

IV.6

Specimen banking as a source of retrospective baseline data and a tool for assessment and management of long-term environmental trends

Antonius A.F. Kettrup and Petra Marth

IV.6.1. Introduction

According to the “European Inventory of Existing Commercial Substances” EINECS (after GDCh/BUA, 1987), more than 100,000 different chemical substances are produced worldwide. 1000–2000 new chemicals are entering the market every year in addition to those already in circulation. For most of them we lack sufficient information about their effects on man, animals and plants and about their further reaction fate transport mechanism, and effect on the environment (SRU, 1987). New technologies *always* produce unintended and unpredicted wastes and impacts. According to the single integrated EC waste list (Commission Decisions 2000/532/EC, 2001/118/EC), the majority of waste from organic chemical processes (code 07) and significant part of waste from inorganic chemical processes (code 06) are considered as a hazardous waste. A large number of the European Community legislation in force and in preparation is focused on various chemical waste and waste chemicals. It can be found in the EUR-Lex register 15.10.30.30 “Waste management and clean technology”, Web site: http://www.europa.eu.int/eur-lex/en/lif/reg/en_register_15103030.html.

Usually the introduction of chemicals into the environment represents an irreversible step. A considerable number of chemicals reaching the environment do not degrade at all or only very slowly. They accumulate in the environment and, having been distributed, become ubiquitous of certain pollutants, e.g. PCBs, herbicides and chlorinated insecticides. Persistent biologically active chemicals, even at concentrations below our ability to analyze or detect, can pose serious pervasive and possible irreversible threats to human health and the integrity of the biosphere (Lewis, 1988).

Numerous industrial countries have passed laws to assess the hazards of chemicals for man and the environment. EC legislation on chemicals is collected in the register EUR-Lex 15.10.20.50 “Chemicals, industrial risk and biotechnology” in the Web site http://www.europa.eu.int/eur-lex/en/lif/reg/en_register_15102050.html. Among others, it comprises the Council Directive 76/769/EEC (1976) relating to restrictions on the marketing and use of certain dangerous substances and preparations, with numerous current amendments, first one of 2002 (*OJ L* 183 12.07.2002), as well as four lists of priority substances established by the Decision No 2455/2001/EC of the European

Parliament and of the Council in the field of water policy, and Commission Regulations (EC) No 1179/94 (1994), No 2268/95 (1995) and No 143/97 (1997) as foreseen under Council Regulation (EEC) No 793/93 (1993). The principles for assessment of risks to man and environment of different chemicals were laid down in Commission Directive 93/67/EEC (1993), Regulations (EC) No 1488/94 (1994), as well as in Commission recommendations, e.g. 1999/721/EC (1999) and C(2001)439.

Investigations on forecasting the impacts of chemicals are afflicted by special costs and problems (Fig. IV.6.1) (Wagner, 1994a,b).

Living organisms and their associations are enormously heterogeneous, complex and open systems are characterized by poorly defined boundaries. Under the best circumstances, we can evaluate most quantitative features of biological systems only for certain properties and only by using certain samples or subsets of the class. Results are always statistical in nature and extrapolation from the sample to the class is necessary. Many environmental factors that cannot be reproduced or represented in the laboratory or by models can modify the environmental behavior and effects of chemicals. The error in

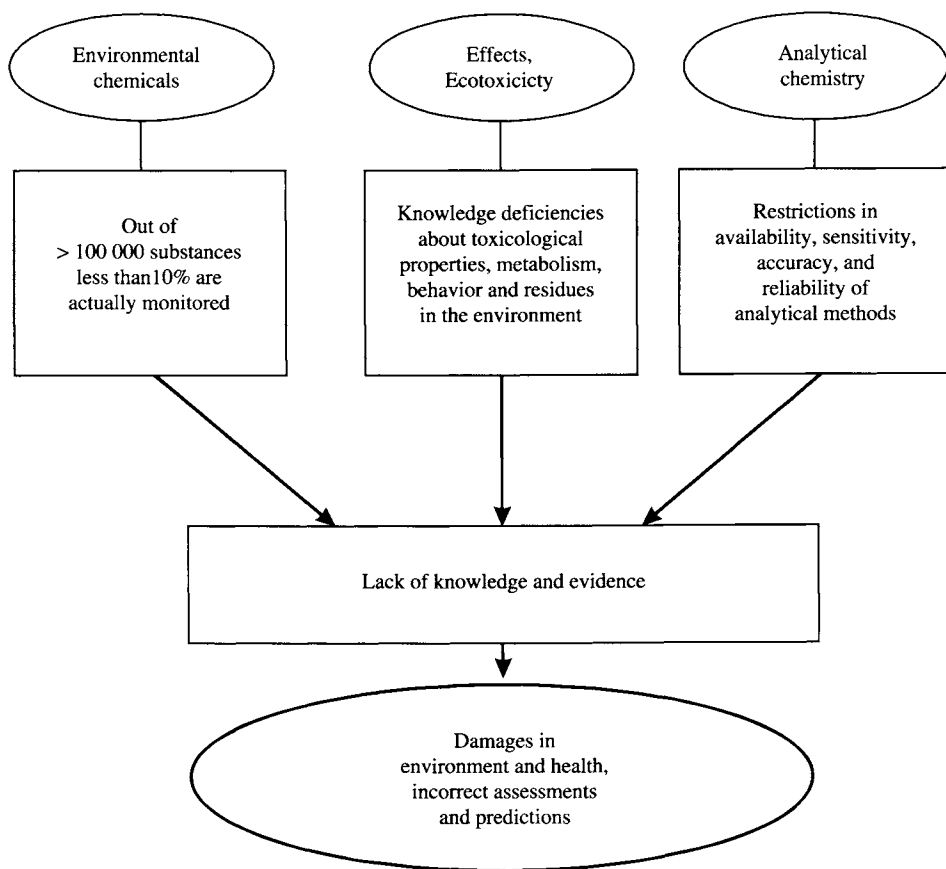


Figure IV.6.1. Deficits in assessment and control of environmental chemicals (after Wagner, 1994a,b).

forecasts based upon trend extrapolations, without knowing the relationships of environmental variables may become enormously enlarged with time. Recognizing the actual form of a trend among reasonable alternatives is difficult and often subjective. Thus, the level of uncertainty of most forecasts and assessment of chemical impacts upon man and the environment is often quite high. Even today, we repeatedly confront serious and unexpected consequences of our technologies, products and wastes. Politicians and administration can only develop effective damage protection and risk contaminant strategies, if they have reliable scientific information available.

IV.6.2. Bioindicators

The idea that organisms can provide an indication of the quality of their environment is widespread and at least as old as agriculture (Thalium, 1588). It is not possible to establish any clear definition for the term “bioindicator” considering the large amount of published literature. The following definitions are suggested congruent to many European authors (Wittig, 1993):

- *Bioindication* is the use of an organism (a part of an organism or a society of organisms) to obtain information on the quality (of a part) of its environment. Organisms that are able to give information on the quality (of a part) of its environment are bioindicators.
- *Biomonitoring* is the continuous observation of an area with the help of bioindicators, which in the case may also be called biomonitors. With the aid of organisms trends in time and space concerning the distribution and ecological effects of environmental chemicals can be observed by a semi-quantitative evaluation of the results.

Biological samples, from the environment, are mainly used and analyzed as representatives for larger entities or similar or related environmental compartments. This requires the selection of standardized (bio)indicator systems, which react with known specifics and sensitivity to environmental chemicals and have the capability of spatial and/or temporal integration. Such indicator systems can be efficiently and reproducibly analyzed and evaluated vicariously for the total entity of sensitive targets in the environment to be observed, which are often extremely variable with respect to the space, time and physiology. Bioindicator systems can be applied in such cases, where potential integral effects of complex or unknown immission types have to be detected and quantified. Such effects may occur on different levels from specific organs of single organisms up to whole ecosystems. Bioindicators are also preferred, where they offer advantages due to their high sensitivity towards a broad spectrum of substances or because of their ability to accumulate a substance over an extended period or to integrate its influence in an area of known and relevant size. This is the case, if the sensitiveness of available analytical methods for dangerous substances is too low to find them in other environmental media (air, water and soils).

In addition to the concentrations of toxic substances and their metabolites biological specimens can also be analyzed for essential components and a broad spectrum of possible biochemical, physiological, morphological and/or genetic effects. Organisms and biological communities normally do not react to single components or substances in

their environment. They show the effects of the totality of all the acting substances and environmental factors. Decisive for the use of biological specimens is their ecotoxicological relevance that means the relevance or indicative function of the found effects for other living organisms and communities including men.

IV.6.3. Idea of environmental specimen banking

With respect to effects of pollutants, the acquisition of reliable information regarding their quantities and distribution under natural conditions requires a systematic program of environmental monitoring in which concentrations of hazardous chemical substances are measured in suitable environmental specimens of various trophic stages and food chains. But actual monitoring of the environment can only be as good as our present knowledge, analytical capabilities, and quality assurance and quality control allow. From among the multitude of substances found in the environment only those can be monitored, which have already been recognized to be hazardous. The present assessment of the measured environmental concentrations of hazardous substances – and thus, the quality of regulatory decisions – suffers from the fact that no results are available on pollutant burdens of former times or that the data which are available are ambiguous (Kayser et al., 1982).

Before this background at the beginning of the 1970s the idea of using biological samples as reference material to furnish proof of environmental pollution was put forward by Frederick Coulsten of the Albany Medical College, Albany, New York and Friedhelm Korte of Institute of Ecological Chemistry, GSF-Forschungszentrum, Munich-Neuherberg.

In an environmental specimen bank (ESB) carefully selected, relevant environmental samples are stored systematically at temperatures below -150°C immediately after collection. In these conditions either no, or as small as possible, chemical changes occur over a long period of time. Baseline levels of contaminants in the environment can be established by taking samples with use of known approved methods and preserving them at the present time for future demand in ecological–chemical research. Long-term storage of samples with indicator functions represents a necessary complement to the actual monitoring of the environment and a safety net in the assessment of chemical risk.

A systematically established archive of frequently collected representative environmental specimen samples fulfill the following important functions (Kayser et al., 1982; Wise and Zeisler, 1984, 1985; Lewis, 1988; Keune, 1993):

- They may be used for the determination of the environmental concentrations of those substances, which, at the time of storage, were not recognized to be hazardous or which at present cannot be analyzed with adequate accuracy (retrospective monitoring).
- They may serve as reference samples for the documentation of the improvement of analytical efficiency and for the verification of previously obtained monitoring results.
- Early detection of environmental increases in hazardous chemicals thought to be under control is possible. Also, the effectiveness of restrictions, regulations or management practices that have been applied to the community, the environment, or to the manufacture, distribution, disposal or use of toxic chemicals can be assessed.
- Depending upon the analysis and evaluation of stored materials ESB can save considerable time and money when unexpected impacts are observed.

- Sources of chemicals may be identified. Often, by the time a chemical is recognized as a health or environmental problem it is sufficiently widespread to defy identification of the principal sources or pathways.
- ESB can offset the lack of reliable data on pollutant burdens of earlier times because inconsistencies or ambiguities among available data usually limit assessments and regulatory decisions.

In Germany the Federal Minister for Research and Technology supported a comprehensive pre- and pilot phase of ESB between 1976 and 1984. During this period the technical feasibility regarding the sampling of different species, handling and shipping of samples, deep-freezing, homogenization, ultra trace analysis, packing materials, logistics, storage temperature and documentation was confirmed (Boehringer, 1988). The results were so encouraging that in 1985 the German government decided to set up a permanent ESB under the responsibility of the Federal Ministry for the Environment, Nature Conservation and Reactor Safety (BMU), coordinated by the Federal Environmental Agency (Umweltbundesamt). Two specimen banks are subsumed under the general heading of the German ESB:

- The Specimen Bank for Environmental Specimens at the Institute of Applied Physical Chemistry of the Research Center Jülich (KFA).
- The Specimen Bank for Human Organ Specimens at the Institute of Pharmacology and Toxicology of the University of Münster.

The work is distributed among five institutions depending on their special scientific capabilities. In Table IV.6.1 the participating institutions of the German ESB and their responsibilities are summarized.

Table IV.6.1. Participating institutions of the German ESB.

Institution	Task
KFA Research Center Jülich, Institute of Applied Physical Chemistry	Specimen Bank for Environmental Specimens, central banking facilities, logistics, element analysis, sampling of marine specimens
University of Münster, Institute of Pharmacology and Toxicology	Specimen Bank for Human Tissues, sampling, characterization, storage and analysis of human samples, data banking system
GSF Research Center Neuherberg Institute of Ecological Chemistry	Analysis of CHC
Biochemical Institute for Environmental Carcinogens, Grosshansdorf	Analysis of PAHs
University of the Saarland, Saarbrücken Institute of Biogeography	Selection and characterization of areas and specimen types, sampling of terrestrial and limnic specimens, ecological questions
Fraunhofer-Institute of Environmental Chemistry and Ecotoxicology	ERGO Ltd., Hamburg

In the meantime the national ESBs in the several countries and international cooperation of ESBs in the Federal Republic of Germany, USA, Canada, Japan, Finland, Sweden, Norway and Denmark have been established (Wise and Zeisler, 1985; Wise et al., 1988; Zeisler et al., 1992; Stoepler and Zeisler, 1993; Kubin et al., 1997; Pugh, 2001).

IV.6.4. Realization

IV.6.4.1. Sampling

IV.6.4.1.1. Selection of sampling areas

Sampling areas have been chosen as to form a national Network of Ecological Assessment Parks coordinating environmental specimen banking with long-term ecological research and environmental monitoring (Lewis, 1985, 1987; Paulus et al., 1990; Lewis et al., 1993). An overall concept has been developed by a committee of experts under the auspices of the BMU, taking into consideration different types of ecosystems with corresponding representative sampling areas according to the following criteria:

- stability of utilization,
- assured long-term use,
- sufficient minimal size,
- availability of suitable samples,
- practicability, e.g. accessibility, public ownership (National Park), no conflict with the protection of biotopes and species, high level of information, nearby suitable institutions for research.

The list of, at present, 14 areas (Fig. IV.6.2) comprises the major ecosystems and habitat types that occur within the Federal Republic of Germany including

- limnic and marine ecosystems,
- urban industrial ecosystems,
- forest and agricultural ecosystems and
- semi-natural ecosystems.

Since 1985, continuous sampling has been carried out every 2 years but it should be converted into a 1 year sampling frequency to utilize the analytical, biometric and meteorological data much more effectively in the sense of real-time monitoring.

IV.6.4.1.2. Selection of specimen types

The selection and assignment of representative specimen species of the terrestrial, limnic and marine ecosystems for the ESB was undertaken by a committee of experts in consideration of the above-mentioned indicator functions so that a broad spectrum of different types of matrices (all trophic levels) and media (air, sediment and soil) with environmentally relevant concentrations of xenobiotics is available (see Figure IV.6.2)

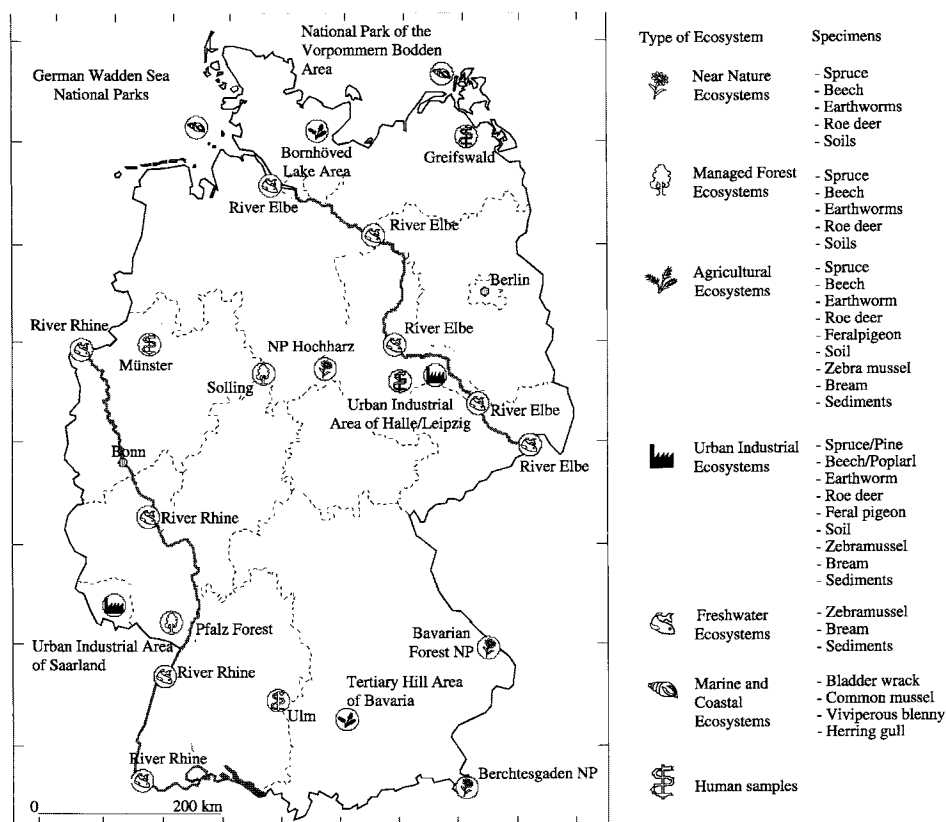


Figure IV.6.2. Sampling areas and specimens of the German ESB-program.

(Lewis, 1985; Lewis et al., 1993). The following requirements must be fulfilled for using a matrix as bioindicator:

- The chemicals must be accumulated comparable to levels occurring in the environment.
- Contamination trends in the environment must correspond to those in the matrix.
- The matrix should have a widespread distribution and must be available in time and place to a sufficient extent.
- The organism should be sedentary and easy to identify.
- The species should accumulate the pollutant without being killed or rendered incapable of reproduction.

IV.6.4.1.3. Standard operation procedures

Standardized sampling guidelines or standard operating procedures (SOP) are the basis for the comparability, reliability and repeatability of the banked samples. They contain detailed instructions for the

- selection of sampling sites and specimens,
- sampling,
- providing cover for repeatability of sampling,
- area and sample characterization,
- sample treatment and long-term storage,
- documentation of sampling and storage conditions,
- chemical analysis,
- data processing and evaluation and
- quality assurance (Wagner, 1994a,b).

Sampling of biological and other environmental specimens is always influenced by factors which may modify the exposure as well as the accumulation behavior of the specimen types in relation to xenobiotics, e.g. by climatic factors, weather conditions and changes in the population sampled or in the structure of the whole ecosystem (Wagner, 1994a, 1995; Klein and Paulus, 1995).

Ecological and biometrical sample characterization provides basic information about changes in the quality of the sampled material and its comparability with previous and following samples from the same area or the same specimen type sampled in other areas. Biological sample characterization can also give information about ecological and ecotoxicological effects to the population sampled. Figure IV.6.3 demonstrates changes

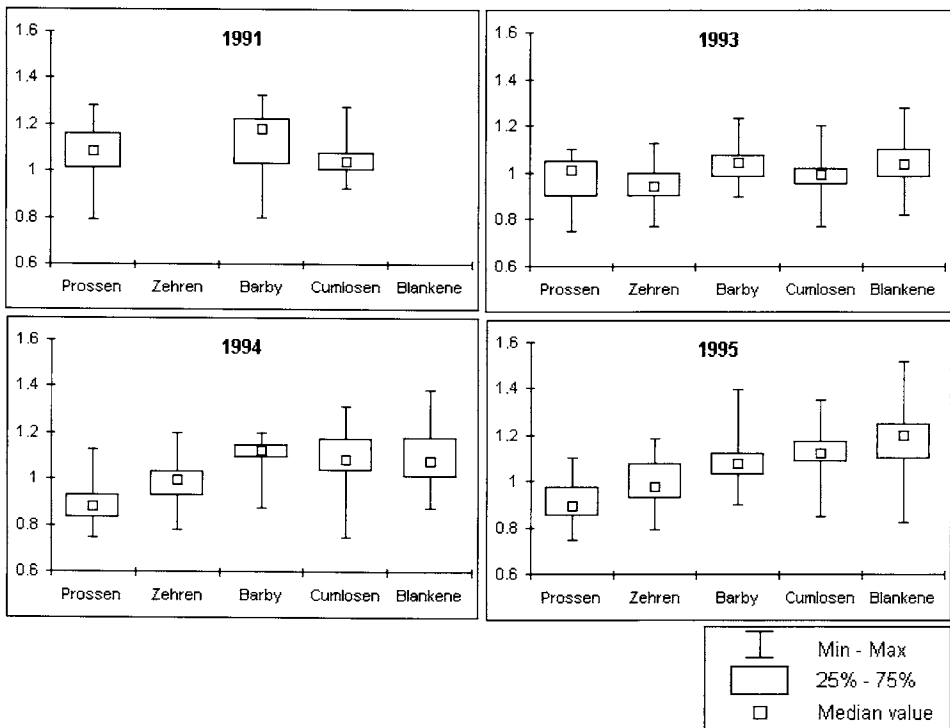


Figure IV.6.3. Condition index of breams caught along the Elbe River.

in the condition index, the relation between the body weight and the length of bream caught in different sites of the Elbe River from 1991 to 1995. While the values from 1991 show high variability, standardization of the sampling to a defined age class brought better reproducible values demonstrating clear increase of the condition index downstream the Elbe River and also increasing values in some of the sampling sites (Wagner et al., 1996).

Another key for the understanding and evaluation of analytical results is the land use structure of the sampling area and its annual changes. This means that not only the potential sources of chemical pollution have to be detected, characterized and monitored but also sources of anthropogenic nutritional and physical disturbance. Figure IV.6.4 shows a computer map of the urban industrial agglomeration of the Saar region in western Germany as an example for the land use mapping in extended sampling areas as a basis for the detection of temporal changes and the interpretation of analytical results. However, in agricultural areas the land use pattern has to be mapped much more in detail and actualized each year to recognize the potential or specific emissions and effects originating from each unit of used area (Müller et al., 1996).

In environmental specimen banking samples of different specimen types and ecosystems are frequently sampled, characterized, processed and stored with considerable effort to maintain the precautions necessary for deferred analysis on initially unknown substances or parameters. Quality assurance is therefore an absolute demand and an

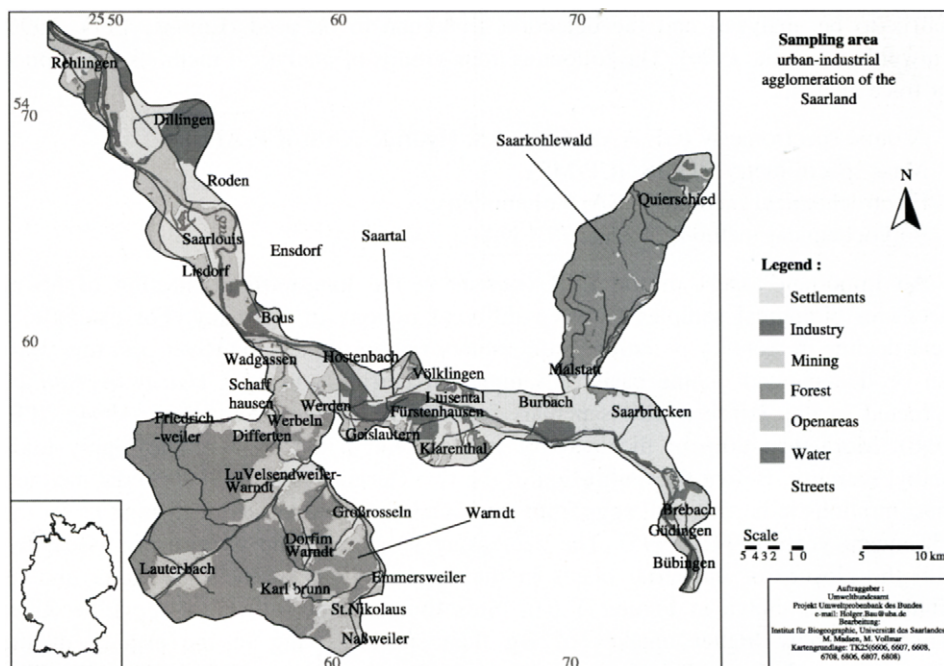


Figure IV.6.4. Computer map of the industrial agglomeration of the Saar region.

innovative challenge in ESB. Errors made during the sampling in the field, transportation and sample pre-treatment can seldom be recognized and never corrected afterwards during the following analytical measurements. Thus, the quality assurance system for ESB includes the whole process from planning, sampling, ecological and biometrical characterization, packing, transportation, storage, homogenization and subsampling up to the analytical procedures and the evaluation of the results (Klein et al., 1994; Paulus et al., 1995).

A flow chart of the entire preparation procedure for the final long-term storage of an environmental specimen is shown in Figure IV.6.5. On average 2.5 kg of material per specimen per sampling site was collected producing nearly 250 standardized subsamples of approximately 10 g each.

IV.6.4.2. Analytical sample characterization

The choice of pollutants or classes of pollutants for the analytical sample characterization took place according to ecotoxicological importance.

IV.6.4.2.1. Inorganic analysis

The analytical procedures for inorganic analysis of ESB samples were selected with emphasis on trace analysis capability and applicability for very complex biological matrices. During the pilot phase sample preparation was optimized in dependence on the matrix to be analyzed and the detection technique to be used (Emons, 1994, 1996; Umweltbundesamt, 1996). The following four groups of analytical methods are applied for trace analysis:

- Atomic spectrometry (GF-AAS, CV-AAS, Hydride AAS, ICP-AES)
- Mass spectrometry (IDMS, ICP-MS)
- Electrochemical methods (PSA, Voltammetry)
- Radiochemical methods (INAA, PGCNA).

An important aspect of the ESB consists in the long-term monitoring of heavy metals in biological samples between different regions in Germany. For example, a clear decline in mercury pollution in the estuary region of the Elbe River past few years can be documented on the basis of samples of herring gull eggs (*Larus argentatus*) collected in the Trischen bird sanctuary (see Figure IV.6.6) (Schwuger, 1994; UPB, 1996). More than 90% of the mercury was present in the form of the highly toxic methyl mercury. Before the unification of the two German states (1988/89) the mercury concentrations in herring gull eggs from the island Trischen was twice as high as for the subsequent years (1991–1995). The decreasing temporal trend is probably associated with the closure of industrial plants in the upper regions of the Elbe River and its tributaries. As shown in Figure IV.6.6, birds living in the estuary of the Elbe River (Trischen) show higher uptakes of Hg than species living in the estuary of the Weser River (Mellum). This demonstrates the influence of mercury input from the Elbe River into the North Sea.

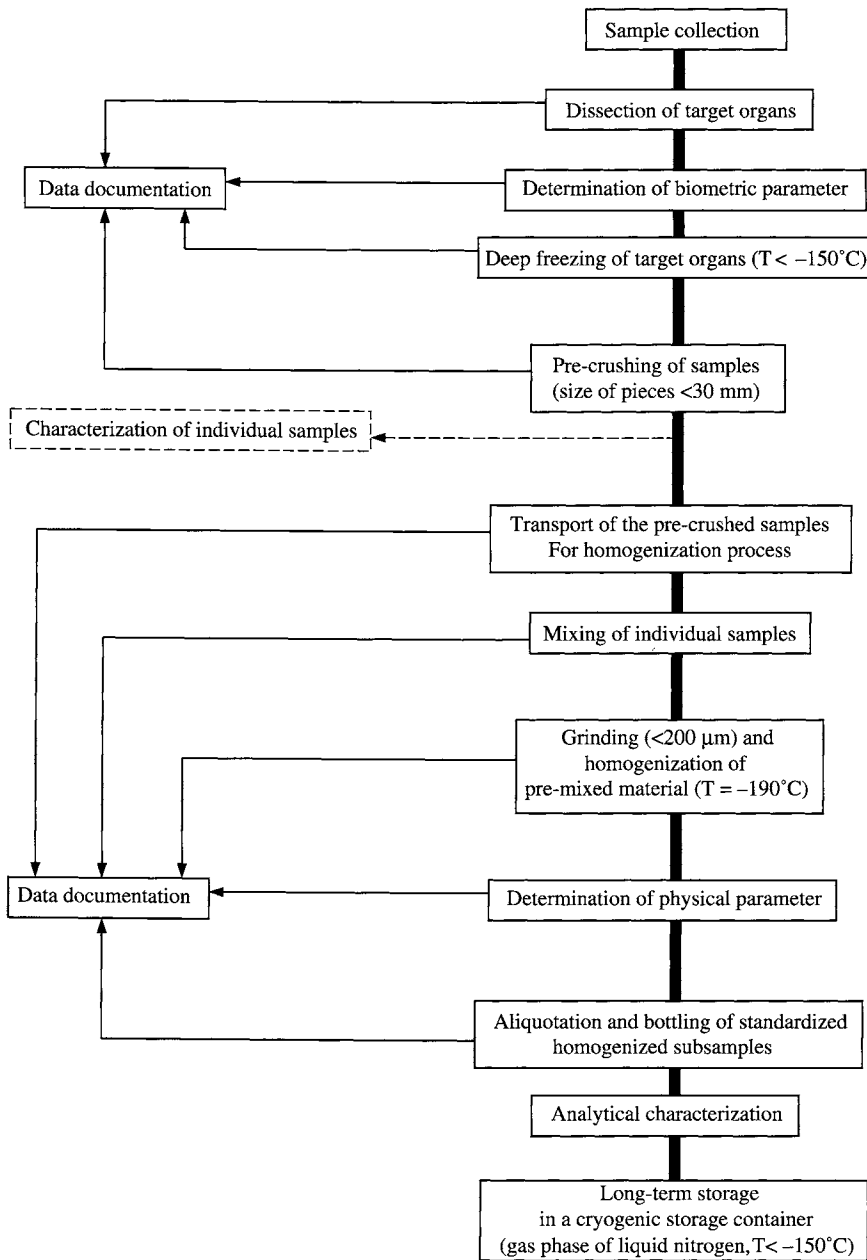


Figure IV.6.5. Flow chart of the sample preparation steps of the German ESB.

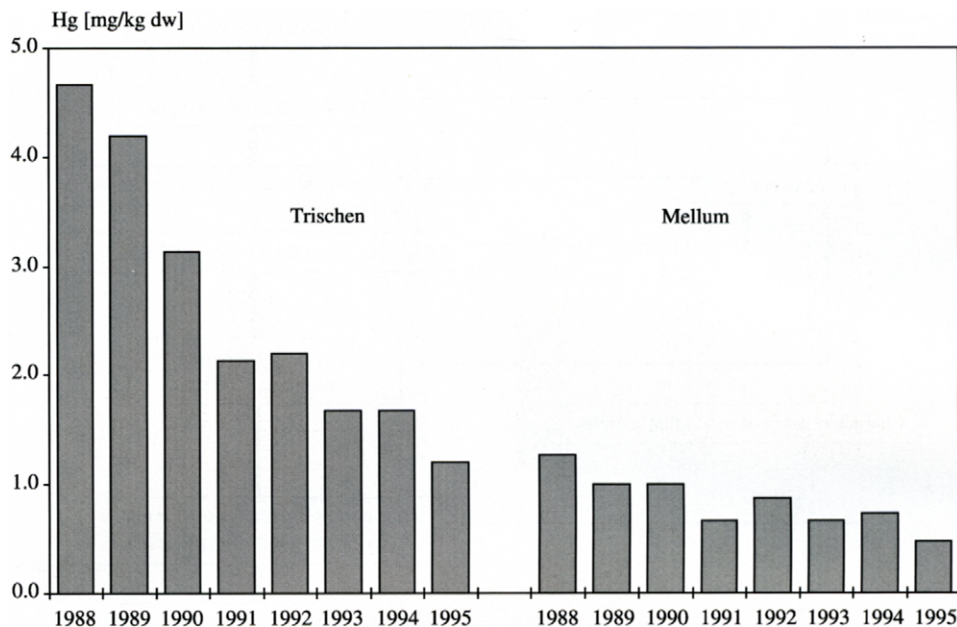


Figure IV.6.6. Temporal trend of mercury concentration in herring gull eggs from the islands Trischen and Mellum (after UPB, 1996).

IV.6.4.2.2. Polycyclic aromatic hydrocarbons (PAHs)

The determination of PAHs is of very high importance because they are considered to be the most relevant class of environmental carcinogens (Grimmer, 1993). PAH immission sources result from incomplete fossil fuel combustion, wood burning and waste gases from industrial and household combustion processes. The continuous measurement of PAH concentration in various selected environmental matrices representing the terrestrial, aquatic and atmospheric ecosystem provides the opportunity to recognize trends in the environmental pollution.

For PAH determination sample homogenates are extracted with toluene or cyclohexane by Soxhlet. Interferences are removed by liquid/liquid distribution and chromatography on silica and Sephadex LH20. The detection of PAHs is carried out by gas chromatography equipped with FID or MS (SIM) detectors (Grimmer et al., 1996).

Spruce and pine sprouts are passive sampler reflecting the atmospheric pollution by PAHs (Jacob et al., 1996). For example, the benzo[a]pyrene (B[a]P) concentration of spruce sprouts from the industrialized area of Saarland decreased by a factor of 3 within 10 years (see Figure IV.6.7).

The same trend in pine sprouts has been observed for a sampling area in East Germany (Dübener Heide) during the period 1991–1995 with B[a]P declining from 3.5 to 1.5 $\mu\text{g}/\text{kg}$. In principle, similar results were also obtained with poplar leaves from Halle, whereas samples from Leipzig showed no consistent temporal trend (Fig. IV.6.8). These findings show that the reduction of pollution by technical improvements such as modern

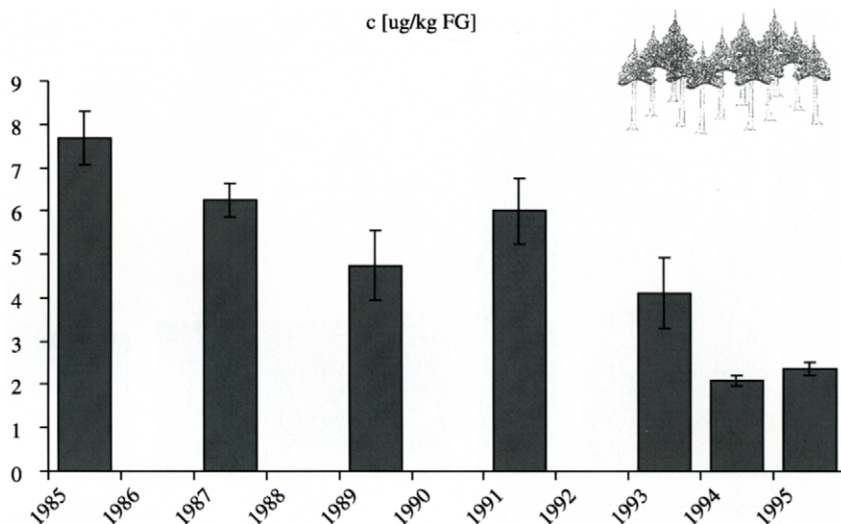


Figure IV.6.7. Time-dependent decline of the B[a]P concentration of spruce sprouts from Saarland-Warndt during 1985–1995.

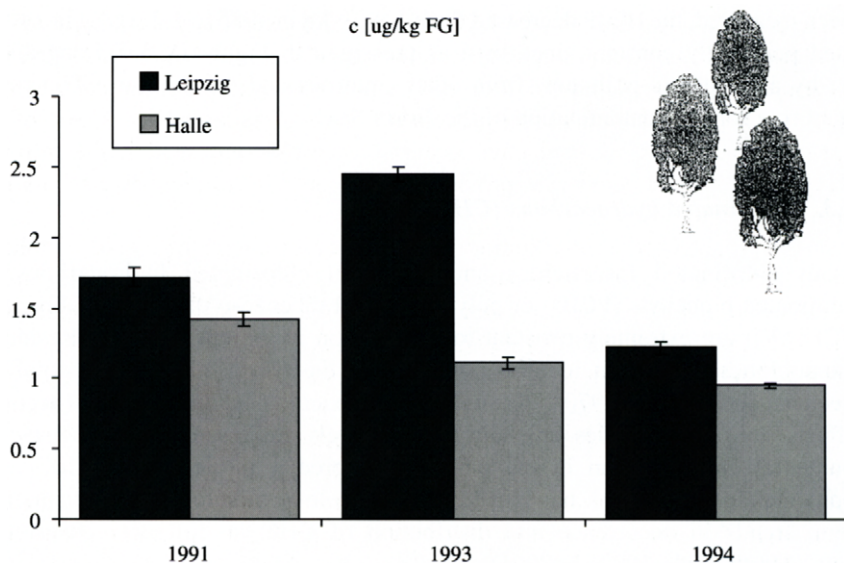


Figure IV.6.8. Temporal trend of the B[a]P concentration in poplar leaf homogenate from two different sampling locations of Dübener Heide (East Germany) during 1991–1994.

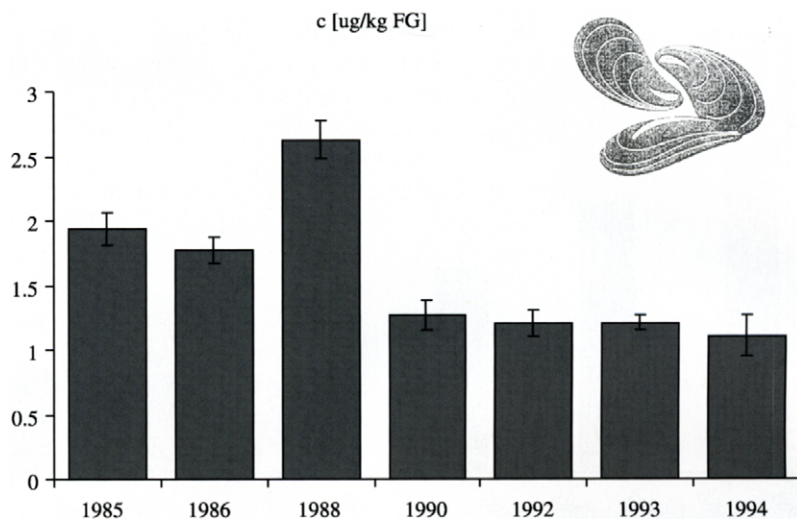


Figure IV.6.9. Temporal trend of the B[a]P concentration of mussel homogenate from Eckwarderhörne (North Sea) from 1985 to 1994.

vehicle conceptions as well as improved domestic and industrial combustion devices in the past were successful.

Mussels (*Mytilus edulis*) as a bioindicator of the marine environment for the North Sea (Eckwarderhörne) exhibited likewise a decline of the PAH concentration although to a lesser extent (Jacob et al., 1996). With the exception of 1988 when higher concentrations have been measured, the B[a]P decreased from 1.9 $\mu\text{g}/\text{kg}$ in 1985 to 1.2 $\mu\text{g}/\text{kg}$ in 1990 and remained practically constant since then as presented in Figure IV.6.9. Long-distance transfer by atmospheric pollutants from other countries and the dilution effect play an important role for the contamination of the North Sea.

IV.6.4.3. Chlorinated hydrocarbons (CHC)

Numerous chlorinated insecticides and industrial chlorinated hydrocarbons (e.g. polychlorinated biphenyls (PCBs) or polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F)) are extremely resistant to degradation in the environment. Residues of these xenobiotics have been identified throughout the world, although most of them have been banned since the 1970s. Because of their toxicological properties and accumulation effects, long-term studies on their residue levels are essential to understand the environmental contamination in the past and to predict future trends. The German environmental specimen bank has played a crucial role in monitoring and evaluation of long-term trends in occurrence and distribution of CHC in different ecosystems in Germany (Marth et al., 1999a,b, 2001).

For the CHC determination the samples are mixed with anhydrous sodium sulfate/sea sand to form a free flowing product, which is extracted in a column with

n-hexane/acetone (2:1 v/v). Clean up is performed with gel-permeation and high performance liquid chromatography. Quantification is carried out by high-resolution gas chromatography (HRGC) equipped with electron capture detection using two columns of different polarity. More details about the applied method are given by Oxyinos et al. (1992).

Herring gull eggs are a suitable bioindicator for lipophilic xenobiotics since they express the local and temporal environmental conditions (Oxyinos et al., 1993). The time series of herring gull eggs at the sampling locations on the island of Mellum and Trischen are shown in Figure IV.6.10. The location influenced by the Elbe River (Trischen) exhibited higher concentrations than the location Mellum, which is influenced by fluxes of pollutants in the river Weser. The significantly higher DDE concentrations in eggs from Trischen in 1989 can be explained by DDT applications in the former GDR in the late 1980s.

Figure IV.6.11 summarizes the PCDD/F levels of herring gull eggs from 1988 to 1993 at Trischen and Mellum (Schramm et al., 1996). A decreasing trend of the contaminants is obvious. This development shows the result of the legislative actions to minimize the dioxin emissions, e.g. the ban on sea burning of hazardous waste.

Investigations of sediments and fish (bream) along the Elbe River (Fig. IV.6.12) in 1991 have shown that the eastern sampling sites (Prossen, Dresden) have been heavily contaminated by hexachlorobenzene (HCB), octachlorostyrene (OCS), DDT-metabolites and PCBs (Fig. IV.6.13) (Oxyinos et al., 1995). This observation is probably a result of the considerable pollution of the Elbe River by the industrialized areas (e.g. Pardubice, Neratovice, Usti) of the former CSFR (Nesmerak, 1993). Sediments at the station Dresden are highly contaminated because they were strongly subjected to effluents of a pulp mill and chemical industries until 1991 (IKSE, 1992). A 30% decrease of the CHC concentrations in bream muscle tissue was found between station Dresden and Vockerode, further downstream, the total CHC burden remained nearly constant. Elevated levels of hexachlorocyclohexane isomers (HCHs) in bream muscles and livers were observed downstream between Aken and Heinrichsberg due to the influxes of the Mulde and Saale Rivers as well as discharges from pesticide plants located in Magdeburg (Marth et al., 1996).

The comparison of the CHC-pattern (Fig. IV.6.14) in bream livers exhibits significant distinctions between the different ecosystems. DDT-metabolites and PCBs contributed to one-third, respectively, to the total CHC burden of bream livers from the limnic ecosystem of the Elbe River. PCBs were the major organochlorine contaminants (70%) in breams of a typical industrialized area (Saarland) in contrast to agricultural areas of East Germany, where DDT-metabolites were the dominant pollutants (80%). This is caused by the continued application of DDT in the former GDR after DDT was banned in Western Europe.

These investigations have proved usability of herring gull eggs and breams as bioindicators for monitoring temporal and spatial trends of CHC (Marth et al., 2000).

Significantly higher concentrations of DDT-metabolites were found in pigeon eggs from Leipzig (East Germany). Fortunately, a decreasing trend of these pollutants can be observed (Fig. IV.6.15).

Besides CHC, other chlorinated compounds were studied in samples of the German environmental specimen bank, e.g. chlorinated phenols (CP) (Martens et al., 1999).

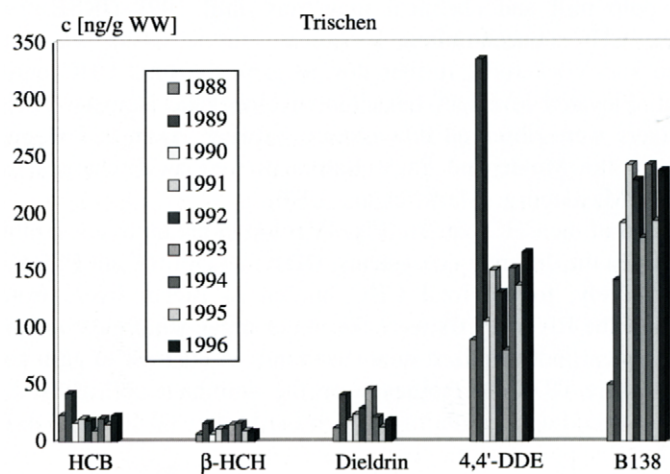
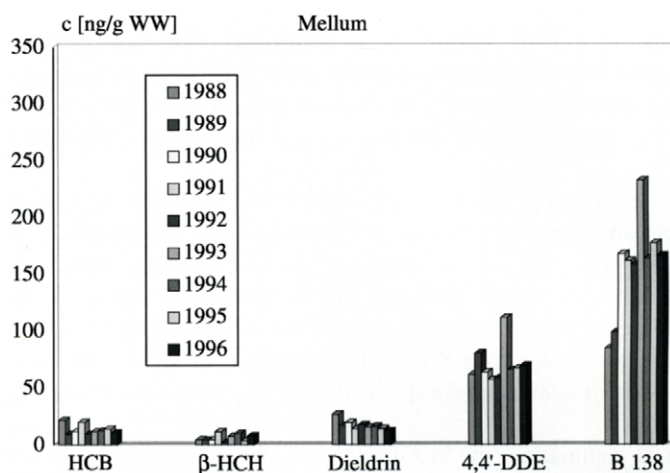


Figure IV.6.10. Temporal trend of selected CHC in herring gull eggs from the islands Trischen and Mellum.

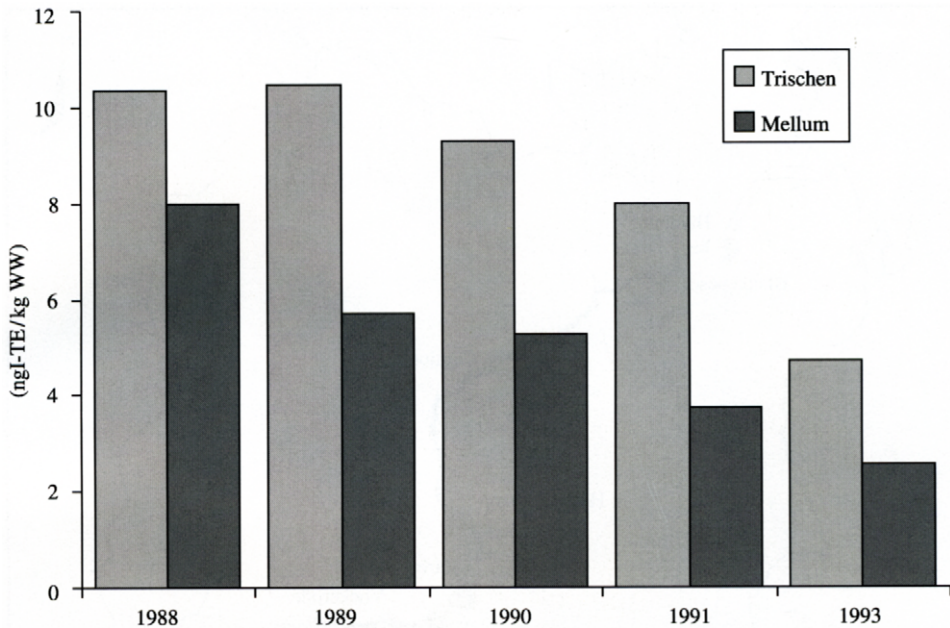


Figure IV.6.11. Temporal trend of PCDD/F-ITE in herring gull eggs from the islands Trischen and Mellum.

IV.6.5. Conclusion and future perspectives

Twenty years of practical experience in environmental specimen banking have demonstrated that the concept of long-term storage of biological specimens for the retrospective analysis has contributed to traditional environmental pollution monitoring as an important complement. The ESB can serve as a valuable resource for the assessment of long-term trends of pollutants affecting human and environmental health, in particular for those pollutants that have been unnoticed (Kettrup et al., 1999).

The results fulfill the proposed goals of the ESB as far as political and administrative decisions concerning the emission levels or other regulations on pollutants can be confirmed by their decrease in the environment (e.g. introduction of unleaded fuel, ban on sea burning of hazardous waste). Illegal applications of chemicals could have been identified, e.g. DDT applications in the former GDR.

The generated SOP (Umweltbundesamt, 1996) for analytical and sampling procedure are well documented and similar to the demands of Good Laboratory Practice or the European Standards for Analytical Work. Thus, in this field it became possible to identify and to avoid possible errors in the future. Due to high quality standard of sampling and analysis the collection is of particular value for future analytical work on environmental contaminants of which we presently know very little.

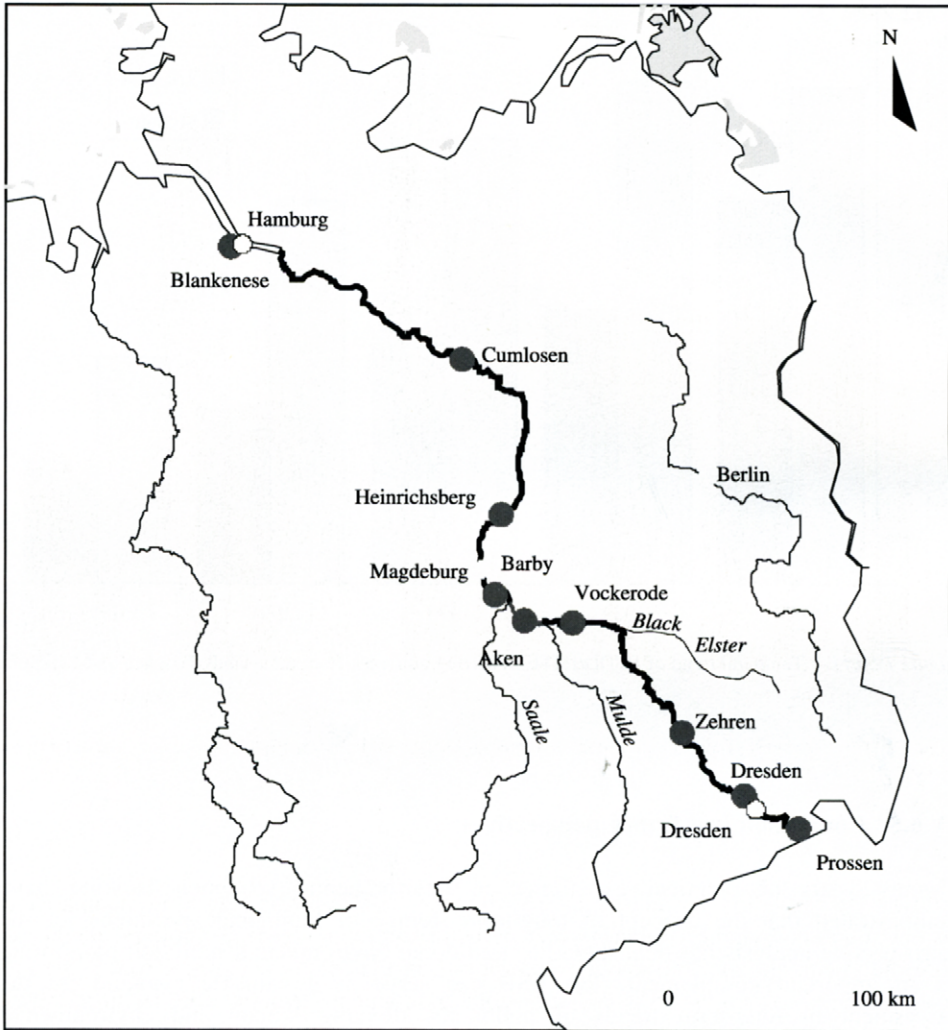


Figure IV.6.12. Sampling sites of bream along the Elbe River.

The increasing amount of generated data now allows the verification of many relationships discovered in the past in the environmental research with very high accuracy that are bioaccumulation, biomagnification, distribution, transport and at least degradation of chemicals. The history of the conceptual development of the ESB has resulted in a pool of knowledge that can be used for future decisions and recommendations for researchers and politicians (Marth and Kettrup, 1998; Kettrup et al., 1999).

As a new instrument for science, administration and management, ESB can support analytical and environmental research and monitoring generally in many ways and make it more effective and reliable, e.g. supply reference materials for environmental

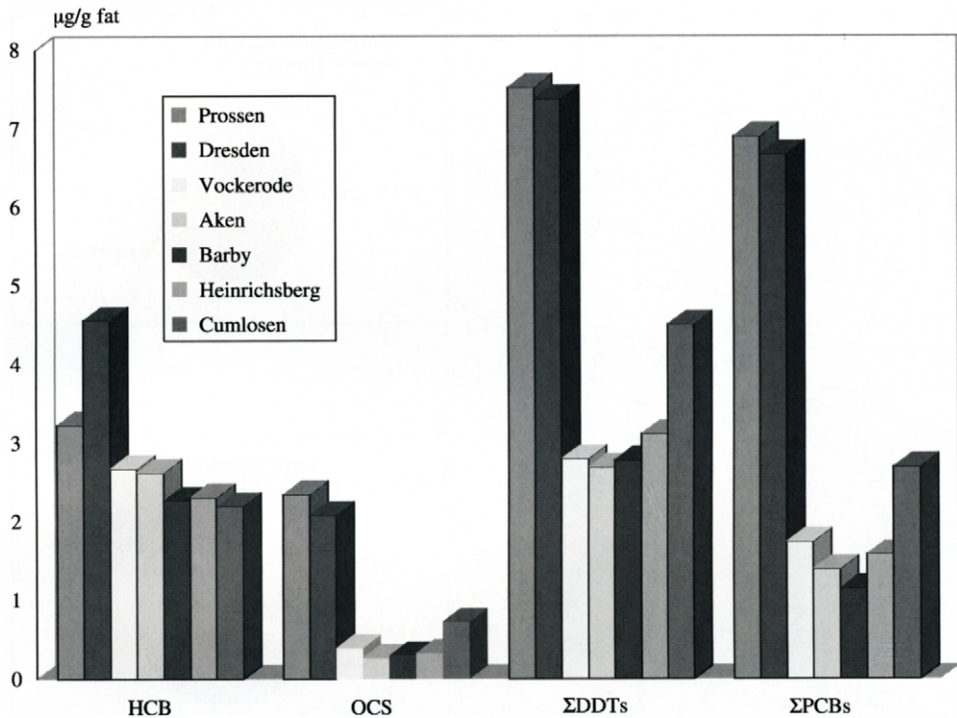


Figure IV.6.13. Levels of major contaminants related to fat content in bream muscle tissue from the Elbe River in 1991.

analysis, preservation of authentic records as an archive for long-term comparisons of environmental change, securing and perpetuation of evidence in biology, medicine, forensic medicine, biotechnology, deposition or conversion of problematic wastes, environmental planning, risk and exposure assessment (Zenick and Griffith, 1995; Subbramanian and Iyengar, 1997; Kettrup et al., 1999).

For future scientific activities the following aspects are recommended:

- Extension of the set of chemicals that are analyzed.
- Selection of the most important bioindicators to minimize costs and labor.
- Creation of a pattern library for sources and bioindicators by normalizing and archiving chromatographic raw data and applying multivariate statistics.

Other countries contribute with their research/monitoring programs to the extension of analyzed chemicals or environmental matrices of specific or common interest, e.g. Swedish EPA within environmental specimen banking program screened in environmental samples: in 1999 – chlorinated paraffins and extended list of metals; in 2000 – flame retardants HBCD and TBBPA; in 2001 – organic tin compounds, phosphorylated flame retardants, highly fluorinated compounds, antimony, octylphenol, triclosan, pesticides and CPs. In urban areas, studied matrices comprised such

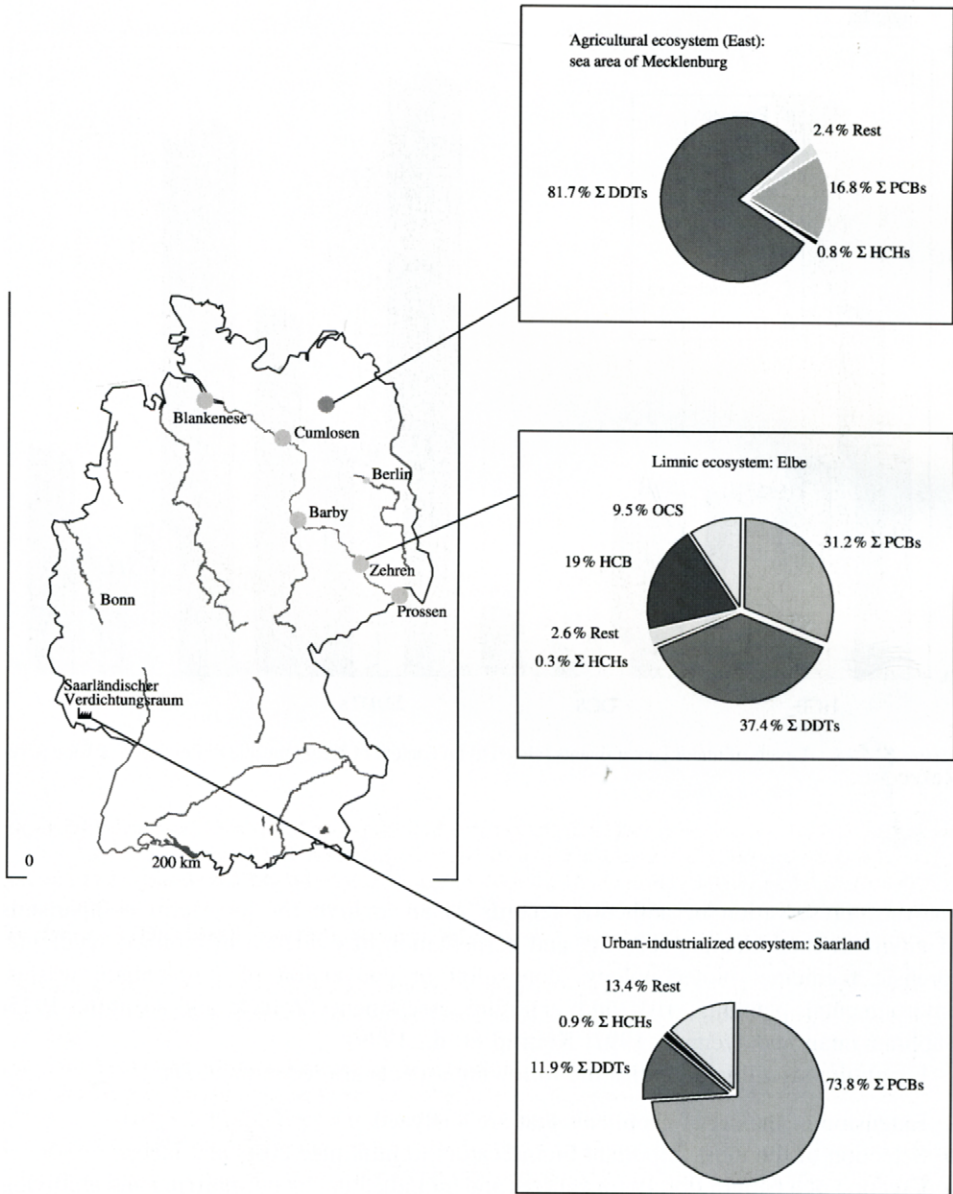


Figure IV.6.14. Comparison of different ecosystems with bream (*Abramis brama*) as bioindicator.

problematic waste as fly ash (current studies) and sewage sludge (started in 2002) (Hedlund, 2001).

One of the major tasks for the ESB in the future will be to make ties to ESBs of other nations and in cooperation with these establish an international forum for exchange of information and samples.

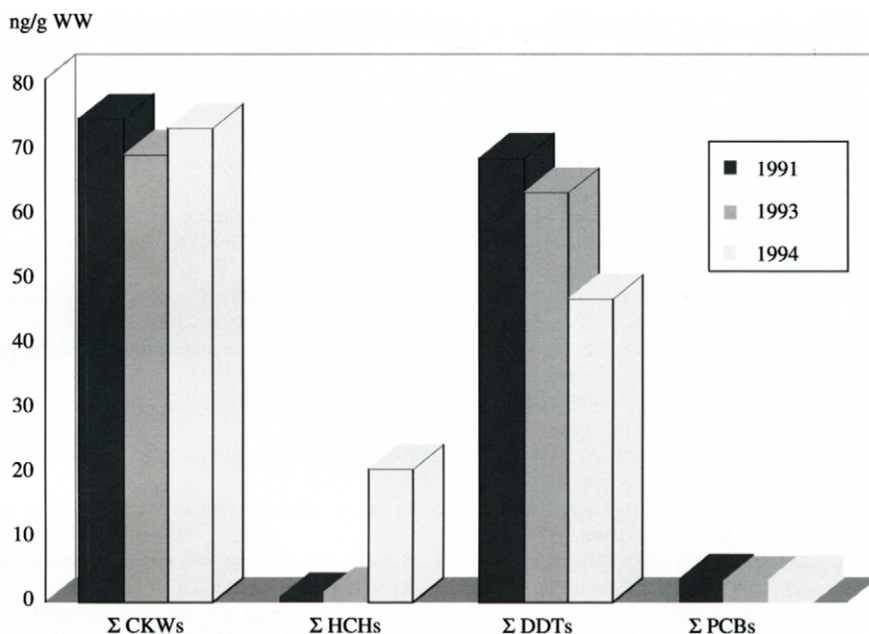


Figure IV.6.15. Temporal comparison of CHC in pigeon eggs from Leipzig.

References

- Boehringer, U.R., 1988. Umweltprobenbank: Bericht und Bewertung der Pilotphase. Bundesministerium für Forschung und Technologie, Umweltbundesamt Berlin, Springer, Berlin (in German).
- EC, 2000/532/EC: Commission Decision of 3 May 2000 replacing Decision 94/3/EC establishing a list of wastes. Basic Act 32000D0532, OJ L 226 06.09.2000, p. 3.
- EC, 2001/118/EC: Commission Decision of 16 January 2001 amending Decision 2000/532/EC as regards the list of wastes. OJ L 47 16.02.2001, p. 31.
- EC, 2455/2001/EC: Decision of the European Parliament and the Council of 20 November 2001 establishing the list of priority substances in the field of water policy and amending Directive 2000/60/EC. OJ L 331 15.12.2001, p. 5.
- EEC, 76/769/EEC: Council Directive of 27 July 1976 on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations. Basic Act 31976L0769, OJ L 262 27.09.1976, p. 201.
- Emons, H., 1994. Inorganic analysis within the German Environmental Specimen Bank. Bilateral Seminars of the International Bureau, 19, 195–204 (Forschungszentrum Juelich GmbH).
- Emons, H., 1996. Environmental specimen banking – aspects of metal determination and distribution. *Fresenius J. Anal. Chem.*, 354 (5/6), 507–510.
- EUR-Lex – Directory of Community Legislation in Force: Analytical Register 15.10.20.50. Chemicals, Industrial Risk and Biotechnology, Web site: http://www.europa.eu.int/eur-lex/en/lif/reg/en_register_15102050.html.
- EUR-Lex – Directory of Community Legislation in Force: Analytical Register 15.10.30.30. Waste management and clean technology, Web site: http://www.europa.eu.int/eur-lex/en/lif/reg/en_register_15103030.html.
- GDCh/BUA, 1987. Altstoffbeurteilung, p. 32 (in German).
- Grimmer, G., 1993. Relevance of polycyclic aromatic hydrocarbons as environmental carcinogens. In: Garrigues, P., Lamote, M. (Eds), *Polycyclic Aromatic Compounds 13th Int. Symp.* PAH, 1–4 Oct. 1991 Bordeaux, Gordon and Breach, London, pp. 31–41.

- Grimmer, G., Hildebrandt, J., Jacob, J., Naujack, K.-W., 1996. Standard Operating Procedure for the analysis of polycyclic aromatic hydrocarbons (PAH) in various matrices. In: Umweltbundesamt (Ed.), Federal Environmental Specimen Bank: Standard Operating Procedures for Sampling, Transport, Storing, and Chemical Characterization of Environmental Specimens and Human Organic Specimens, Erich Schmitt Verlag, Berlin, pp. 18, Chap. 12 (in German).
- Hedlund, B., 2001. Toxic Substances Coordination. Environmental Monitoring. Swedish EPA, 2001, Web site: <http://www.internat.environ.se/documents/isses/monitor/modoc/screen.htm>.
- IKSE – Internationale Kommission zum Schutz der Elbe, 1992. Erste Auswertung der Abwasserlasten von industriellen Direktleinleitern aus drei ausgewählten Industriezweigen im Einzugsgebiet der Elbe im Jahre 1991 gegenüber 1989, in German.
- Jacob, J., Grimmer, G., Hildebrandt, J., 1996. Long-term decline of atmospheric and marine pollution by polycyclic aromatic hydrocarbons (PAH) in Germany. *Chemosphere*, 34, 2099–2108.
- Kayser, D., Boehringer, R.U., Schmidt-Bleek, F., 1982. The environmental specimen banking project of the Federal Republic of Germany (pilot phase). *Environ. Monit. Assess.*, 1, 241–255.
- Kettrup, A., Marth, P., 1998. Specimen banking as an environmental surveillance tool. In: Schüürmann, G., Markert, B. (Eds.), *Ecotoxicology*, Wiley, Chichester, pp. 413–436.
- Kettrup, A., Schramm, K.-W., Marth, P., Oxyinos, K., Schmitzer, J., 1999. Specimen banking as an environmental surveillance tool. *Ann. Chim.-Rome*, 89, 489–498.
- Keune, H., 1993. Environmental specimen banking (ESB): an essential part of integrated ecological monitoring on a global scale. *Sci. Total Environ.*, 139/140, 537–544.
- Klein, R., Paulus, M. (Eds.), 1995. *Umweltproben für die Schadstoffanalytik im Biomonitoring-Standards zur Qualitätssicherung bis zum Laboreingang*, Gustav Fischer Verlag, Jena (in German).
- Klein, R., Paulus, M., Wagner, G., Müller, P., 1994. Das ökologische Rahmenkonzept zur Qualitätssicherung in der Umweltprobenbank des Bundes. *Beitragsserie in der UWSF - Z- Umweltchem. Ökotox.*, 6, 221–232 (in German).
- Kubin, E., Lippo, H., Karhu, J., Pokolainen, J., 1997. Environmental specimen banking of nationwide biomonitoring samples in Finland. *Chemosphere*, 34 (9/10), 1939–1944.
- Lewis, R.A., 1985. Richtlinien für den Einsatz einer Umweltprobenbank in der Bundesrepublik Deutschland auf ökologischer Grundlage, Universität des Saarlandes, Saarbrücken (in German).
- Lewis, R.A., 1987. Guidelines for environmental specimen banking with special reference to the Federal Republic of Germany: ecological and managerial aspects. U.S. Department of the Interior, National Park Service. U.S. MAB Report, 12, 1–182.
- Lewis, R.A., 1988. Remarks on the status of environmental specimen banking in relation to health and environmental assessment. In: 11th U.S.–German Seminar of State and Planning on Environmental Specimen Banking. Bayreuth, Bavaria, May 1–3, 1988.
- Lewis, R.A., Horras, C., Paulus, M., Klein, B., 1993. Auswahl ökologischer Umweltbeobachtungsgebiete in der Bundesrepublik Deutschland. In: Likens, G.E. (Ed.), *An Ecosystem Approach to Aquatic Ecology*, Springer, New York (in German).
- Martens, D., Schramm, K.-W., Kettrup, A., 1999. Chlorinated phenols (CP) in samples of the environmental specimen bank of Germany. *GSF-Bericht* 02/99, P.13, 401–404 (GSF Neuherberg).
- Marth, P., Kettrup, A., 1998. Umwelt- und Humanprobenbanken. *Landsberg, Ecomed Verlagsgesellschaft* 13. Erg. Lfg. 5/98, IV-7, 1–19 (in German).
- Marth, P., Schramm, K.-W., Henkelmann, B., Wolf, A., Oxyinos, K., Schmitzer, J., Kettrup, A., 1999a. Die Rolle der Umweltprobenbank in der Umweltüberwachung am Beispiel von chlorierten Kohlenwasserstoffen in ausgewählten Matrices. *UWSF-Z. Umweltchem. Ökotox.*, 11, 89–97.
- Marth, P., Schramm, K.-W., Oxyinos, K., Schmitzer, J., Kettrup, A., 1999b. Occurrence and distribution of chlorinated hydrocarbons in different ecosystems in Germany. *GSF-Neuherberg, GSF-Bericht* 02/99, O C2, 66–71.
- Marth, P., Martens, D., Schramm, K.-W., Schmitzer, J., Oxyinos, K., Kettrup, A., 2000. Environmental specimen banking. Herring gull eggs and breams as bioindicators for monitoring long-term and spatial trends of chlorinated hydrocarbons. *Pure Appl Chem.*, 72, 1027–1034.
- Marth, P., Martens, D., Schramm, K.-W., Schmitzer, J., Oxyinos, K., Kettrup, A., 2001. The German Environmental Specimen Bank: Application in trend monitoring of chlorinated hydrocarbons. In: Johnston, J.J. (Ed.), *Pesticides and Wildlife*. ACS Symposium Series 771, American Chemical Society, Washington, DC, pp. 68–81.

- Müller, P., Wagner, G., Paulus, M., Klein, R., 1996. Biological Environmental Specimen Banking as Precondition for Intelligent Environmental Monitoring, BESBE-2, Stockholm.
- Nesmerak, I., 1993. Kontamination der Elbe aus dem Gebiet der Tschechischen Republik und der Moldau mit organischen Schadstoffen. In: Heinisch, E., Kettrup, A., Wenzel-Klein, S. (Eds), Schadstoffatlas Osteuropa, Ecomed-Verlag, Landsberg, pp. 167–170 (in German).
- Oxynos, K., Schmitzer, J., Dürbeck, H.W., Kettrup, A., 1992. Analysis of chlorinated hydrocarbons (CHC) in environmental samples. In: Rossbach, M., Schladot, J.D., Ostapczuk, P. (Eds), Specimen Banking, Springer, Berlin, p. 127.
- Oxynos, K., Schmitzer, J., Kettrup, A., 1993. Herring gull eggs as bioindicator for chlorinated hydrocarbons. *Sci. Total Environ.*, 139/140, 387–398.
- Oxynos, K., Schramm, K.-W., Marth, P., Schmitzer, J., Kettrup, A., 1995. Chlorinated hydrocarbons- (CHC) and PCDD/F-levels in sediments and breams (*Abramis brama*) from the River Elbe (contribution to the German Environmental Specimen Bank). *Fresenius J. Anal. Chem.*, 353, 98–100.
- Paulus, M., Horras, C., Klein, B., Lewis, R.A. 1990. Vertiefte Auswahl von Probenahmeregionen für die Umweltprobenbank und ökologische Beratung zu ihrem Betrieb. Umweltforschungsplan des Bundesminister für Umwelt, Naturschutz und Reaktorsicherheit. Anschlußbericht zum BMU-Forschungsvorhaben 10808001, Saarbrücken, in German.
- Paulus, M., Klein, R., Zimmer, M., Jacob, J., Rossbach, M., 1995. Die Rolle der biometrischen Probencharakterisierung in der Umweltanalytik am Beispiel der Fichte (*Picea abies*). Beitragsserie in der UWSF -Z- Umweltchem. Ökotox., 7, 236–244 (in German).
- Pugh, R., 2001. The national biomonitoring specimen bank. *CCEHBI Quarterly*, 2, 1–4 (Web site: <http://www.chbr.noaa.gov/Newsletter/volume2/issue1/nbsb.html>).
- Schramm, K.-W., Kettrup, A., Schmitzer, J., Marth, P., Oxynos, K., 1996. Environmental Specimen Bank – a useful tool for prospective and retrospective environmental monitoring. *TEN*, 3, 43–49.
- Schramm, K.-W., Marth, P., Wolf, A., Hahn, K., Oxynos, K., Schmitzer, J., Kettrup, A., 1999. Verteilungskoeffizienten chlorierter Kohlenwasserstoffe zwischen Muskulatur und Leber bei Fischen. *UWSF-Z. Umweltchem. Ökotox.*, 11, 277–280 (in German).
- Schwuger, M.J., 1994. Environmental Specimen Bank of the Federal Republic of Germany – significance of surfactants. *Bilateral Seminars of the International Bureau*, 19, 159–194 (Forschungszentrum Juelich GmbH).
- SRU (Rat der Sachverständigen für Umweltfragen), 1987. Umweltgutachten 1987, Deutscher Bundestag, Drucksache 11/1569 und Verlag Kohlhammer, Stuttgart/Mainz, in German.
- Stoeppler, M., Zeisler, R. (Eds), 1993. Biological environmental specimen banking. *Sci. Total Environ.*, BESB special issue, 139/140.
- Subbramanian, K.S., Iyengar, G.V. (Eds), 1997. Environmental Biomonitoring. Exposure Assessment and Specimen Banking. *ASC Symposium Series 654*, ACS Publ., Washington, DC, p. 298.
- Thalius, J. 1588. *Sylvia Hercynia, Sive Catalogus Plantarum Sponte Nascentium in Montibus*. Frankfurt/M.
- Umweltbundesamt (Ed.), 1996. *Federal Environmental Specimen Bank: Standard Operating Procedures for Sampling, Transport, Storing, and Chemical Characterization of Environmental Specimens and Human Organic Specimens*, Erich Schmitt Verlag, Berlin (in German).
- UPB, 1996. *Jahresbericht der Bank der Umweltproben 1995*, Forschungszentrum Jülich GmbH, Jülich, in German.
- Wagner, G., 1994a. Biologische Umweltproben. In: Stoeppler, M. (Ed.), *Probenahme und Aufschluß*, Springer Labormanual, Berlin, (in German).
- Wagner, G., 1994b. Environmental specimen banking (ESB) in the Federal Republic of Germany – an instrument for long-term environmental monitoring, assessment, and research. In: Alef, K., Blum, W., Schwarz, S., Riss, A., Fiedler, H., Hutzinger, O. (Eds), *Ecoinforma '94*, 5, Bayreuth, pp. 457–462.
- Wagner, G., 1995. Basic approaches and methods for quality assurance and quality control in sample collection and storage for environmental monitoring. *Sci. Total Environ.*, 176, 63–71.
- Wagner, G., Klein, R., Nentwich, K., Paulus, M., Sprengart, J., Wüst, R., Müller, P., 1996. *Umweltprobenbank des Bundes: Beiträge zur Probenahme und Probenbeschreibung. Jahresbericht 1995*, Saarbrücken, in German.
- Wise, S.A., Zeisler, R., 1984. The pilot environmental specimen bank program. *Environ. Sci. Technol.*, 18, 302A–307A.
- Wise, S.A., Zeisler, R., 1985. The U.S. pilot environmental specimen bank program. In: Wise, S.A., Zeisler, R. (Eds), *International Review of Environmental Specimen Banking*. *Nat. Bureau of Standards Spec. Pub.* 706, Gaithersburg, MD, pp. 34–35.

- Wise, S.A., Zeisler, R., Goldstein, G.M. (Eds), 1988. Progress in Environmental Specimen Banking. NBS Special Publication 740, U.S. Dept. of Commerce, U.S. Government Printing Office, Washington, DC.
- Wittig, R., 1993. General aspects of biomonitoring heavy metals by plants. In: Markert, B. (Ed.), *Plants as Biomonitors—Indicators for Heavy Metals in the Terrestrial Environment*, VCH, Weinheim, pp. 3–27.
- Zeisler, R., Koster, B.J., Wise, S.A., 1992. Specimen banking at the National Institute of Standards and Technology. Analytical Approaches as Related to Specimen Banking, Nat. Bureau of Standard Spec., Gaithersburg, MD.
- Zenick, H., Griffith, J., 1995. The role of specimen banking in risk assessment. *Environ. Health Perspect.*, 103 (Suppl. 3), 9–12.