and assessment.

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