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PROCEEDINGS

Effect of monitoring strategies and reference data of the German Environmental Specimen Banking Program

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Abstract The constitution of the German Environmental Specimen Bank (ESB) has started in 1985, subsequent to a successful pilot study concerning the feasibility. Since that time, a multitude of technological and methodical standards have been developed, which allow for a high quality of the storage-samples and of the specimen characterization. While the storage-samples are kept for retrospective analysis, by now, already comprehensive data on the material-developing in the environment are available due to a real time monitoring of selected environmental chemicals over a period of up to twenty years. Thus, spatial and temporal trends can be described. Since the state of knowledge on critical tissue concentrations in the sublethal range is extremely low at present, it is however not possible to accomplish a direct assessment of relevancy of the substance concentrations. Hence, within the scope of the German ESB Program, the following strategies on assessment of relevancy are observed: use of biomarkers, histopathological examinations, biometric specimen characterization, use of ecological indicator groups, and development of a reference system with analytical and biometric data. Thus, for example endocrine effects in male breams in the river Saar, which correlate directly to operational discharges from municipal sewage plants, could be detected. By histopathological examinations, fibrotic and necrotic tissue adaptations on the gonads had been ascertained cumulatively, which unambiguously imply a restricted fertility of the male breams. In the river Rhine, an improved growth along the timeline could be described on the basis of biometric characterization of breams, which is regarded as rate for the reaction to all structural and material changes in the water body. Presently, with the development of a reference system based on the data collected in the scope of the Environmental Specimen Bank, a basis for the assessment of monitoring results with accumulation indicators is provided.

Key words Environmental Specimen Banking • effect monitoring • reference system • critical tissue concentration • bioaccumulation • biometric specimen characterization

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Introduction

The Environmental Specimen Bank (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, Dessau. It 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. To achieve its 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 (Fig. 1) and their storage in accordance with high quality standards. Therefore, the samples are frozen in the gas phase above liquid nitrogen on site directly subsequent to the biometric specimen characterization and ecological/bio-geographical description.

During the process of chemical characterization, 6 out of 200 subsamples from each sampling site are

Map of environmental specimen bank sampling areas

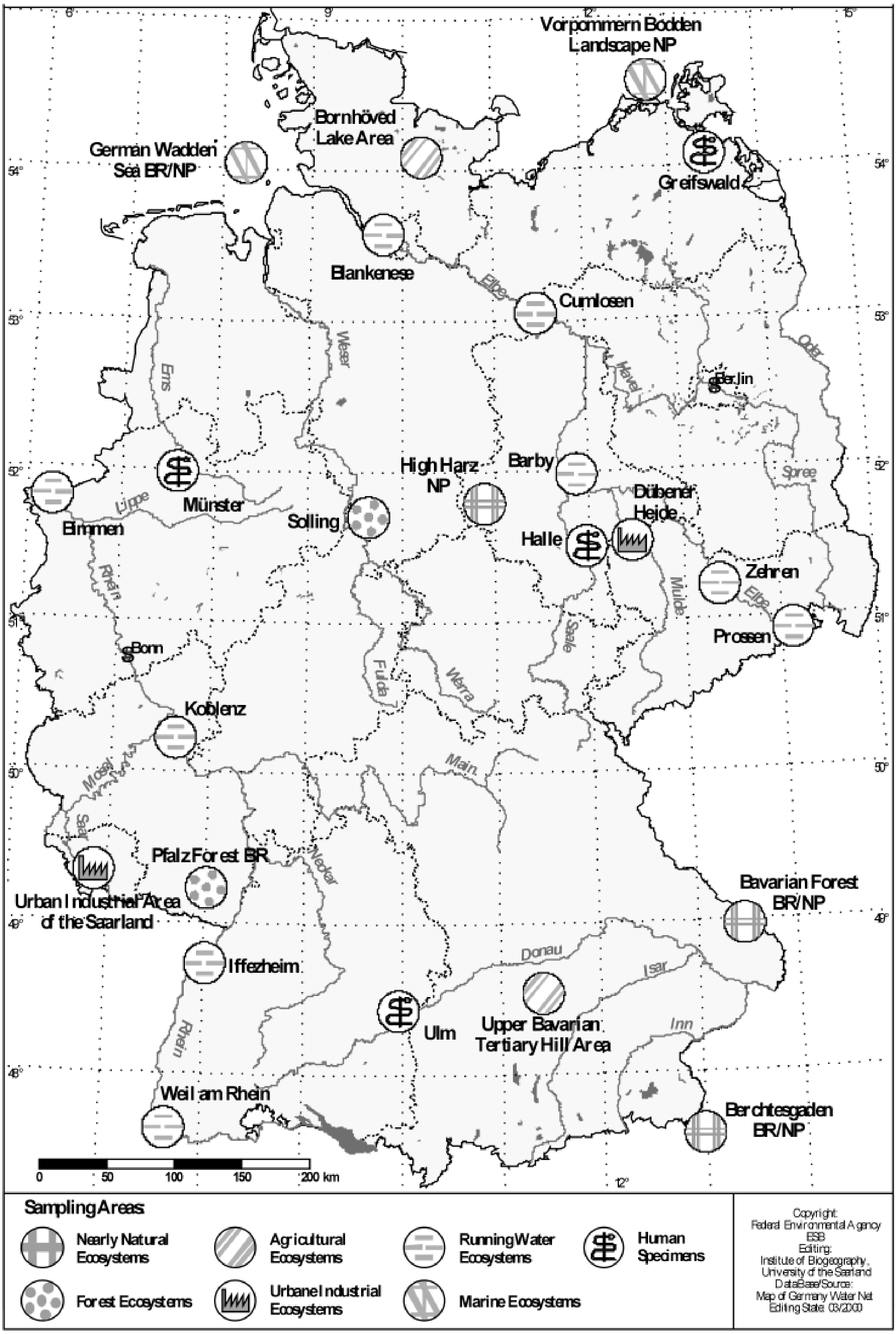

Fig. 1. Sampling areas of the German ESB Program.

analyzed for inorganic substances, chlorinated hydrocarbons and polycyclic aromatic hydrocarbons [6]. Meanwhile exist time-series of high-quality data gained from the biometric and analytical specimen characterization, which partly refer back to more than twenty years and allow for spatial comparisons as well as for the description of temporal changes of numerous environmental chemicals in Germany. Online data retrieval in all hitherto existing results is also possible for external interested parties using *www.umweltprobenbank.de*. Due to the lack of knowledge on critical tissue concentrations in the sublethal range, it is to this day hardly possible, to derive the relevancy for the organisms affected from the substance concentrations ascertained in environmental samples.

All the more complicated is it, to use them as relevance indicators for populations, biocoenoses or even entire habitats [5]. Because also in the foreseeable future no sufficient state of knowledge on critical tissue concentrations can be expected, we apply in the scope of the ESB the following assessment strategies:

- use of biomarkers,
- histopathological examinations to identify pollutant-caused tissue adaptations,
- biometric specimen characterization to demonstrate the pollutant effects on the sample-collectives,
- constitution of a species information system and use of ecological indicator groups to identify changes in biocoenoses and habitats,
- development of a reference system with analytical and biometric data.

With the selection of the above mentioned strategies is accounted for the fact, that statements concerning the effects of chemical substances on biological systems are principally testable on every level of the biological organisation – from the molecule to the biocoenosis.

Biomarkers

Biomarkers can be defined for various biological systems on lower levels of the biological organisation, at which, however, presently the establishment of appropriate and reliable markers varies depending on the system. Right this aspect provides a further starting point to integrate samples for biomarker studies already now into the ESB, because this allows for analysing them retrospectively at a later date with improved markers.

In this context, we have methods on sampling and storing of specific compartments of breams exemplarily tested. Blood plasma, the most important medium of transport, and spleen, as a representative of body tissue, were chosen. Both compartments are taken as specimens during the routinely conducted samplings in the rivers Saar, Rhine, Elbe, Mulde, Saale and Danube since 2002. Up to now, tests for vitellogenin (Vtg), as a comparatively reliable biomarker for hormonal effects, have been carried out exemplarily with the blood samples and have been checked for analogous effects on the male breams at the sampling sites of the Environmental Specimen Bank. The existence of Vtg in male breams, which normally do not build up this precursor substance of the york proteins, is regarded

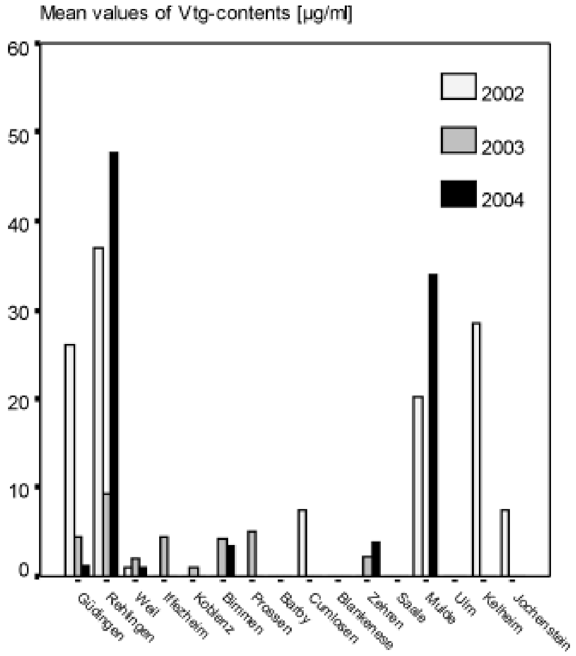

Fig. 2. Mean values of the vitellogenin concentrations in blood plasma of male breams for the years 2002–2004.

as positive proof for endocrine disrupting substances in the environment. The analyses have been carried out by means of an ELISA-Assay.

The hitherto existing results in comparison with all sampling sites are depicted in Fig. 2. Out of it appears that the male breams from both sampling sites in the river Saar (Güdingen and Rehlingen), from the sampling site in the river Mulde (region Halle–Leipzig–Bitterfeld), and from the sampling site in the Danube near Kehlheim show conspicuously increased Vtg-values. In a continuative study could be shown by Klein *et al.* [1], that these increased values are closely spatially and presumably as well causally associated with operational discharges from municipal sewage plants.

Histopathological examinations

As pointed out earlier, the analysis of vitellogenin in blood plasma of male fish provides an excellent possibility to demonstrate the existence and effect of suchlike chemicals. But from this cannot certainly be derived, whether or not the Vtg-values, found especially in the rivers Saar, Mulde, and Danube (near Kehlheim), comprise an impaired fertility. Therefore 159 gonad-specimens from the breams assayed in 2002 had been histologically examined. The formation of ovarian follicles within the male gonads is regarded as definite evidence of endocrine effects [2–4, 7]. Alongside, further structural changes had been checked. At this, it was ascertained, that only two of the animals examined, showed endocrine effects in the structure of the male gonads. But alongside, a multitude of fibrotic and necrotic appearances was found. With fibrosis is meant in this connection the presence of connective tissue strands as a cyst-like formation with amorphous content. In case of a significant development, an impaired

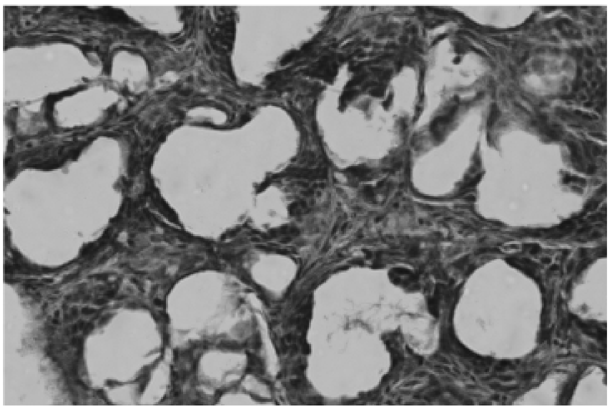

Fig. 3. Necrotic tubuli in the gonads of a male bream (*Abramis brama*) from the Danube near Kelheim.

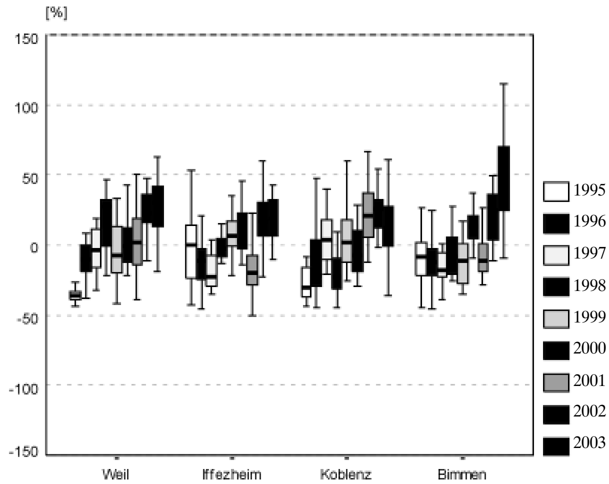

Fig. 4. Box&Whisker-Plots of the percentage deviation of the weights of breams, caught in the Rhine between 1995 and 2003, from the weights expected.

functionality of the gonads can be assumed. The same is true for a significant development of necrotic areas, as depicted in Fig. 3 with a bream from the Danube near Kehlheim.

Suchlike structural alterations were found particularly in breams from the rivers Saar, Saale, and Mulde, which indicates that the animals in these regions are exposed to a general burden by environmental chemicals, which has already led to pathological effects.

Biometric specimen characterization

The biometric specimen characterization is performed by investigations during the sampling with the aid of standardized data sheets. Biometric investigations provide irretrievable data about the effects of hazardous substances on environmental samples.
These include, for example:

- in case of the Norway Spruce (*Picea abies*), the Scots Pine (*Pinus sylvestris*), the Red Beech (*Fagus sylvatica*), and the Lombardy Poplar (*Populus nigra 'Italica'*): the proportion of necrotic needle/leave areas in the total needle/leave surface and for conifers additionally the needle coverage;
- in case of the Herring Gull (*Larus argentatus*) and the Domestic Pigeon (*Columba livia* f. domestica): the fresh weight of the eggs, the size of the eggs, the thickness of the egg shells and the herefrom derived Ratcliffe-Index;
- in case of the fish species Bream (*Abramis brama*) and Eelpout (*Zoarces viviparus*): the weight-length-relationship, the liver-body-weight-relationship (hepatosomatic index, HSI), and the relative variances in weight and length along the timeline.

Considering for example the weight- and length-variances in the Rhine, it was possible to ascertain, that, since the start of the samplings, the breams in all four sampling sites are characterized by increasing accretion (Fig. 4). In this connection it is assumed that the accretion – with a certain time delay – is suited as a measurement for the reaction of the breams to all structural and material changes in the water body. It could be revealed, that the rates of accretion and the weight-length-relationships are important indicators for the overall situation.

Species-information-system and ecological indicator groups

At present, within the German ESB program an information system is built up, which is able to gather along the timeline changes in populations and biocoenoses of selected species-groups, which serve as indicators for habitat-state and habitat-changes. Therewith it is possible to establish direct correlations to the data on chemical media, collected during the routine operation of the ESB, and to come to conclusions concerning its relevance on the biotic environment.

As indicator groups we chose the carabide beetles (Carabidae) and the vascular plants on the forest-sampling sites of the ESB, and for the limnic sampling sites the fish. Besides the description of the species numbers and the density of individuals per area, the extraction of functional indicator factors from these primary data is of great importance for the identification and assessment of changes along the timeline. To this belongs the characterization of the biocoenoses on the basis of ecological requirement types, as for example for the ground beetles on the basis of their habitat preferences, yearly rhythmicity, and flight dynamics (see Fig. 5) as well as the characterization by means of appropriate indices, for instance the "evenness", the "species-diversity", and the "species-turnover-rate".

ESB-reference-system

Owing to the lack of knowledge on critical tissue concentrations, the elaboration of reference values is a common approach in order to make an assessment system for substance concentrations in biota available. At this has to be pointed out, that reference values are not toxicologically founded, but statistically derived values, which provide a basis for the description of the general background burden. The background burden results from the circumstance, that the most environmental pollutants are ubiquitously spread in our days

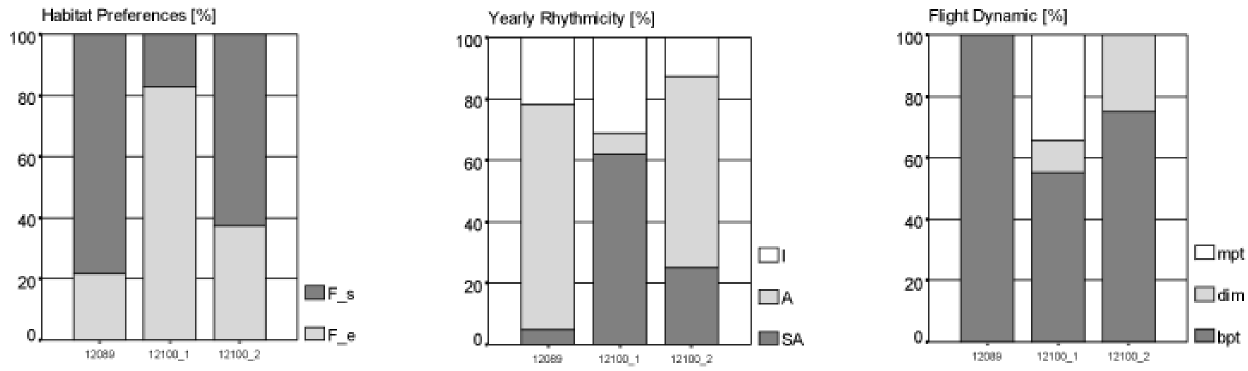

Fig. 5. Structure of the Carabide beetle coenoses in the sampling area 'Solling', on the basis of habitat preferences, yearly rhythmicity, and flight dynamics of the registered individuals.

and are traceable about the world in environmental media, in plants, animals, foodstuff, and in human-biological materials. A pollution load exceeding the background burden does not necessarily mean that this will lead already to biological alterations or even toxic reactions.

But, provided that a suitable data basis is available, the elaboration of such reference systems offer at present the only possibility of creating a direct assessment basis for substance concentrations ascertained in biota.

The Federal Environmental Specimen Bank (ESB) is targeted on an national integrating monitoring of all ecosystem compartments. By selecting sets of specimen types which incorporate all main trophic levels, the comprehensive monitoring of a broad pollutant spectrum on an ecosystematic level is possible. For this reason the data and specimens generated here, can be used as reference concentrations and reference specimens, and bring out basic assessment criteria for the interpretation of pollutant concentrations in other monitoring studies.

In 2005, for some specimen types and substance groups, first reference values had been developed and discussed at Trier University on the data basis of the Environmental Specimen Bank. The base consists of Box&Whisker-Plots, as common in the descriptive statistics, which had been compiled for the individual biometric parameters and substance groups combined for all areas under investigation in the ESB program within the selected period. At this, the latest values of the previous sampling years, which are free of temporally vectored developments and documented with data from all sampling sites, are used as reference period.

For the evaluation of the reference values is assumed, that the values within the first quartile represent the lower concentration level (low concentrations), the interquartile range with 50% of the values between the 25th and the 75th percentile is equivalent to a medial concentration level (medial concentrations), whereas the values above the 75th percentile are considered as high (high concentrations, see Table 1 and Fig. 6, exemplarily for the bream (*Abramis brama*)). For biometric data, nutrients, essential trace elements, natural potential pollutants and xenobiotics, a differentiated evaluation according to its respective importance or function is carried out.

These reference values mark the distribution of the mentioned parameters in representative areas of Germany, in which the following main ecosystem types are included: urban-industrial ecosystems, agricultural ecosystems, forest ecosystems, near-natural woodland ecosystems, limnic ecosystems, coastal ecosystems.

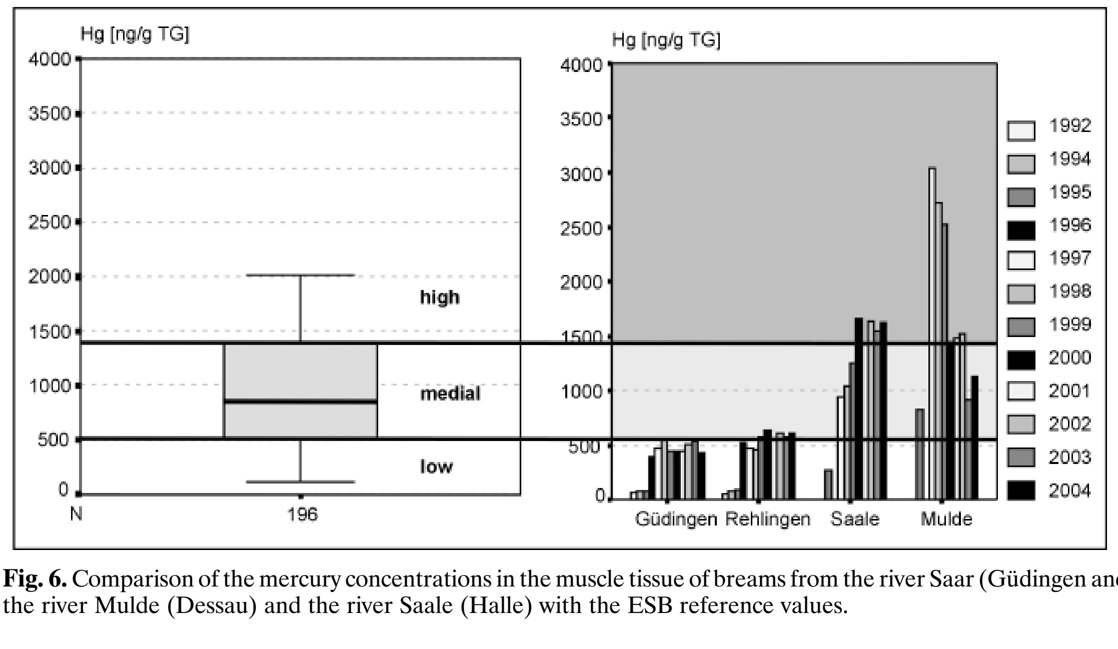

Fig. 6. Comparison of the mercury concentrations in the muscle tissue of breams from the river Saar (Güdingen and Rehlingen), the river Mulde (Dessau) and the river Saale (Halle) with the ESB reference values.

Table 1. ESB reference values for chemical constituents in the muscle tissue of breams (*Abramis brama*)

	Unit	Concentration		
		low	medial	high
Fat content	%	<2.30	2.30–5.04	>5.04
Water content	%	<77.2	77.2–79.8	>79.8
Cu	μg/g DW	<1.08	1.08–1.48	>1.48
Hg	ng/g DW	<520	520–1404	>1404
Tl	ng/g DW	<1.49	1.49–8.76	>8.76
Pb	ng/g DW	<56	56–124	>124
As	μg/g DW	<0.37	0.37–0.54	>0.54
Se	μg/g DW	<1.65	1.65–2.46	>2.46
PCB(z)	ng/g fat	<15.3	15.3–72.2	>72.2
HCB	ng/g fat	<97	97–1375	>1325
a-HCH	ng/g fat	<3.8	3.8–31.8	>31.8
g-HCH	ng/g fat	<14.8	14.8–33.3	>33.3
b-HCH	ng/g fat	<5.5	5.5–84.3	>84.3
OCS	ng/g fat	<7.3	7.3–343	>343
pp-DDE	ng/g fat	<358	358–3851	>3851
Dieldrin	ng/g fat	0.0	0.0–20.8	>20.8
pp-DDD	ng/g fat	<89	89–1569	>1569
pp-DDT	ng/g fat	<2.5	2.5–34.0	>34.0
op-DDT	ng/g fat	<12	12–200	>200
PCB 28	ng/g fat	0	0–139	>139
PCB 52	ng/g fat	<126	126–457	>457
PCB 101	ng/g fat	<319	319–767	>767
PCB 118	ng/g fat	<172	172–475	>475
PCB 153	ng/g fat	<594	594–1635	>1635
PCB 138	ng/g fat	<727	727–1931	>1931
PCB 180	ng/g fat	<327	327–925	>925

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