EFFECT-DIRECTED IDENTIFICATION OF BIOAVAILABLE TOXIC ORGANIC COMPOUNDS IN CONTAMINATED SEDIMENTS

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List of abbreviations

A	ASE
ACN	acetonitrile
ant	anthracene
ASE	Accelerated Solvent Extraction
B	Bitterfeld
b[*a*]ant	benzo[*a*]anthracene
b[*b*]fluo	benzo[*b*]fluoranthene
b[*k*]fluo	benzo[*k*]fluoranthene
b[*ghi*]per	benzo[*gh*i]perylene
b[*a*]pyr	benzo[*a*]pyrene
b[*e*]pyr	benzo[*e*]pyrene
chry	chrysene
CN	cyanopropyl silica
db[*ah*]ant	dibenzo[*ah*]anthracene
DCM	dichloromethane
DDD	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene (Dichloro-Diphenyl-Dichloroethane)
DDE	1,1-bis-(4-chlorophenyl)-2,2-dichloroethene (Dichloro-Diphenyldichloro-Ethylene)
DDT	1,1,1-trichlor- 2,2-bis- (4-chlorophenyl)ethan (Dichloro-Diphenyl-Trichloroethane)
DI	Desorption Index
DNA	deoxyribonucleic acid
dw	dry weight
EC	Effect Concentration
EDA	Effect-Directed Analysis
F	fraction
f	fluorescence
flu	fluorene
fluo	fluoranthene
F_{rap}	rapidly desorbing fraction
F_{slow}	slowly desorbing fraction
$F_{veryslow}$	very slowly desorbing fraction
HCB	hexachlorobenzene
a-HCH	alpha- hexachlorocyclohexane
b-HCH	beta- hexachlorocyclohexane
d-HCH	delta- hexachlorocyclohexane
e-HCH	epsilon- hexachlorocyclohexane
HPLC	High Performance Liquid Chromatography
HOC	Hydrophobic Organic Chemical
i[*cd*]pyr	indeno[1,2,3-*cd*]pyrene

k_{rap}	rate constant for rapid desorption [h^{-1}]
k_{slow}	rate constant for slow desorption [h^{-1}]
$k_{veryslow}$	rate constant for very slow desorption [h^{-1}]
M	Most
metho	methoxychlor
mpara	methyl-parathion
MW	molecular weight
naph	naphthalene
NO	nitrophenyl silica
P	Přelouč
pe	parent extract
PG	population growth
PGC	porous graphitized carbon
phen	phenanthrene
PNA	*N*-phenyl-2-naphthylamine
prom	prometryn
pyr	pyrene
S_0	initial concentration of sediment contaminants [µg/g(sediment)]
S_t	amount sorbed to the sediment [µg/g(sediment)] at a given time t
sed	sediment
SIM	selected ion mode
T	TENAX®
TIC	Total Ion Current
TOC	Total Organic Carbon
t_{rap_eq}	time required to remove an amount of the respective compound that is equivalent to F_{rap}

1 General introduction and objectives

The environment is increasingly affected by changes due to growing urbanisation and industrial production including industrial and private emissions, the application of pesticides in the agriculture and the discharge of waste waters by rivers for example. Once in the environment, chemicals can be distributed via air (smoke, SO_x, NO_x) and water (industrial and urban discharge, pesticides from agricultural land-use) or accumulate in soils, sediments and the food web posing a threat for the health and well-being of humans as well as of the ecosystem. Due to increasing numbers of chemicals, today about 36 million organic and inorganic chemicals are known [1], the environmental compartments are loaded with complex mixtures of numerous chemicals, whereas the environmental relevance of a chemical is determined by the mass released, prevalent conditions in the related compartments and transfer routes. Compound properties like solubility, volatility, lipophilicity as well as the acute and chronic toxicity, persistence and accumulation in food chains will strongly affect the fate and behaviour of chemicals in the environment and thus the potential to cause adverse effects [2].

In the year 2000, the Water Framework Directive became operative with the goal to improve and protect the status of aquatic ecosystems as well as to advance the sustainable use of the resource water [3]. In Germany, about 62 % of the water bodies are in an insufficient ecological and chemical status having high concentrations of priority pollutants or an altered composition of the environmental quality elements fish, algae, macrophytos and invertebrates with losses of sensitivity [4]. Anderson & Kumblad [5] stressed the importance of the benthic habitat as it concentrates the majority of the biomass and primary production in water bodies. Sediments contain highly differentiated microhabitats forming multiple ecological niches with a high biodiversity [6] being characterized by complex interactions between different species like algae, polychaetes, copepods, nematodes as well as bacteria, diatoms and plants like seaweed [7]. As a substrate for a variety of organism to live in and on, sediments are crucial as habitat providing food for aquatic vertebrates (e.g. worms and amphipods) as well as for economic important species, like lobsters, mussels and shrimps. The decomposition of organic material, nutrient cycling and transport to overlying waters as well as carbon storage are main goods and services of sediment ecosystems [8, 9]. As pelagial organisms assimilate an extensive part of their nutrient demand from benthic organisms, these two habitats are linked with respect to food webs as well as the energy flow.

Sediments are formed through erosion of agricultural land, channel banks as well as urban roads [10] consisting of fine-, medium- and coarse grain material. Surfacial run-off, deposition from the air and sewage discharge are main input routes that strongly influence the composition as well as the pollutant load of the particles. Sediments consist of a quite complex structure combining inorganic and organic components. Weathered stone, clay mineral as well as iron, aluminium and manganese hydroxide characterize the inorganic, mineral part, whereas products of plant decomposition and humic substances are main components of the organic matter [11]. The fraction as well as the linkage of clay mineral, metal hydroxides and humic substances are specific for each sediment strongly influencing the behaviour of pollutants within the sedimentary phase.

Malmqvist & Rundle [12] stated that the overriding pressure on running water ecosystems stems from the increase in the human population with concomitant increases in urban development, industry, agricultural activities as well as water abstraction, diversion and damming. The discharge of urban and industrial waste waters, the extensive agricultural land-use in catchment areas and accidentally releases lead to increasing concentrations of pollutants and nutrients over the past century. With respect to the composition of sediment, three general ways of interaction between the solid phase and an organic molecule can occur: (i) partitioning into the natural organic phase, (ii) intermolecular forces, like van der Waals-, dipole-dipole-forces or hydrogen bonding, lead to adsorption of organic molecules on the mineral surface and (iii) chemical sequestration if the solid surface and the organic molecule have reactive groups (surfacial carbonyl groups and amino groups of the molecule) [13] leading to a retention of these substances. Depending on the physico-chemical properties of the compound and the solid phase, sediments can concentrate pollutants and act as a sink for a broad range of compounds.

According to the Water Framework Directive (WFD) European rivers should achieve a good ecological status until 2015. Therefore, the assignment of effects in aquatic ecosystems to those stressors that cause the effects is crucial to achieve the goals of the WFD [14]. Environmental samples can contain a broad range of substances making it impossible both to identify all contaminants and to link the presence of individual compounds to observed adverse effects. Therefore, the sample complexity can be reduced stepwise with the goal to separate toxic from non-toxic components and link observed effects to the presence of individual substances. That is what is normally done in an Effect-Directed Analysis (EDA) [15] and was used to answer the question:

What contaminants contribute to observed adverse effects of sediment extracts from river Elbe basin towards green algae *Scenedesmus vacuolatus*?

Sediments act as sinks for a broad range of pollutants generated by human activities like pesticides or polycyclic aromatic compounds [16-18]. But the total concentration of sediment contaminants is a poor predictor of actual risk and toxic effects [19] since a fraction of contaminants is strongly bound on sediment organic matter and thus not available for the uptake into organisms. Bioavailability is a complex process including desorption, diffusion and partitioning processes. Sediment, compound as well as biota properties determine the bioavailability of sediment contaminants resulting in a specific dose in biota at a given concentration in sediment. The concept of bioaccessibility was introduced as a reasonable simplification. Being independent of biota properties and behaviour the bioaccessible fraction solely encompasses that fraction that might desorbs within a selected time frame of hours or days [20]. TENAX®, an organic polymer, is commonly used to selectively trap the bioaccessible fraction of small amounts of sediment (normally 1 g dry weight) [21, 22]. To introduce the concept of bioaccessibility into an EDA for a more realistic risk assessment of contaminated sites a greater amount of sediment needs to be extracted for subsequent fractionation steps, biotesting and chemical analysis. Chapter 3 wants to answer the question:

How can high quantities of sediment be extracted with TENAX® to gain a sufficient amount of sample for a subsequent EDA?

In fresh sediments chemical and microbiological degradation processes occur causing false-positive as well as false-negative results when determining the ecotoxicological risk on the basis of bio assays. Freeze-drying is one common method for sediment conservation, but known to change the contaminant bioaccessibility. Chapter 4 deals with the question:

Does freeze-drying influence the desorption behaviour and thus bioaccessibility of sediment-associated contaminants as determined by TENAX® extraction?

Bioaccessibility is assumed to have a great influence on the prioritization of contaminated sites with respect to posed risk. The TENAX® extraction method was shown to be suitable to selectively extract the bioaccessible fraction. As EDA is a valuable

approach to identify causes of adverse effects, an EDA was performed on the basis of TENAX® derived extracts of three sediment samples to answer the question:

Does the introduction of the concept of bioaccessibility into an EDA influence the prioritization of contaminated sites with respect to the posed risk?

2 Effect-Directed Analysis of sediment-associated algal toxicants at selected hot spots in the river Elbe basin

2.1 Introduction

Sediments often contain complex mixtures of toxicants that might pose a risk to ecosystem and human health. Investigations of the hazard originating from environmental samples are often based on known and a priori selected contaminants such as the priority pollutants defined by the Water Framework Directive [23]. This has the disadvantage to overlook toxicants beyond the target list. Non-target analysis of environmental samples enables the investigation of a broad range of substances, but is time consuming and laborious due to the multitude of substances. Even if all substances are identified, in the most cases no toxicological information is available.

Biological analysis is an established approach to assess the (eco)toxicological hazard of environmental samples since it provides an integrative parameter covering the effects evoked by all compounds contained. However, no conclusions on responsible toxicants can be drawn. That means for example, required remediation procedures can not be chosen solely on the basis of biotesting without knowledge about the relevant compounds. These facts clearly reflect the limitations of chemical and biological methods to correlate causes and effects when used individually.

The Effect-Directed Analysis (EDA), an approach that appeared for the first time in the early 1980s, comprises physico-chemical fractionation, biological testing and chemical analysis (Figure 2-1). The concept of an EDA is based on a sequential reduction of the complexity of toxic mixtures, generally using chromatographic fractionation procedures to separate toxic from non-toxic compounds. The response of a bio assay is used to direct the analytical pathway towards identifying the chemical compounds causing the observed response [24].

EDA of environmental samples has been shown to be a valuable approach for the identification of contaminants in the environment that exhibit toxic effects to a multitude of organisms [15, 16, 25]. Applying such a procedure poses the possibility to compare pollution patterns and to identify individual toxicants causing measurable effects. This helps to identify emission sources and to explain impacts on ecosystems [26, 27].

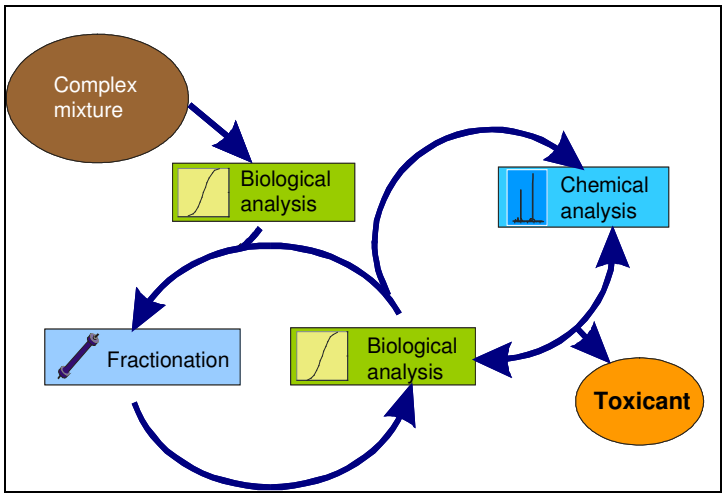

Figure 2-1: *Scheme of an Effect-Directed Analysis according to Brack [15].*

The confirmation of identified toxicants, as last step of an EDA, is crucial to assure that the identified compounds in fact cause the observed effects. The responsible compound might be present below the detection limit or masked by other compounds requiring a conclusion on the contribution of identified toxicants to the overall toxicity [28]. The confirmation may be based on the recently developed Index of Confirmation Quality (ICQ) comparing the sample toxicity with the toxicity of a corresponding artificial mixture or joint effect predictions over the whole range of effect levels [29].

Benthic algae are the dominant primary producers in many rivers [30]. They play a key role in river ecosystems as the basis of the aquatic food webs but also by impacting the mobility of nutrients and contaminants [31]. Benthic algae are in direct contact to sediments, which are a sink for a broad range of environmental toxicants. Since algae are highly sensitive to many toxicants [32] this multiple exposure situation may pose a risk to primary production.

The aim of the present study was to identify major algal toxicants in three sediments from industrial areas of the river Elbe. Therefore, the work was organized in 3 steps. Firstly, the sediments were extracted with Accelerated Solvent Extraction (ASE) and fractionated on three coupled normal-phase HPLC columns to reduce the complexity of the samples. In the second step, parent extracts, HPLC fractions as well as recombined samples were tested for adverse effects using unicellular green algae *Scenedesmus vacuolatus* as test organism. Thirdly, chemical analysis of effective fractions was performed to provide information about the identity and quantity of potential toxicants. On that basis, artificial mixtures were prepared and tested in the algae assay to confirm the identified toxicants.

2.2 Material and Methods

2.2.1 Sediment extraction and clean up

Freeze-dried sediments from sampling sites at Přelouč (P), Most (M) and Bitterfeld (B) were extracted using Accelerated Solvent Extraction (ASE) at 50 °C and 103 bar with acetone and dichloromethane (25:75 v/v). The volume of the extracts was reduced by the means of rotary evaporation. Elemental sulphur and macromolecular matrix components were separated applying gel permeation chromatography (GPC). Prior to cleaning of the sediment extracts blanks were run and checked for background disturbance. A detailed description of the extraction and clean up procedure can be found in chapter 6.2.1 and 6.2.3, respectively. For cleaned raw extracts the term parent extracts (pe) is used.

2.2.2 Fractionation

The parent extracts were fractionated on coupled cyanopropyl silica (CN), nitrophenyl silica (NO) and porous graphitized carbon (PGC) HPLC columns [33]. The separation is based on the polarity (CN-column), the number of aromatic rings (NO-column) as well as the degree of chlorination and planarity (PGC-column) of the compounds. For further details see chapter 6.2.4.

2.2.3 Biological analysis

Blanks, parent extracts, fractions and recombined extracts of the fractions were tested for the inhibition of a synchronous culture of unicellular green algae *Scenedesmus vacuolatus* in growth. Samples were provided in dimethyl sulphoxide (DMSO) with a maximum concentration of 0.1 % in the test system, which proved to be of no detectable effects. The toxicological endpoint was the inhibition of population growth during one generation cycle lasting 24 h according to the procedure described elsewhere [34]. Slight modifications were made using fluorescence measurement at t_0 and t_{24h} (Backscat Fluorometer, Haardt, Kiel, Germany) instead of cell counting. To correct for background fluorescence due to sample components, samples without algae were measured as well. The exact test procedure and conditions are described in chapter 6.3.

For effective samples complete concentration-response curves were recorded. Therefore, geometric dilution series of the DMSO samples were prepared exhibiting effects between 10 and 90 % inhibition described with at least 4 data points. Experimental

data of tested extracts and fractions were fitted using a three parameter Hill function (Eq. 2-1), which assumes a logistic distribution of the data.

$$E = \frac{a}{1 + \left(\frac{x_{50}}{c} \right)^{p}}$$

Eq. 2-1

E denotes the fractional effect ($0 \leq E \leq 1$) and c the concentration (g SEq/L(medium)). The parameters of the models (a, x_{50}, p) were estimated using SigmaPlot 10.0 software (Systat Software Inc., San José, CA, USA). The fitting was based on the Marquardt-Levenberg algorithm.

2.2.4 GC-MS analysis

Compounds were identified and quantified using a gas chromatograph equipped with a mass selective detector (GC-MS). The oven was programmed from 50 to 300 °C with 4 °C/min holding this temperature for 6.5 min. The identification of unknown compounds was performed in total ion current (TIC) modus on the basis of retention time and mass spectra of respective standard compounds. Standard compounds were purchased from Fluka (Buchs, Switzerland), Aldrich (Deisenhofen, Germany), Riedel-de Haen (Seelze, Germany), Chiron (Trondheim, Norway) and Promochem (Wesel, Germany) in purest quality and are listed in the appendix. Quantification was performed in selected ion mode (SIM) on the basis of an external calibration. A detailed description of the analytical method can be found in chapter 6.2.5.

2.2.5 Confirmation

Confirmation of the identified toxicants was performed on the basis of artificial mixtures prepared according to the identified compounds and their concentrations. Complete concentration-response relationships were prepared and compared to those of original fractions. 95 % confidence intervals were estimated using SigmaPlot Software.

To obtain a quantitative measure of the agreement between the efficiency of original fractions and their artificial mixtures, the Index of Confirmation Quality (ICQ) was calculated applying an approach on the basis of complete concentration-response curves rather than of single values [29]. The ICQ value demonstrates the ratio of the

effective concentration of the original fraction ($EC_x^{original}$) and the artificial mixture (EC_x^{mix}) at one effect level x (Eq. 2-2).

$$ICQ = \frac{EC_x^{original}}{EC_x^{mix}}$$

Eq. 2-2

The index x ranges from 10 % inhibition to the maximal observed effect of the respective fraction. Effective concentrations were calculated as described in chapter 6.3.

2.3 Results

2.3.1 Parent extracts

Parent extracts (pe) from Přelouč (P), Most (M) and Bitterfeld (B) inhibited algal growth when tested in concentrations between 0.1 and 150 g SEq/L(medium) (Figure 2-2). A concentration-dependent response could be observed for all three samples varying in the position, the slope and the maximal observed effects of the curves. Sediments of Přelouč and Most were effective in the same concentration range, whereas effective concentrations of Bitterfeld extracts were one order of magnitude greater. This was also reflected by the calculated EC_{30} values (Table 2-1) of 1.78 g SEq/L (Přelouč), 6.56 g SEq/L(medium) (Most) and 55.89 g SEq/L(medium) (Bitterfeld). As the curve steepness was characterized by the p-value of the Hill function, M^{pe} evoked the steepest concentration-response curve with a p-value of 0.62, whereas curves of P^{pe} and B^{pe} were with p-values of 0.50 and 0.42 plainer (Table 2-1).

Maximal observed effects were lower than 100 % in all cases accounting only for 46 % (P^{pe}), 69 % (M^{pe}) and 41 % (B^{pe}), respectively. Testing parent extracts at higher concentrations was not reasonable since the sample solubility would have been exceeded in DMSO as well as in the water phase. The biological analysis of parent extracts should provide information about the ecotoxic potential of the tested sample and the concentration range of interest. The recording of complete concentration-response curves, including the plateau phase at upper end, was not necessary.

Experiments were designed to describe effects between 10 and 90 % if possible and run in triplicate to ensure the statistical safety of the data. The highest standard deviations account for 15 % (P^{pe}), 27 % (M^{pe}) and 17 % (B^{pe}) when considering only effects greater than 10 % inhibition.

Main characteristics of concentration-response curves are their position and steepness. Fitting enables the accurate mathematical description of the curves and thus the comparison of certain curves on the basis of characteristic parameters such as EC_{50} and slope-values (p). Several models exist being capable to describe a curve in the high effect domain, the steepest section or the low effect domain. For the present work the accurate description of the steepest section of the curve rather than the exact modelling of the low or high effect domain should be achieved. As can be seen in Figure 2-2, the Hill model was able to accurately describe the experimental data.

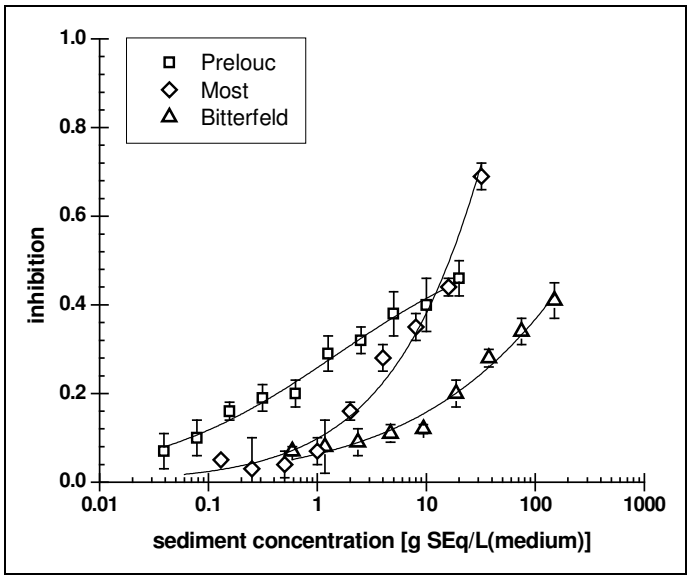

Figure 2-2: *Inhibition of algal growth induced by the sediment extracts of Přelouč, Most and Bitterfeld. Mean values and standard deviations are given as symbols and error bars, respectively. Fitted functions are presented as solid curves.*

The observed algal growth inhibition revealed the presence of toxicants. For a correlation of observed effects and sample components, parent extracts were analysed with GC-MS. Figure 2-3 presents the chromatogram of Přelouč parent extract as an example. Complex chromatograms were detected containing hundreds of peaks. Such a chromatogram clearly illustrated the limitations of chemical analytical methods to establish cause-effect relationships when used exclusively. The identification as well as quantification of all components was impossible bearing the risk to overlook toxicants especially if they were present only at low concentrations. Further steps were necessary to reduce the complexity of the parent extracts to successfully correlate observed effects and compounds present.

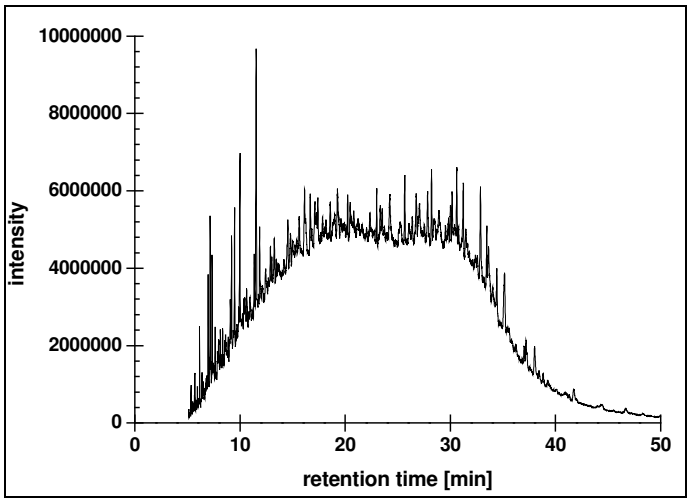

Figure 2-3: *GC-MS chromatogram of Přelouč parent extract measured in TIC mode.*

2.3.2 Biological analysis of fractions

Parent sediment extracts of the different sampling locations were separated into 18 fractions characterized by the substance classes listed in Table 6-2.

Algal reproduction was mainly inhibited by F8 to F11 (Figure 2-4) typically containing PAHs and nonpolar polycyclic aromatic compounds, whereas for fractions coeluting with chlorinated compounds (F2 to F5), small ring PAHs (F6 and F7) and more polar compounds (F13 to F18) effects were generally lower than 30 %. Only P15 and B14 inhibited algal reproduction about 40 % and 80 %, respectively. P10, M10 and B14 are the most toxic fractions for the respective sampling location exhibiting effects of 88 %, 72 % and 80 %, respectively. Effects evoked by these fractions were higher than effects of the related parent extracts of 47 % (Přelouč), 72 % (Most) and 43 % (Bitterfeld) tested at same concentrations.

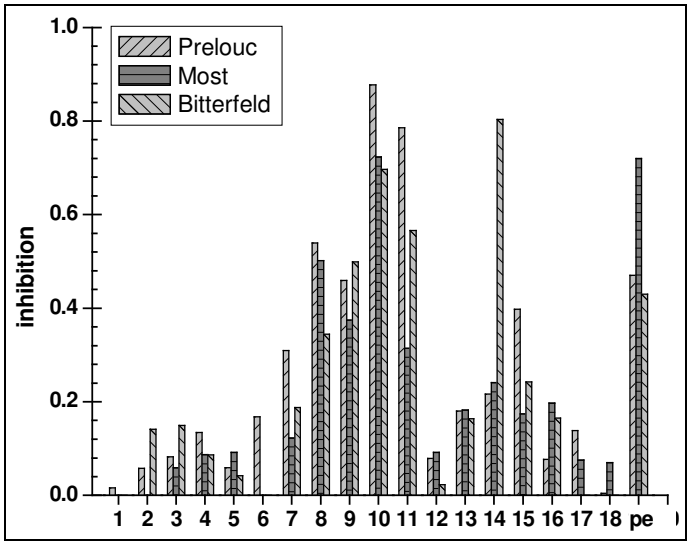

Figure 2-4: *Screening of HPLC fractions 1 to 18 of Přelouč, Most and Bitterfeld sediment concerning the growth inhibition of Scenedesmus vacuolatus. Fractions were tested in the following concentrations: Přelouč (32 g SEq/L(medium)), Most (32 g SEq/L(medium)) and Bitterfeld (156 g SEq/L(medium)). Effects of the related parent extracts (pe) are given as well tested at same concentration like the fractions.*

Testing the fractions at one concentration yielded a relative ranking of the fractions with respect to their toxicity. Based on these results, for F8 to F11 of all three sampling locations, P15 as well as B14 complete concentration-response relationships were prepared. A 1:2 dilution series was sufficient to have at least 4 data points between 10 % and the maximal observed inhibition. Only for B14 a 1:1 dilutions series was necessary to accurately describe the concentration-response relationship. These fractions were subjected to chemical analysis as well for an identification of potential toxicants.

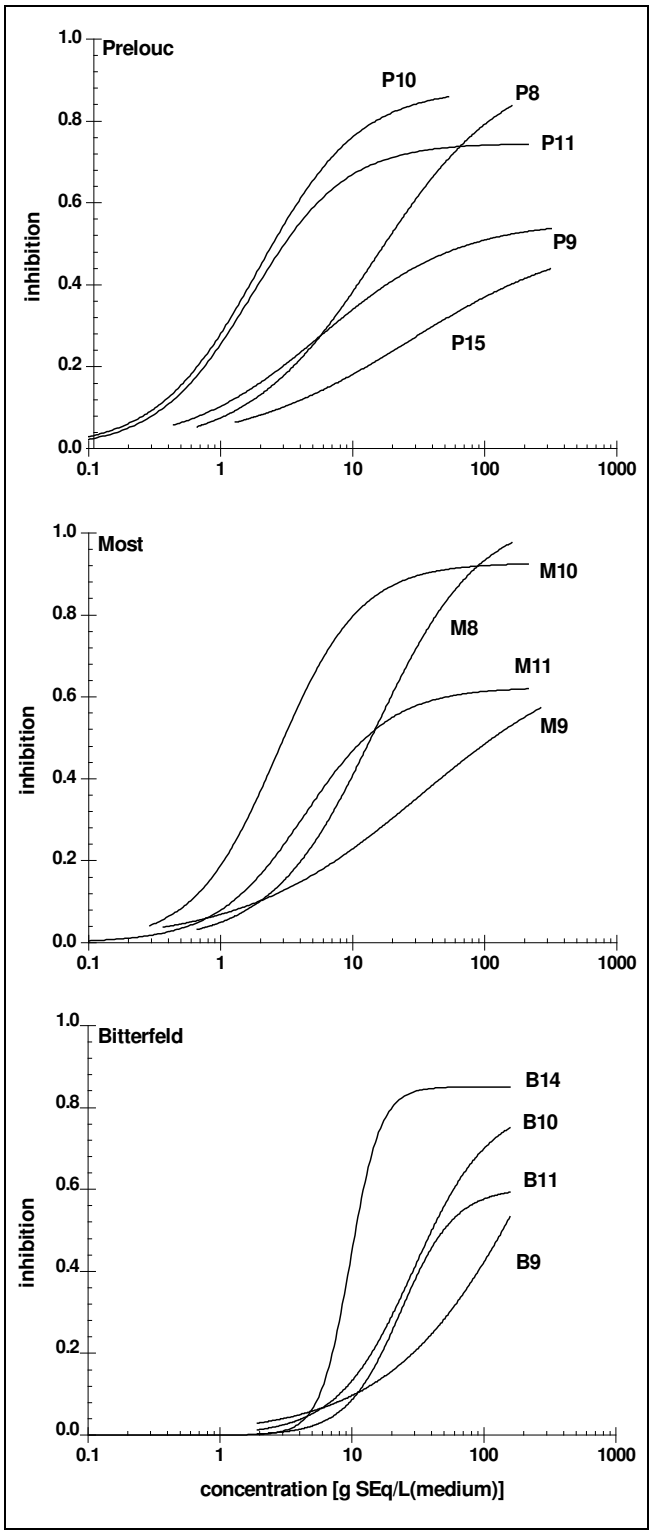

Figure 2-5: *Concentration-response relationships of HPLC fractions that proved to have an inhibition greater than 30 % in the former screening test. Fitted curves on the basis of the Hill function are presented.*

All fractions exhibited sigmoid concentration-response relationships when tested in concentrations between 0.1 and 1000 g SEq/L(medium) differing in the position, the steepness and the maximal observed effects (Figure 2-5). Přelouč and Most fractions were

effective in the same concentration range, whereas the effective concentrations of Bitterfeld fractions were one order of magnitude greater. With p-values of 3.79 and 0.64, as a numerical value of the curve steepness, the steepest curve was recorded for B14, whereas M9 evoked the plainest curve (Table 2-1). The maximal effects ranged from only 54 % (P9) to 95 % (M8).

Most toxic fractions on the basis of the calculated EC_{30} values were P10 (1.09 g SEq/L(medium)), M10 (1.57 g SEq/L(medium)) and B14 (8.27 g SEq/L(medium)) (Table 2-1).

Table 2-1: *Concentration-response relationships of parent extracts (pe,) recombined samples (remix) and effective fractions with respect to algal growth inhibition. Experimental data were fitted using Hill function (Eq. 2-1) showing the square of correlation coefficient (R^2), the concentrations [g SEq/L] inhibiting algal growth about 30 % (EC_{30}), the slope of the curve (p), the maximal observed inhibition (a) as well as the number of data points (N) and controls (C).*

	N (C)	EC_{30} [g SEq/L]	a	p	R^2
Přelouč					
pe	30 (17)	1.78	0.57	0.50	0.93
remix	20 (17)	2.65	0.60	0.71	0.96
P8	18 (12)	6.53	0.94	0.90	0.99
P9	21 (12)	7.06	0.56	0.83	0.95
P10	23 (12)	1.09	0.88	1.14	0.97
P11	25 (18)	1.23	0.75	1.22	0.97
P15	18 (12)	41.28	0.53	0.65	0.98
Most					
pe	24 (18)	6.56	4.55	0.62	0.95
remix	18 (6)	8.72	0.43	1.60	0.96
M8	18 (12)	6.56	1.05	1.11	0.96
M9	20 (12)	19.37	0.72	0.64	0.90
M10	21 (12)	1.57	0.93	1.38	0.99
M11	24 (12)	4.08	0.62	1.31	0.95
Bitterfeld					
pe	27 (16)	55.89	2.11	0.42	0.95
remix	18 (5)	39.47	0.45	1.12	0.93
B9	13 (12)	55.04	1.49	0.76	0.96
B10	15 (12)	20.69	0.81	1.51	0.98
B11	14 (12)	23.28	0.60	2.11	0.98
B14	21 (18)	8.27	0.85	3.79	0.96

To exclude toxic procedural artefacts and procedural losses, sub samples of F1 to F18 were recombined (remix) and tested. Remix EC_{30} values were in good agreement with that of the related parent extracts deviating not more than a factor of 2. Only p values,

characterizing the slope, indicated steeper concentration-response curves for the recombined samples.

2.3.3 GC-MS analysis of effective fractions

The fractionation procedure enables the separation of certain substance groups as can be seen in Figure 2-6. For example, P10 includes PAHs with a mass of 252. As only few peaks were detected revealing that the complexity of the parent extract was significantly reduced by the fractionation step.

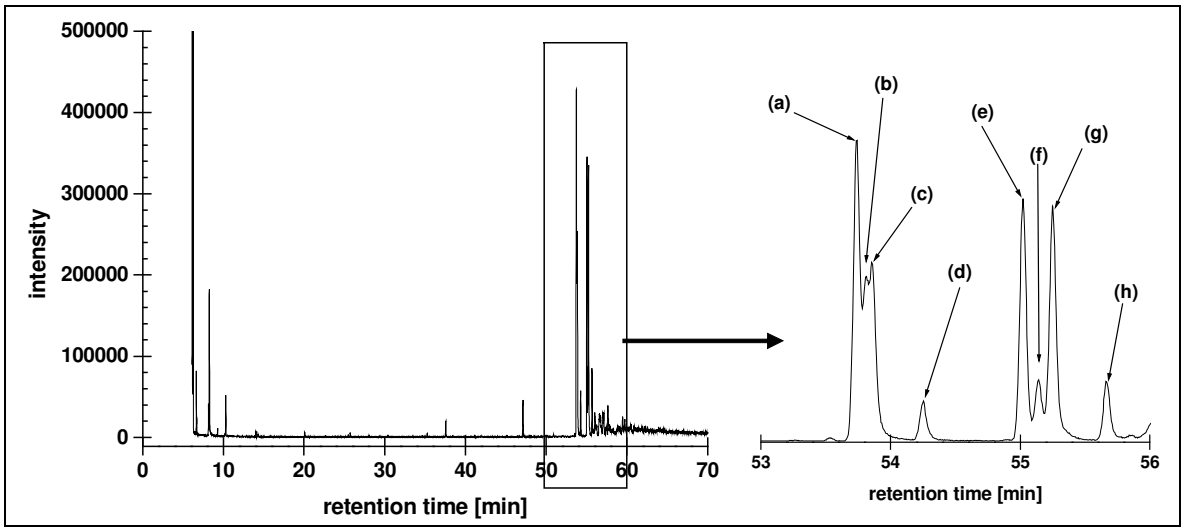

Figure 2-6: *GC-MS chromatogram of P10 measured in SCAN mode. The detail on the right side presents the identified compounds: (a) benzo[b]fluoranthene, (b) benzo[j]fluoranthene, (c) benzo[k]fluoranthene, (d) benzo[a]fluoranthene, (e) benzo[e]pyrene, (f) benzo[a]pyrene D12 (internal standard), (g) benzo[a]pyrene, (h) perylene.*

Chemical analysis in fractions 8 to 11 detected PAHs with increasing number of aromatic rings as well as polyaromatic oxygen and sulphur heterocycles (Table 2-2) well in agreement with the compounds expected on the basis of the fractionation procedure (Table 6-2). *N*-phenyl-2-naphthylamine was found as a main constituent of B14. This compound was identified in former investigations as one main contaminant in Bitterfeld sediment being specifically toxic to green algae [16]. Additionally, in P15 7H-benzo[*de*]anthracen-7-one was identified.

Table 2-2: *Identified compounds and respective concentrations [µg/g] in the effective fractions. Concentrations refer to dry weight of the sediment.*

		Přelouč **[µg/g]**	***Most*** **[µg/g]**	***Bitterfeld*** **[µg/g]**
F8	**fluoranthene**	1.773	1.098	0.136
	pyrene	0.882	1.161	0.117
	benzo[*b*]naphtho(2,3-*d*)furan	./. [(a)]	0.043	./.
	11H-benzo[*b*]fluorene	0.236	0.135	0.015
	benzo[*b*]naphtho(1,2-*d*)thiophene	0.051	0.025	./.
	benzo[*b*]naphtho(2,3-*d*)thiophene	0.046	0.018	0.003
F9	**benzo[*ghi*]fluoranthene**	0.044	0.104	0.024
	benzo[*a*]anthracene	0.859	0.482	0.060
	chrysene	1.103	0.622	0.077
F10	**benzo[*b*]fluoranthene**	0.842	0.475	0.071
	benzo[*j*]fluoranthene	0.373	0.226	0.029
	benzo[*k*]fluoranthene	0.406	0.194	0.027
	benzo[*a*]fluoranthene	0.158	0.104	0.013
	benzo[*e*]pyrene	0.644	0.386	0.047
	benzo[*a*]pyrene	0.664	0.433	0.054
	perylene	0.177	0.172	0.014
F11	**indeno[1,2,3-*cd*] fluoranthene**	./.	0.011	./.
	indeno[1,2,3-*cd*] pyrene	0.442	0.246	0.049
	benzo[*ghi*]perylene	0.516	0.015	0.050
	dibenzo[*ah*]anthracene	./.	0.360	./.
F14	***N*-phenyl-2-naphthylamine**	./.	./.	0.586
F15	**7H-benzo[*de*]anthracen-7-one**	1.178	./.	./.

[(a)] ./. means not detected

Přelouč fractions contained polycyclic aromatic compounds in concentrations of 0.04 to about 1.8 µg/g sediment (dry weight) (Table 2-2). The concentrations in Most fractions were quite similar to those in Přelouč. Bitterfeld fractions contained the smallest concentrations of PAHs with 0.003 to 0.1 µg/g (dry weight).

Greatest concentrations in all three samples were found for fluoranthene, pyrene and 7H-benzo[*de*]anthracen-7-one (only Přelouč) followed by chrysene, benzo[*b*]fluoranthene, benzo[*a*]pyrene and benzo[*e*]pyrene with 0.4 to more than 1 µg/g (dry weight) for Přelouč and Most fractions. Concentrations in Bitterfeld fractions were generally about one order of magnitude smaller.

2.3.4 Confirmation

To confirm the correlation between the measured effects and the identified compounds, artificial mixtures composed as shown in Table 2-2 were prepared and tested. Grote et al. [29] introduced the Index of Confirmation Quality (ICQ) (Eq. 2-2) as a quantitative measure of the similarity or dissimilarity between effects of the original sample and an artificial mixture for effect levels greater than 10 % inhibition (Figure 2-7). If smaller effects should be confirmed as well, it has to be ensured that the fitting model is able to accurately describe the experimental data in the low effect range.

Three cases can occur: (i) ICQ values equal 1 indicating a full accordance of concentration-response curves of the artificial mixture and the original sample and thus a full confirmation of identified toxicants, (ii) ICQ values smaller than 1 indicating that the artificial mixture was less toxic than the sample leaving unexplained toxicity and (iii) ICQ values greater than 1 indicating that the artificial mixture was more toxic. The vertical line at 1 marked the effect of the original sample and thus the toxicity that has to be explained.

Observed ICQ values covered a range from 0.15 to 5.06 (Figure 2-7) reflecting all three former mentioned cases. For P8, P9, P11, M8, B9 and B14 having ICQ values between 0.15 and 2.21, the majority of the ICQ values was smaller than 1 indicating a lower toxicity in the artificial mixture as compared to the original sample. With the exception of P11 and B14, the calculated ICQ values depended on the considered effect level and equalled 1 only at high effect levels. The opposite hold for B10 with ICQ values of 0.86 to 2.85. For P10, P15 and M9 to M11 the ICQ values covered a range from 0.45 to 5.06 indicating an underestimation at low effect levels as well as an overestimation at high effect levels. High ICQ values of 4.77, 4.96 and 5.06 as achieved by P15, M10 and M11, respectively were caused by an ongoing rise in effects caused by the artificial mixture, while effects caused by the original sample increase just indiscernible. With ICQ values between 1.3 and 3.3, the concentration-response curve of the artificial mixture of B11 is completely shifted to lower concentrations overestimating the toxicity of the original sample.

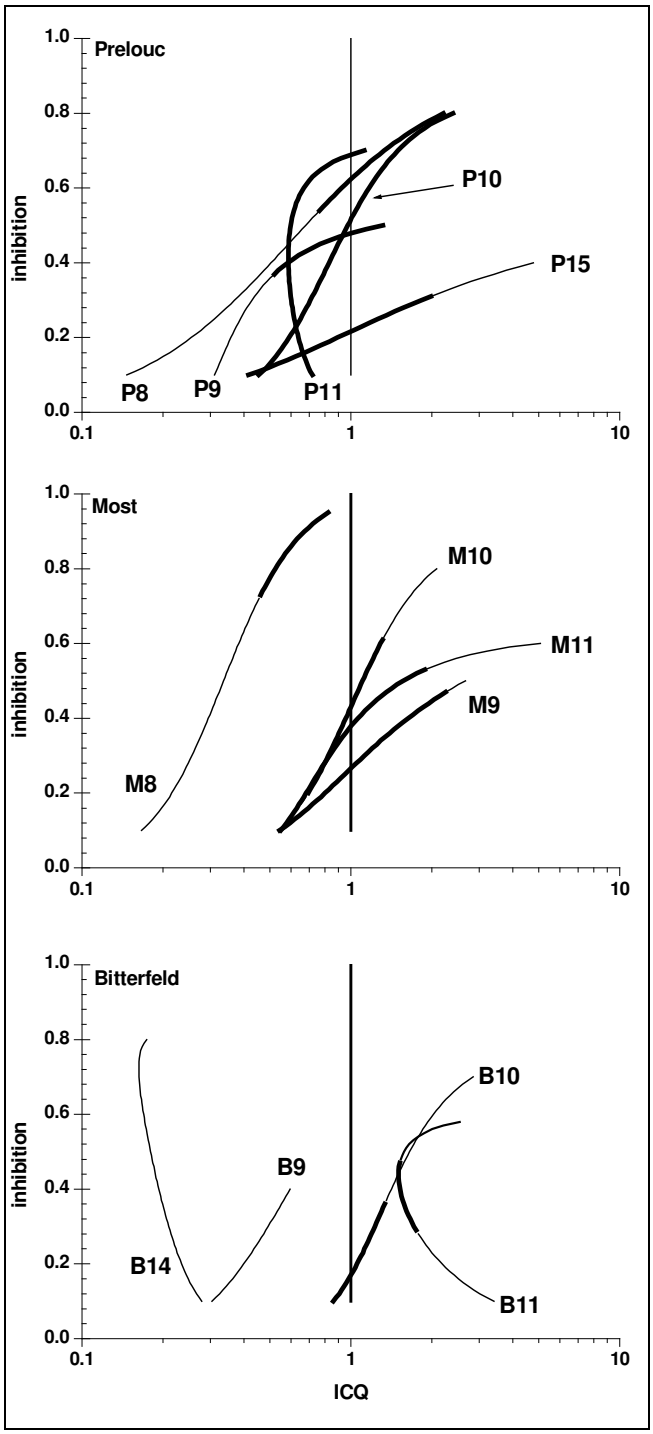

Figure 2-7: *Index of Confirmation Quality (ICQ) for the tested artificial mixtures of effective ASE fractions of Přelouč, Most and Bitterfeld location. The vertical line at 1 marks the original sample presenting the toxicity, which has to be explained. Bold intercepts denote domains of related concentration-response curves having overlapping 95 % confidence intervals suggesting no significant differences between the efficiency of compared samples. The grey boxes indicate the ICQ range of 0.5 to 2 assuming a successful confirmation.*

The evaluation of ICQ values is nontrivial, since no criterion exists to correlate the accordance of concentration-response curves with calculated ICQ values and thus the

success of a confirmation procedure. In agreement with Arrhenius et al. [35] it was suggested that a mixture of neat standards simulating the composition of a sample did not fully explain the effect of a sample if concentration-response curves of the sample and the artificial mixture were significantly different, i.e. 95 % confidence intervals did not overlap. Since this overlap could depend on the effect level under consideration (like P8, M8), the decision whether all relevant toxicants had been identified depended on the effect level as well. If the overlap of confidence intervals was translated to the ICQ curves (Figure 2-7) bold lines indicated "confirmed" while hair lines indicated that significant contributions to toxicity remained unexplained. Despite the sample and experiment specificity of ICQ ranges, the results suggested that a domain of ICQ > 0.5 can be regarded as a reasonable overall estimate for overlapping 95 % confidence intervals. Thus, an ICQ > 0.5 was defined as "toxicants confirmed" while an ICQ < 0.5 indicated "significant toxicity unexplained".

For all fractions ICQ values depended on the effect level. With the exception of P11, B11 and B14, ICQ plots indicated that the portion of effect that can be explained with the identified toxicants increased with the effect levels. Based on the settings above, identified toxicants were regarded as confirmed for P10, P11 and P15 over the whole range of effect levels and in P8 and P9 for effect levels above 40 % inhibition. At lower effect levels significant portions of effects cannot be explained by the identified toxicants. In Most sediment extract toxicity of fractions M9 to M11 could be explained by the identified toxicants while M8 toxicity can only be explained at high effect levels above an inhibition of 70 %. In the case of Bitterfeld, the identified toxicants in B10 and B11 were confirmed to be responsible for the measured effect, while in B9 and B14 a significant contribution of non-identified compounds had to be assumed. For M10, M11, B10 and B11 artificial mixtures of identified toxicants seemed to overestimate the toxicity of the fractions at high effect levels.

2.4 Discussion

Sediments taken from three hot spots of the river Elbe basin were exhaustively extracted, fractionated as well as biologically and chemically analysed to identify major algal toxicants. It was shown that polycyclic aromatic compounds as well as heterocycles containing oxygen and sulphur were responsible for observed effects of all three locations. That is consistent with results of several groups identifying PAHs as major pollutants in Elbe sediments [36, 37]. Site-specific toxicities were detected with *N*-phenyl-2-

naphthylamine and 7H-benzo[*de*]anthracen-7-one in Bitterfeld and Přelouč sediment, respectively. *N*-Phenyl-2-naphthylamine has been used as a stabilizer for organic polymers with specific toxicity to algae that was suggested to act intracellular as a reactive compound in cell membranes [38]. 7H-benzo[*de*]anthracen-7-one as identified in P15, was detected in atmospheric aerosols, too [39] and assumed to be formed during combustion processes. Furthermore, that compound occurs as an intermediate for the synthesis of vat and disperse dyes [40]. The facts that Přelouč is known for dye production and P15 is deeply red coloured strengthen the identification of 7H-benzo[*de*]anthracen-7-one. Concerning human toxicity it is known to cause anaemia [41], but data with respect to its ecotoxic potency is rare.

Interestingly, effects of the most toxic fractions P10, P11, M10, B10, B11 and B14 were higher than effects caused by the respective parent extract (Figure 2-4). It was shown that the separation of interacting compounds, as achieved by the fractionation, can lead to an enhanced toxicity compared to the unfractionated samples [42]. Toxic procedural artefacts were excluded by testing the recombined samples (Table 2-1). The lower toxicity of parent extracts compared to fractions thereof may be caused by the following reasons.

Firstly, matrix components, as contained in the parent extracts, might cause a complexation of pollutants being responsible for a delayed up take as well as a reduced accumulation [43, 44] causing either a reduced slope or an effect shift to higher concentrations. Additionally, the biological analysis of parent extracts is problematic due to their high background fluorescence hampering fluorescence analysis.

Secondly, according to Raoult's Law, the solubility of a compound present in an ideal mixture is equal to the solubility of the pure compound multiplied by its mole fraction in the mixture. Fractionation increases the mole fraction of the components remaining in the mixture by removing others. Thus, their solubility is increased. In cases where solutions are close to saturation for some of the toxic compounds, this may enhance toxicity. The application of Raoult's Law on the dissolution of mixtures of lipophilic compounds in water was shown by Ghosh et al. [45].

Thirdly, matrix components are known to reduce the bioavailability of toxicants in aqueous test systems [44]. Fractionation might remove these matrix compounds causing a higher bioavailability of the components and therefore a shift of the concentration-response curves to lower concentrations when testing fractions.

A fourth possible explanation might be the potency of PAHs as major components of the sediment fractions to exhibit enhanced toxicity if exposed to UV light. Exposure to

light with a low intensity UV contribution is inevitable for toxicity testing towards green algae [46]. Matrix compounds like humic substances and dissolved organic matter are able to absorb short wavelengths of the light provided for algal growth. Removal of matrix components by the fractionation procedure may enhance the phototoxicity of these compounds. Several reasons were shown to cause either a reduced bioavailability or greater concentrations of toxicants in aqueous test systems hampering the comparison of parent extracts and fractions with respect to their ecotoxic potency. Whereas the influence of matrix components on bioavailability and fluorescence analysis can be reduced by more efficient clean up methods such as membrane-assisted techniques [47], the effects of Raoult's Law cannot be avoided. Thus, testing parent extracts solely helps to estimate the concentration range of interest for further investigations.

Toxicity analysis of three sediment samples based on fractionation and subsequent biotesting with green algae revealed astonishingly similar patterns although the samples were collected from industrial areas with rather different production lines. Maximum effects were found in fraction 10 characterized by PAHs with a molecular weight of 252 and lipophilicities of log K_{OW} = 6 to 7. Toxic potency of PAH fractions decreased with decreasing molecular weight and lipophilicity. PAHs have been frequently identified as major toxicants in contaminated sediments [42, 48]. Interestingly, the present study confirmed their relevance at industrial sites even if the variety of industrial productions seems to suggest different pollution patterns and key toxicants. The predominance of PAHs may be due to the fact that large industrial sites often emit PAHs in relevant amounts independent of what is actually produced there, for example through handling of petroleum products, incineration processes and traffic. These emissions might be often less controlled than the emission of (by)-products or wastes. However, the example of *N*-phenyl-2-naphthylamine and 7H-benzo[*de*]anthracen-7-one shows also that production-specific toxicants may play a crucial role for observed effects as well.

Polycyclic aromatic hydrocarbons, heterocyclic compounds containing oxygen and sulphur as well as *N*-phenyl-2-naphthylamine and 7H-benzo[*de*]anthracen-7-one were identified (Table 2-2) causing the observed effects on *Scenedesmus vacuolatus*. The confirmation was based on artificial mixtures and ICQ values as a novel, recently published tool for the quantitation of the difference between the extract and mixture toxicity [29]. In agreement with the previous study [29] ICQ values for most fractions increased with increasing effect levels. This indicates that at high effect levels the identified toxicants explain more of the toxicity than e.g. at effect levels of 10 or 20 %

inhibition. The specific modes of action of single components might determine the provoked effects at lower effect levels reflecting the influence of the specific composition of the tested mixture [29].

ICQs above a value of 1 were observed for the first time. The reason for this behaviour is still unknown. However, it may be hypothesized that the absence of unidentified compounds and matrix components in the artificial mixture that may lower solubility and bioavailability in the original fractions may enhance ICQs.

The interpretation and evaluation of ICQ values with respect to the question when to decide for "toxicants confirmed" or "significant toxicity unexplained" is still a matter of debate. A statistical approach based on the overlap of 95 % confidence intervals of related concentration-response curves eases the decision from which distance deviations gain in importance. In the present study this resulted in an ICQ domain between 0.5 and 2 where confidence intervals of the original fraction overlap with those of the artificial mixtures for most samples and thus there is no strong indication of overlooked toxicants. Defining an ICQ range of a successful confirmation eases the evaluation of ICQ values without further investigations of the concentration-response curves. On the basis of this criterion in general the identified polycyclic aromatic compounds confirmed well the cause of the observed effects of P10, P11 and M9 to M11, B10 and B11. Despite ICQ values of up to 4.96 and 5.06, compounds of M10 and M11 are considered as confirmed as well, since deviations occur only at high effect levels due to higher maximal effects of the artificial mixtures. The absence of matrix components rather than wrongly identified substances and concentrations determined are suggested to cause these deviations. For P8, P9, M8, B9 and B14 the confirmation is regarded as not successful since the calculated ICQ values are completely or partly beyond the suggested limits. With *N*-phenyl-2-naphthylamine as the main toxicant of B14, the observed effect cannot exclusively be linked to the presence of this single compound.

7H-benzo[*de*]anthracen-7-one was shown to contribute to the effect caused by P15 yielding ICQ values between 0.42 and 4.77. Deviation between the original and artificial sample might be explained by the fact that P15 was deep red coloured, whereas the 7H-benzo[*de*]anthracen-7-one solution was only light yellow coloured. The high background fluorescence hampers fluorescence analysis and coloured components of P15 can absorb wave lengths relevant for algal growth. Further investigations are needed to clarify the influence of the sample colour excluding the presence of additional algae toxicants which might be masked by the high background fluorescence and sample colour of P15.

2.5 Conclusions

Effect-Directed Analyses of three sediment samples from different industrial hot spots of the River Elbe basin agreed in the identification of PAHs as major toxicants to green algae while in Bitterfeld and Přelouč additional toxicants such as *N*-phenyl-2-naphthylamine and 7H-benzo[*de*]anthracen-7-one play an important role. The Index of Confirmation Quality [29] together with a decision criteria based on statistical arguments proved to be a powerful tool for a first and quantitative confirmation of the identified toxicants. Furthermore, making a step from solely effect confirmation to a hazard confirmation, effects of identified toxicants can be tested under environmental conditions and towards higher biological levels such as periphyton or plankton [28]. These results might give an impression about the impact of an identified toxicant on an *in situ* community rather than on single species.

Typically, an EDA is based on total concentrations yielding an estimate about the level of contamination. This might be helpful to determine the distribution pattern of contaminants along the river basin, but is a poor predictor of the potential risk originating from an individual sample. The introduction of the concept of bioaccessibility into an EDA might be an improvement towards a more realistic risk assessment of contaminated sites considering the extent to which contaminants can be taken up or the extent they cause adverse effects.

3 Large volume TENAX® extraction of the bioaccessible fraction of sediment-associated organic compounds for a subsequent Effect-Directed Analysis*

3.1 Introduction

Hydrophobic organic chemicals (HOC) that enter the aquatic environment rapidly become associated with sediment and suspended particles [49, 50] due to the large surface area available for adsorption [51, 52]. Sorption on sediment particles occurs in two stages: (i) an initially fast stage due to adsorption on easily accessible sites of the outer surface and (ii) a slower phase caused by the partitioning to more remote sites and absorption into organic matter [53]. Therefore, the strength as well as the location of sorption mainly determines the bioavailability and thus ecotoxicological risk of HOCs in aquatic environments.

Bioavailability is a complex concept including diffusion, desorption and partitioning processes as well as complex interactions of biological and ecological factors, like habitat properties, feeding behaviour and digestion mechanisms [54] that result in a specific dose in biota at a given concentration in sediment. The concept of bioaccessibility was introduced by Semple et al. [55] as a reasonable simplification since it considers only the kinetics of desorption from sediment at standardised conditions. Desorption of sediment associated compounds was shown to occur in an initial rapid release of molecules sorbed weakly on the outer spheres of sediment particles followed by a slower release of molecules desorbing from the intraparticle poresystem and thus more remote sites. The rate will be controlled by pore and matrix diffusion being much slower than film diffusion determining the fast release. Bioaccessibility discriminates between contaminants that take part in partitioning between sediment and biota in a relevant time frame and those that are enclosed in structures that do not allow rapid desorption.

Bioaccessibility is an operational concept that is independent from biota properties and behaviour. It is based on multiphase desorption kinetics from sediment and assumes the rapidly desorbing fraction as relevant for accumulation in biota [56, 57], while the contribution of slower desorbing fractions is small [58, 59]. The rapidly desorbing fraction can be extracted e.g. with TENAX® [21, 60], cyclodextrin [61, 62] or supercritical fluid

* Parts of this chapter are published as Schwab K, Brack W. 2007. *J Soil & Sediment* 7: 178-186.

extraction [63]. Previous investigations indicate a good correlation between the rapidly desorbing fraction and bioaccumulation in *Lumbriculus variegatus* [64, 65].

Effect-Directed Analysis (EDA) as a combination of physico-chemical fractionation, biological testing, and chemical analysis, was shown to be quite helpful for the identification of effect based key toxicants [14, 15, 66]. EDA focuses on linking measurable effects in environmental mixtures to individual components. For solid samples such as soils and sediments, the initial step of an EDA in general is the extraction procedure that removes soluble compounds from the solid matrix and makes them feasible for subsequent biotesting and chromatography. The extraction step widely determines the composition of the mixture subjected to further EDA. With focus on organic toxicants, in most cases EDA is based on exhaustive extraction of organic compounds [67-69]. This procedure corresponds to what is typically done in chemical analysis, i.e. focusing on total contents of pollutants having the advantage of standardised and reproducible methods with limited expense of time and solvents. With respect to hazard assessment exhaustive extraction represents a kind of worst case scenario, assuming 100 % bioavailability. Total concentrations of pollutants in sediment are poor predictors of hazards and risks [70, 71]. With respect to EDA of mixtures containing compounds with different bioavailability, total extraction might result in an erroneous hazard prioritisation if compounds with high toxicity and low availability are present. Thus, a restriction of the extraction of sediments or soils in EDA studies to bioavailable fractions may significantly enhance the relevance of EDA to hazard and risk assessment.

The targeted extraction of the rapidly desorbing fraction as initial step of an EDA promises a better consideration of bioaccessibility and improved toxicant prioritization. To date, standard protocols (e.g. TENAX® desorption studies) are based on a consecutive extraction of only small amounts of sediment, typically 1 g dry weight [72]. In order to obtain sufficient extract for subsequent biotesting, fractionation and structure elucidation in EDA, a large volume TENAX®-based extraction method was developed that allows for an enhancement of the extracted amount of sediment by a factor of 125.

The EDA-directed large volume extraction procedure for bioaccessible fractions of sediment contaminants was developed in four steps. In the first step desorption kinetics were recorded according to the classical consecutive small volume approach [72]. Desorption characteristics like rapidly, slowly and very slowly desorbing fractions and respective rate constants were determined by relating TENAX®-extracted amounts at a given time to total amounts yielded by Accelerated Solvent Extraction (ASE). This aimed

at determining the time needed to extract a fraction that is equivalent to the rapidly desorbing fraction. Since consecutive extraction procedures are not feasible in EDA, in a second step this extraction time was applied to single extractions of small amounts. Thirdly, an extraction apparatus and a corresponding protocol were developed allowing an up-scaling by a factor of 125, named the large volume approach. Reproducibility and comparability to the small volume approach were tested. Since the success of an EDA depends on the absence of toxic procedural blanks, in a fourth step the toxicity of blanks towards growth inhibition of green algae *Scenedesmus vacuolatus* was analysed, reduced by further clean up and compared with effective concentrations of a sediment extract obtained using the same extraction procedure.

3.2 Material and Methods

3.2.1 Exhaustive sediment extraction

Fresh sediment sampled at Přelouč location was extracted exhaustively with Accelerated Solvent Extraction (ASE) to determine the total concentration of organic pollutants. Sediments were extracted at 100 °C and 103 bar using a mixture of acetone and hexane 1:1 (v/v) as solvents. Prior to the extraction, the wet sediment was properly mixed with the same mass of Isolute HM-N to bind water using a mortar and pestle (see also 6.2.1). The volume of the extract was reduced by means of rotary evaporation followed by a gentle stream of nitrogen.

3.2.2 Sediment extraction with TENAX®

Sediment extraction with TENAX® was a two-step procedure including the extraction of desorbing compounds in sediment suspensions with TENAX® and subsequent extraction of the loaded TENAX® with organic solvents providing samples for further investigations like chemical analysis or biological testing. Fresh sediment from Přelouč was used.

Consecutive sediment extraction- desorption kinetics

Consecutive desorption was determined at room temperature by means of an extraction method described in chapter 6.2.2. A mixture of wet sediment (1 g dry weight),

standard-cleaned TENAX® (0.2 g) and bi-distilled water (70 mL) was shaken vigorously in a 100 mL separation funnel. Hg(II)-acetate was added (1.2 mg) to inhibit microbial activity. TENAX® beads were refreshed at periodic intervals and after the separation from the suspension extracted with 20 mL of a 1:1 (v/v) mixture of acetone and hexane. Desorption kinetics were measured in triplicate.

Desorption was described using a first-order three-compartment model [73] assuming no significant readsorption, independence of modelled fractions and simultaneous desorption of all three compartments:

$$\frac{S_t}{S_0} = F_{rap} \cdot e^{-k_{rap} \cdot t} + F_{slow} \cdot e^{-k_{slow} \cdot t} + F_{veryslow} \cdot e^{-k_{veryslow} \cdot t}$$

Eq. 3-1

Initial sediment-sorbed amounts (t=0) and remaining amounts at a given time t [h] were indicated by S_0 and S_t [µg], respectively. The dimensionless parameters F_{rap}, F_{slow} and $F_{veryslow}$ represent the rapidly, slowly and very slowly desorbing fractions, respectively, with corresponding rate constants k_{rap}, k_{slow} and $k_{veryslow}$ [h^{-1}]. Values for F_{rap}, F_{slow} and $F_{veryslow}$ and their respective rate constants were determined by a non-linear curve fitting on the basis of the Levenberg-Marquardt algorithm using Origin Software allowing the exact description of desorption process.

Single TENAX® extraction- small volume approach

The single extraction in the small volume approach was performed at room temperature using a 100 mL separation funnel. 1 g sediment (dw), 1.5 g standard-cleaned TENAX® and 70 mL water were shaken vigorously for a defined interval. After termination, the TENAX® was separated from the sediment suspension, washes with bi-distilled water and rinsed in a vacuum (filter) flask to remove bulk water. Afterwards, the TENAX® was extracted using 20 mL of a 1:1 mixture of hexane and acetone according to the procedure described in chapter 6.2.2. The volume of the extract was reduced by the means of rotary evaporation.

Large volume TENAX® approach

Prior to the extraction procedure, TENAX® beads were rinsed with water, acetone and hexane according to the standard cleaning procedure (6.2.2). Since the bio assay with

green algae detected measurable effects applying TENAX® blanks, further cleaning steps were nessecary using ASE. Standard- and ASE-clean up procedure are described in chapter 6.2.2.

125 g of sediment (dw) from Přelouč, 180 g of cleaned TENAX® and at least 3 L of deionised water were stirred vigorously at 20 °C for 24 h in a special glass vessel (see chapter 6.2.2, Figure 6-5). The TENAX® was isolated from the sediment suspension and washed with deionized water until the water phase was clear. The loaded TENAX® was directly extracted in the vessel using acetone and subsequently hexane. A detailed description can be found in chapter 6.2.2.

Reproducibility of the fraction removed within 24 h (F_{24h}) of the large volume approach and its comparability to the small approach were investigated. Three replicates were run for the reproducibility study.

3.2.3 Clean up and chemical analysis

TENAX® extracts were re-dissolved in dichloromethane and shaken over night with activated copper to remove elemental sulphur. Sediment extracts gained with ASE required a further clean up on Florisil, alumina and silica gel subsequent to the sulphur removal (see chapter 6.2.3).

Chemical analysis was performed applying a gas chromatograph combined with a mass selective detector (GC-MS) (see chapter 6.2.5). The oven temperature was programmed from 60 to 150 °C with a rate of 30 °C/min followed by an increase to 186 °C with 6 °C/min and subsequent heating to 280 °C with 4 °C/min holding this temperature for 8.5 min. Compounds were quantified in the selected ion mode (SIM) on the basis of the molecular ion using an external calibration.

3.2.4 Biological analysis

TENAX® blanks and the extract gained with the large volume approach were tested for toxicity to green algae *Scenedesmus vacuolatus* after solvent exchange with dimethyl sulphoxide (DMSO) with a maximum concentration of 0.1% in the test system, which proved to be of no detectable effect. Exact test conditions are described in chapter 6.3.

3.3 Results

3.3.1 Desorption kinetics of sediment-associated contaminants

Desorption kinetics were determined by the means of consecutive sediment extraction with TENAX® for a period of 41 days. Seven native polycyclic sediment contaminants of Přelouč sediment were used for these investigations (Table 3-1). By plotting sediment concentrations (S_t) normalized to initial concentration (S_0) versus time [h] a typical three-phasic desorption was observed (Figure 3-1). For clarity, only data for naphthalene, anthracene, fluoranthene and benzo[*b*]fluoranthene were shown in Figure 3-1. Curves of chrysene, benzo[*a*]anthracene and pyrene were comparable to that of fluoranthene, which was printed as a representative of these three compounds. Concentration in sediment decreased rapidly during the first 24 h of extraction followed by a slower release of sediment contaminants. S_t/S_0 ratio, meaning the fraction that remained in the sediment, ranged between 0.49 and 0.87 after 24 h (day 1) of extraction and decreased to values between 0.27 and 0.67 at day 41. Whereas about 50 % of total anthracene amount desorbed within the first day (t24h), during the following 40 days the concentration decreased only about additional 20 %. Thus, the main fraction of anthracene desorbed during the first 24 h of extraction, whereas the subsequently released amount was significantly smaller. Benzo[*b*]fluoranthene exhibited the slowest desorption rate as well as the smallest desorbable fraction of the investigated compounds. Within the first extraction day (24 h) only 13 % of total pollutant load was extracted. Similar to anthracene, the concentration decreased about additional 20 % between day 1 and day 41.

Significant differences in slopes and curve progression (Figure 3-1) indicated compound dependent values for the rapidly, slowly and very slowly desorbing fractions (F_{rap}, F_{slow}, $F_{veryslow}$) and the respective rate constants (k_{rap}, k_{slow}, $k_{veryslow}$) (Table 3-1). Therefore, Eq. 3-1 was fitted to the experimental data (Figure 3-1) allowing an exact mathematical description of desorption processes and a calculation of F_{rap}, F_{slow}, $F_{veryslow}$ and the related rate constants. The values for the rapidly desorbing fractions F_{rap} covered a range of 0.12 for benzo[*b*]fluoranthene to 0.30 for pyrene. 70 to 90% of the PAHs found in the sediment belonged to the slowly and very slowly desorbing pool with very limited bioaccessibility.

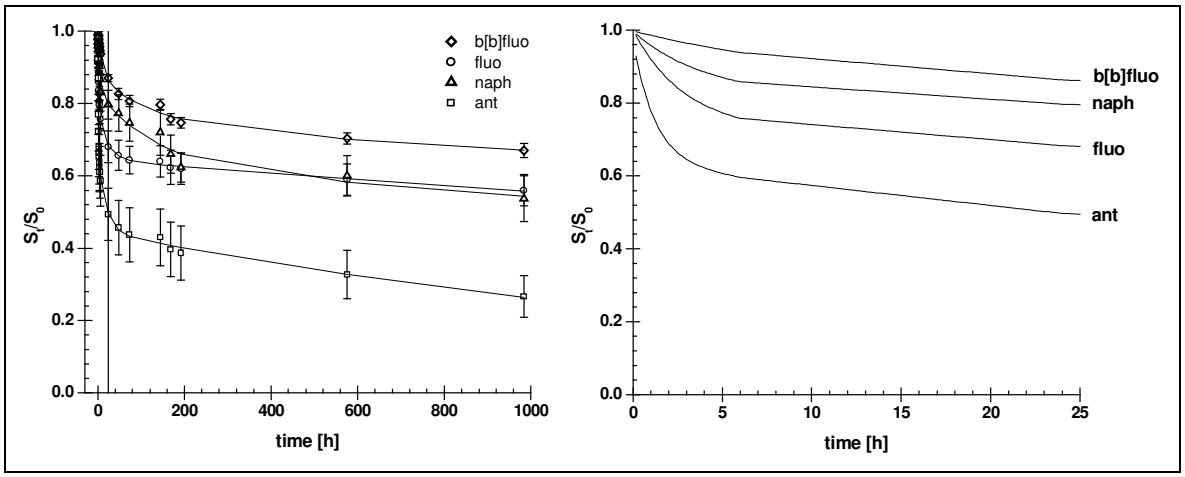

Figure 3-1: *Desorption of native polycyclic sediment contaminants determined by consecutive sediment extraction with TENAX® plotting the sediment concentration (S_t) normalized to initial concentration (S_0) versus time [h]. Benzo[b]fluoranthene (b[b]fluo), fluoranthene (fluo), naphthalene (naph) and anthracene (ant) are shown as representatives. Points mark experimental data including the standard deviation as error bar (n= 3). Fitted functions are given as solid lines. The vertical line in the left graph marks the extraction time of 24 h, what is separately displayed at the right side.*

Calculated rate constants of the rapidly, slowly and very slowly desorbing pool (k_{rap}, k_{slow}, $k_{veryslow}$) were in the range of 10^{-1} h^{-1}, 10^{-2} h^{-1} and 10^{-4} h^{-1}, respectively. Since they differed by one to two orders of magnitude, the rate constants can be clearly assigned to the respective compartment, assuming retarded micropore diffusion as explanation for slow and very slow desorption [53]. Rate constants for the rapid and slow desorption decreased with increasing molecular weight with the exception of naphthalene. Despite the lower molecular weight it desorbed significantly slower. With $k_{veryslow}$ values between 1.0 and $1.6 \cdot 10^{-4}$ h^{-1} no substance dependent pattern can be observed for the investigated compounds. This rule was violated by anthracene with a $k_{veryslow}$ of $5.2 \cdot 10^{-4}$ h^{-1}.

It needs to be stressed here that the extraction of all three fractions proceeds in parallel with different rate constants. Thus, although F_{rap} and t_{rap} can be individually determined for an exact analysis of desorption kinetics [74], F_{rap} can never be extracted individually. The presented procedure focused on the extraction of amounts that were equivalent to F_{rap} (F_{rap_eq}) rather than on the extraction of F_{rap} itself. Thus, on the basis of Eq. 3-1 and the calculated values for fractions and rate constants, a time t_{rap_eq} was determined at which F_{rap_eq} was extracted setting $S_t/S_0 = (1-F_{rap})$. The amount extracted equals that contained in F_{rap} but integrates over all three fractions extracted.

Table 3-1: *Calculated values for the rapidly, slowly and very slowly desorbing fractions (F_{rap}, F_{slow}, $F_{veryslow}$) and their respective rate constants (k_{rap}, k_{slow}, $k_{veryslow}$) determined through fitting of Eq. 3-1 to consecutive desorption data. Standard deviations of 3 replicates are given additionally. t_{rap_eq} indicates the time required to remove an amount of the respective compound that is equivalent to F_{rap}.*

compound	F_{rap}	k_{rap} [10^{-1} h^{-1}]	F_{slow}	k_{slow} [10^{-2} h^{-1}]	$F_{veryslow}$	$k_{veryslow}$ [10^{-4} h^{-1}]	$t_{rap\ eq}$ [h]
naphthalene	0.16 ± 0.05	2.7 ± 0.03	0.21 ± 0.13	0.9 ± 0.5	0.62 ± 0.08	1.4 ± 0.8	8.9
anthracene	0.29 ± 0.04	8.0 ± 0.04	0.20 ± 0.04	5.4 ± 0.4	0.49 ± 0.002	5.2 ± 0.1	2.1
fluoranthene	0.24 ± 0.02	3.8 ± 0.08	0.11 ± 0.02	4.2 ± 0.5	0.64 ± 0.04	1.4 ± 0.2	6.1
pyrene	0.30 ± 0.04	3.2 ± 0.07	0.10 ± 0.02	3.0 ± 1.1	0.60 ± 0.05	1.6 ± 0.2	8.1
benzo[*a*]anthracene	0.23 ± 0.02	1.7 ± 0.01	0.11 ± 0.01	1.8 ± 0.4	0.66 ± 0.01	1.6 ± 0.1	13.2
chrysene	0.22 ± 0.06	1.3 ± 0.05	0.11 ± 0.06	2.0 ± 1.7	0.67 ± 0.02	1.2 ± 0.2	15.7
benzo[*b*]fluoranthene	0.12 ± 0.01	1.0 ± 0.01	0.14 ± 0.01	0.8 ± 0.2	0.74 ± 0.01	1.0 ± 0.3	18.7

In our study, values for t_{rap_eq} covered a range of 2 h for anthracene to 18 h for benzo[*b*]fluoranthene. While a clear trend could be observed indicating a reduction of desorption rates with increasing molecular weight and hydrophobicity, this rule was violated by anthracene. Despite of the higher molecular weight anthracene desorbed significantly more rapidly than naphthalene. No explanation could be found for this behaviour. To address the desorption even of highly lipophilic compounds like benzo[*b*]fluoranthene an extraction time for the single extraction procedure of 24 h was chosen.

3.3.2 Validation of the large volume extraction method

The transferability of the low volume to the large volume approach was tested for the fraction removed within the selected extraction time of 24 h (F_{24h}) as the most relevant parameter with respect to extraction in EDA (Figure 3-2). F_{24h} values ranged from 0.03 to 0.39 for both approaches decreasing with increasing molecular weight of the investigated compounds. F_{24h} values of the small and large volume approach deviated only by 2 to 27 % showing neither a molecular weight dependent pattern nor a systematic over- or underestimation with one of the approaches.

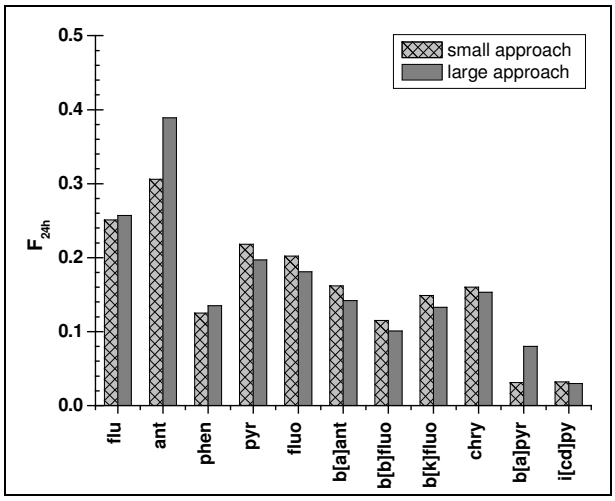

Figure 3-2: *Comparison of small and large volume approach. Displayed are the fractions extracted within 24 h extraction duration (F_{24h}) for small and large volume approach (fluorene (flu), anthracene (ant), phenanthrene (phen), pyrene (pyr), fluoranthene fluo, benzo[a]anthracene (b[a]ant), benzo[b]fluoranthene (b[b]fluo), chrysene (chry), benzo[a]pyrene (b[a]pyr), indeno[c,d]pyrene (i[cd]py)).*

The fraction extracted within 24 h (F_{24h}) was investigated to check the reproducibility of the large volume approach (Figure 3-3). F_{24h} values ranged from 0.03 to 0.57 with relative standard deviations between 4 to 28 % for 3 replicates. The greatest deviation was observed for benzo[*a*]pyrene. For reproducibility studies, the use of sediment from a second sampling campaign might explain significantly higher F_{24h} values of up to 0.57 indicating a variability of sediment properties with time.

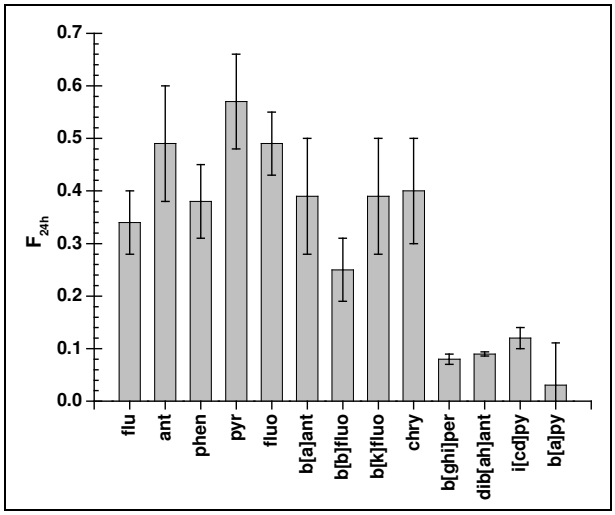

Figure 3-3: *Reproducibility of large volume approach on the basis of the fraction removed within 24 h (F_{24h}). Mean values of three replicates are presented showing the standard deviations as error bars.*

Due to high amounts of TENAX® used, impurities gained in importance, especially in the context of an EDA. To avoid false-positive results, procedural blanks of TENAX® were investigated for adverse effects towards green algae *Scenedesmus vacuolatus*. Population growth of *Scenedesmus vacuolatus* was inhibited by 44 % applying the blank of standard-cleaned TENAX® at a concentration equalling 600 g SEq/L(medium) (Figure 3-4). Further clean up by ASE reduced the observed inhibition of *Scenedesmus vacuolatus* to 21 % tested at the same concentration.

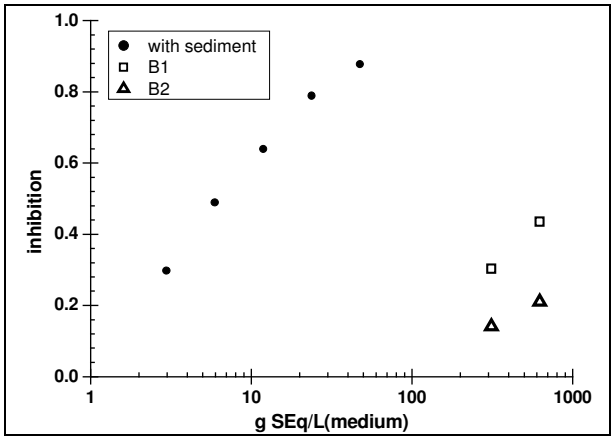

Figure 3-4: *Concentration-response relationship of the TENAX® extract of sediment tested towards growth inhibition of Scenedesmus vacuolatus. Effects of the standard-cleaned (B1) and ASE-cleaned (B2) TENAX® blanks are given as well.*

This reduction of toxicity was paralleled by a significant reduction of the number and intensity of peaks found in GC-MS chromatograms of the blanks of standard- and ASE-cleaned TENAX® (Figure 3-5). The chemical analysis identified several softeners and stabilisers in the sample of the standard-cleaned TENAX® that might have their origin in the production procedure. Reduction or removal of many peaks clearly indicated TENAX® as the origin of these impurities rather than solvents, glass vessels or the GC column. Impurities cannot be fully removed by ASE-cleaning procedure, as seen in the second chromatogram of Figure 3-5.

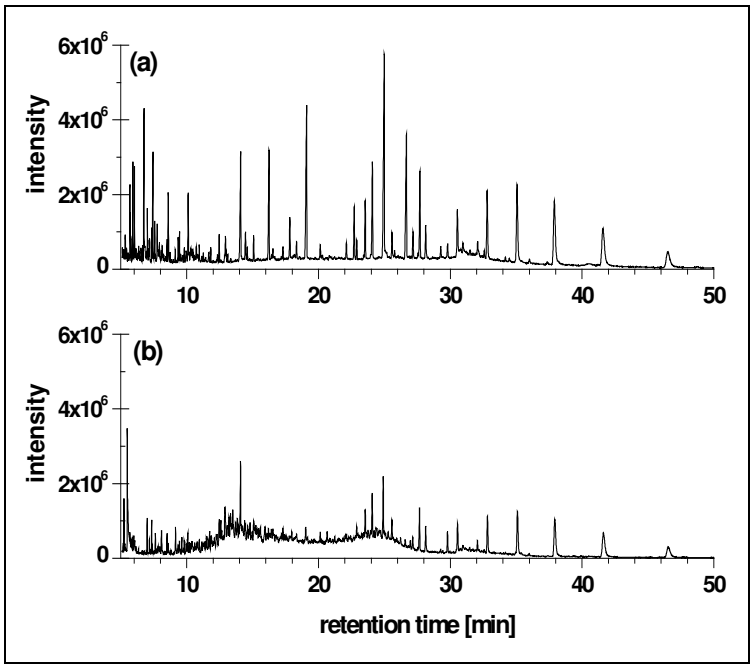

Figure 3-5: *Chromatograms of standard- (a) and ASE-cleaned (b) TENAX® blanks.*

As an example a TENAX® extract from Přelouč sediment was tested towards growth inhibition of green algae *Scenedesmus vacuolatus*. Concentration-dependent effects were observed testing the extract between 3 and 50 g SEq/L(medium) (Figure 3-4) reaching an inhibition of 90 % applying the highest concentration. Effective concentrations of blanks were about three orders of magnitude above that of the sediment extract gained with the same procedure (Figure 3-4). It can be concluded that these blanks did not measurably contribute to observed effects of a sediment extract gained with the same method.

3.4 Discussion

The aim of the study was to investigate the changes occurring through the modification of the test design from a consecutive over a single extraction small volume procedure to a large volume extraction method. Therefore, desorption kinetics of native sediment contaminants were determined through consecutive extraction of sediment with TENAX® to characterize the rapidly, slowly and very slowly desorbing fractions (F_{rap}, F_{slow}, $F_{veryslow}$) as well as the related rate constants (k_{rap}, k_{slow}, $k_{veryslow}$) (Table 3-1). It was demonstrated that F_{rap}, ranging from 12 % to 30 % of the total amount contained, depended on the compound under consideration. Literature values of the rapidly desorbing fractions show great variations ranging from less than 10 % up to over 50 % of the contained

compounds [22, 64]. These great variations can be explained by differences in organic matter composition [75-77], origin of PAHs [78], age and physicochemical properties of pollutants [59]. With a F_{rap} of only 0.16 naphthalene is much less accessible than expected (Table 3-1). This low value can be caused by losses during work due to high volatility of this individual compound. Other groups suggested that the release or degradation of low-molecular-weight PAHs due to weathering of sediment results in a removal of readily accessible fractions and thus a relative increase of the less accessible fractions [79]. Calculated rate constants of the rapidly, slowly and very slowly desorbing pool (k_{rap}, k_{slow}, $k_{veryslow}$) were in the range of 10^{-1} h^{-1}, 10^{-2} h^{-1} and 10^{-4} h^{-1}, respectively being well in agreement with results of other groups [22, 64, 72].

The consecutive extraction of sediment using TENAX® allows the determination of the rapid, slow and very slow release of pollutants. The rapidly desorbing fraction is commonly regarded as an equivalent for the bioaccessible fraction of sediment contaminants. Knowing the time span where F_{rap} is released from the sediment this fraction can be extracted by a single extraction of sediment for a certain extraction time without a TENAX® renewal. The rapidly desorbing fraction deviating in concentration as well as in relative composition from total extracts, becomes accessible for further investigations like chemical analysis or biological testing yielding information about the extent of the bioaccessibility and thus the potential risk originating from the investigated sampling location.

On the basis of the kinetic parameters the calculation of the time needed to extract an equivalent of the rapidly desorbing fraction is possible (Table 3-1). A fixed extraction time of 24 h was selected as a time at which F_{rap_eq} is fully extracted for compounds with a logK_{OW} up to 6.5 (benzo[*b*]fluoranthene). Since less hydrophobic compounds are characterised by higher desorption rates, a slight overestimation of their bioaccessibility must be accepted. A universally valid extraction time for F_{rap_eq} for all components of a complex mixture does not exist since it depends on the individual desorption behaviour of the components (and thus individual pollution patterns) and the composition of the sediment. The selected time can only be a compromise between nearly complete extraction of the rapidly desorbing fraction of highly lipophilic compounds and a limited overestimation of F_{rap_eq} of less lipophilic contaminants. Extraction times applied by other groups accounted for 6 h as well as 30 h aiming to estimate the extent of bioaccessibility [21] or bioaccumulation [64, 80].

Table 3-2 shows the percentage of rapidly, slowly and very slowly desorbing pool remaining in sediment after 24 h of extraction with TENAX®. This may demonstrate that the fraction extracted after t_{rap_eq} numerically equals F_{rap}, however physically it is a mixture of a big share of the rapidly desorbing pool together with low and very low shares of the simultaneously desorbing slow and very slow pools. An alternative way to estimate t_{rap} was recently presented [74]. It considers only the rate constant of the rapid desorption and gives good estimates in kinetic studies. For extraction purposes however, the neglect of the simultaneous desorption from slow and very slow compartment leads to longer extraction times that systematically overestimate bioaccessibility.

Table 3-2: *Percentage of the rapidly, slowly and very slowly desorbing pool remaining in sediment after 24 h of extraction with TENAX®.*

	rapidly desorbing pool	**slowly desorbing pool**	**very slowly desorbing pool**
naphthalene	0.16	80.65	99.67
anthracene	0.00	27.41	98.77
fluoranthene	0.01	36.54	99.66
pyrene	0.04	48.72	99.62
benzo[*a*]anthracene	1.57	64.20	99.62
chrysene	4.85	62.19	99.70
benzo[*b*]fluoranthene	9.94	81.97	99.76

To allow for an estimation of the error made by application of one fixed extraction time, estimated fractions (F_{6h_estim}, F_{24h_estim}) as well as actually measured fractions (F_{6h_meas}, F_{24h_meas}) gained with a single extraction were compared with F_{rap} (Figure 3-6). A good agreement between F_{rap} and F_{24h_estim} was found for high lipophilic compounds. This finding supports the former selected extraction time of 24 h on the basis of the desorption kinetics. As expected for small and medium PAHs a better agreement is observed with 6 h extraction.

Surprisingly, measured fractions extracted after 6 and 24 h (F_{6h_meas}, F_{24h_meas}) increasingly deviate from the estimated fractions with increasing size of PAHs and thus lipophilicity and boiling points. Assuming desorption from the sediment as the rate limiting step [72] and TENAX® as an infinite sink with very rapid absorption kinetics, this deviation cannot be explained. Desorption from sediment should be neither effected by the amount of TENAX® (1.5 g for single extraction instead of 0.2 g for the sequential approach) nor by its frequent renewal. Thus, the systematically higher extraction efficiency

with increasing molecular size of PAHs for single extraction with higher amounts of TENAX® suggests the absorption by TENAX® rather than desorption from sediment as rate limiting step for these compounds. TENAX® particles are complex structures of a highly lipophilic matrix filled with air and surrounded by water. Thus, rate limitations could occur (i) in the water phase due to limited diffusion for increasingly hydrophobic compounds at lower TENAX® amounts, (ii) in the TENAX® matrix itself due to reduced diffusion with increasing molecular size from limited TENAX® particle surface or (iii) in the gas phase with decreasing vapour pressure of the compounds. Our experiments are not able to discriminate between these types of rate limitations. However, they could be an indication that the basic assumption of desorption as rate limiting step in TENAX® extraction might be violated for some compound groups even if standard procedures are applied.

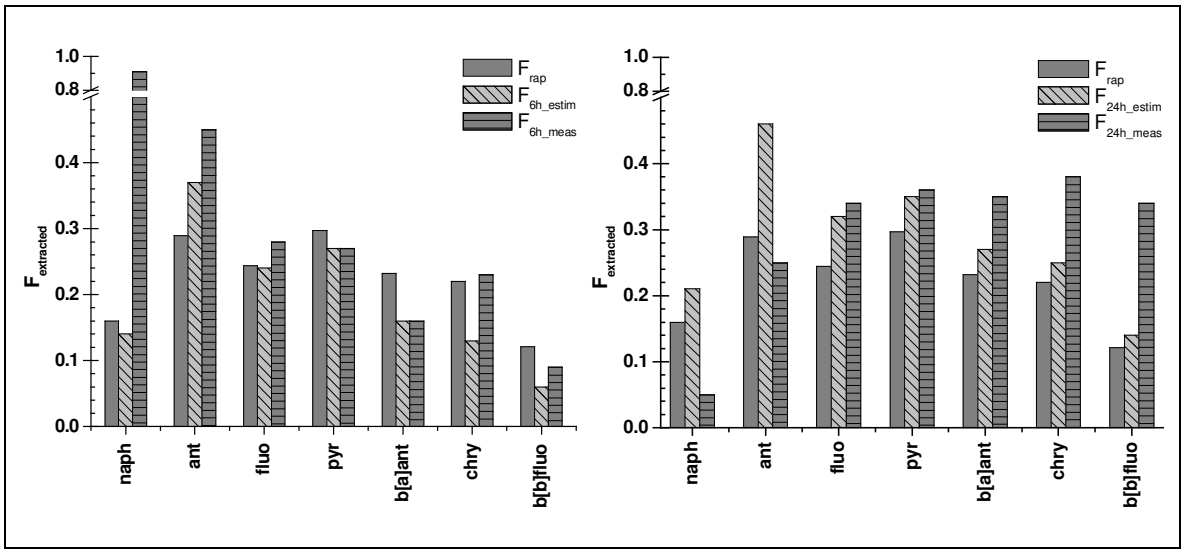

Figure 3-6: *Estimated and measured fractions extracted within 6 h (F_{6h_estim}, F_{6h_meas}) and 24 h (F_{24h_estim}, F_{24h_meas}) from Přelouč sediment. These fractions are compared to F_{rap}. Values for F_{meas} were corrected for the recovery of the individual compound.*

Geometrical and agitation properties might influence desorption rates and affect the transferability of data gained with the small volume approach to the large volume glass vessel. Since the performance of full kinetic studies with the large volume approach is impractical because of the enormous consumption of TENAX® and solvents, only F_{24h} values were compared (Figure 3-2). The good agreement of F_{24h} values in both approaches supports the application of the large volume approach based on the concept derived from

the consecutive approach with small volumes. Observed deviations reflect acceptable variations due to sediment inhomogeneity and analytical deviations.

Only for benzo[*a*]pyrene relative standard deviations of F_{24h} were greater than 30 % (Figure 3-3). Being mainly sequestered on soot particles, the inhomogeneous distribution of contaminants in sediment could have the biggest influence on the results. There are no indications that non-reproducible sorption to TENAX® occurs.

Biological analysis of the TENAX® extract of the Přelouč sediment taken as an example indicated concentration-dependent effects towards *Scenedesmus vacuolatus* (Figure 3-4). This provides a good basis for subsequent EDA and stresses the potential of the large volume TENAX® approach to extract sufficient amounts of toxicants for effect assessment and EDA.

3.5 Conclusions

For the consideration of bioaccessibility in EDA a large volume TENAX® extraction method was presented. Good agreement with the established consecutive small volume extraction allows a sufficiently precise extraction of the rapidly desorbing fraction of a broad range of compounds within a uniform extraction time of 24 h. Although there are several other solid phases applicable for extracting bioaccessible fractions, including solid-phase micro-extraction fibres SPME [81] and semi-permeable membrane devices SPMD [82], TENAX® has unique properties for the depletive extraction of bioaccessible fractions of large amounts of sediment. High surface area, excellent sorption properties and thus a quick removal of lipophilic compounds from the water phase [83], the applicability at different scales and the ease of separation of sediment particles from the adsorbent are strong points of the method. High costs of TENAX®, toxicity and chemicals associated with the blanks due to production residues, high solvent consumption and the special equipment needed, such as glass vessel and ASE, may be shortcomings of the method. Since desorption kinetics are compound dependent, every extraction procedure based on rapidly desorbing fractions can be only a compromise that does not fully reflect bioaccessibility of all compounds to the same degree, although the error seems to be acceptable for the range of lipophilic compounds that have been assessed here. Absolute hazard assessment of sediments based on toxicity testing of TENAX® extracts requires an additional calibration e.g. with body burdens of exposed organisms.

TENAX®-based large volume extraction of rapidly desorbing fractions provides a novel tool in EDA and is believed to allow a better hazard assessment of sediments and key toxicant prioritization. Whether identified key toxicants and their relative contribution to total effects will actually be significantly altered by the new extraction approach will be evaluated in chapter 5.

4 The influence of freeze-drying on the desorption of sediment associated contaminants

4.1 Introduction

Approaches that focus on the direct assessment of unchanged sediments such as bioaccessibility-directed extraction or sediment contact tests face the problem that fresh sediments are known to undergo degradation processes and recommendations for the storage of fresh sediments are very heterogeneously ranging from only a few days [84] up to several weeks [85]. Three problems arise from storing fresh sediments: (i) the microfauna might cause a biological degradation of pollutants, (ii) the release of ammonia through microbiological activity [86] and (iii) chemical degradation through hydrolysis or oxidation, being responsible for false-positive and false-negative test results. Becker & Ginn [87] investigated the toxicity of several sediment samples towards amphipod, polychaete and bacteria over a storing time of 16 weeks. They observed an increase as well a decrease of toxicity depending on the sediment sample as well as on the test organism under consideration. In contrast, Defoe and Ankley [88] did not observe any changes within test results storing sediment up to 101 weeks at 4 °C. The alteration can depend on the level of the contamination as well, since effects of storage time have been greater for low to moderate contaminated sediments, whereas storage of heavily contaminated sediments did not greatly alter observed effects [87]. These results suggest that the changes are unpredictable and depend on the applied test organism as well as the contamination level.

Freeze-drying encompasses the water removal at decreased temperatures having the advantage to circumvent the loss of thermo labile or volatile compounds. Furthermore, the microfauna is killed that normally controls ammonia production and biological degradation. Through water removal chemical degradation is lowered as well. However, freeze-drying has been shown to possibly cause an increase in toxicity, elevated DOC levels as well as an elevated leaching of PAHs in the water phase [84, 89] assuming an alteration of the release process as well as of sediment properties.

The TENAX® extraction method is a common method to selectively trap the rapidly desorbing fraction as an estimate of the contaminant bioaccessibility [21, 90]. A good correlation between the extracted fraction and BSAF values indicate the suitability to simulate the distribution between sediment and organism [60]. You et al. was able to show

that the TENAX® method can be used as an easier, faster and cheaper surrogate for whole organisms assays [65] posing the possibility to detect changes in the desorption behaviour and thus bioaccessibility of sediment associated contaminants avoiding time consuming and laborious sediment contact assays.

The objective of the present study was to expand the current knowledge about the influence of freeze-drying on the desorption behaviour of sediment-associated contaminants as determined by TENAX® extraction. Four sediments of river Elbe and tributaries were selected and extracted with TENAX® for 6 h, 24 h and 30 h reflecting common times to extract the bioaccessible fraction [21, 91]. The influence of freeze-drying was determined by correlating the amount extracted from fresh sediment to that of a dried subsample.

4.2 Material and Methods

4.2.1 Sediment samples

To investigate the influence of freeze-drying on the desorption behaviour of sediment-associated contaminants sediments from Přelouč, Most, Bitterfeld and Magdeburg were used. Fresh sediments were stored at 4 °C in amber glass bottles until required. Prior to the extraction procedure with TENAX® the required sediment amount was freeze-dried.

4.2.2 Sediment extraction

To determine total contents of organic pollutants, wet sediments were extracted using Accelerated Solvent Extraction (ASE). Wet sediments were carefully mixed with the same mass of Isolute HM-N to bind water. Extraction was carried out at 100 °C and 103 bar with a mixture of acetone and hexane (1:1 v/v) as solvent. The volume of the extracts was reduced using a rotation evaporator (for more details see chapter 6.2.1).

To investigate the desorption behaviour of sediment associated organic contaminants, only single TENAX® extractions at a well defined time were performed. Therefore, the fresh and freeze-dried sediment (1 g dry weight), TENAX® (1.5 g) and bi-distilled water (70 mL) were vigorously shaken for 6 h, 24 h and 30 h in a 100 mL separation funnel without introducing fresh TENAX®. After termination, TENAX® beads were separated from the sediment suspension, rinsed with water and transferred to a

vacuum flask to remove bulk water. Thereafter, TENAX® beads were extracted with a mixture of acetone and hexane (1:1 v/v). Experiments were carried out in triplicate. Prior to desorption experiments fresh TENAX® beads were cleaned according to the standard cleaning procedure. For a detailed description see chapter 6.2.2.

To better compare the amount extracted from fresh and freeze-dried sediment the Desorption Index (DI) was calculated:

$$DI = \frac{F_{freeze-dried}}{F_{fresh}} - 1$$

Eq. 4-1

whereas the dimensionless parameters $F_{freeze\text{-}dried}$ and F_{fresh} are the fractions extracted with TENAX® using freeze-dried and fresh sediments, respectively. Fractions are calculated dividing the concentration in the TENAX® extract [µg/g (sed dw)] by the concentration in the ASE extract [µg/g (sed dw)].

4.2.3 Clean up and chemical analysis

ASE as well as TENAX® extracts were firstly shaken with activated copper to remove elemental sulphur. As ASE extracts were coloured, a second clean up step was necessary consisting of adsorption chromatography on Florisil. An exact description can be found in chapter 6.2.3. Samples were re-dissolved in toluene for the chemical analysis.

Components were analyzed applying a gas chromatograph combined with a mass selective detector (GC-MS). The oven temperature was programmed from 60 to 150 °C with a rate of 30 °C/min followed by an increase to 186 °C with 6 °C/min and subsequent heating to 280 °C with 4 °C/min holding this temperature for 8.5 min. Compounds were identified in the Total Ion Current (TIC) Mode on the basis of the retention time and mass spectra. Quantification was performed using an external calibration utilizing standard compounds. Standards as well as samples were measured in the selected ion mode (SIM). A detailed description can be found in chapter 6.2.5.

4.3 Results

A single TENAX® extraction was performed for 6 h, 24 h and 30 h, respectively for sediments from Přelouč, Magdeburg, Most and Bitterfeld. Comparing the fraction extracted from fresh and freeze-dried sediment, the Desorption Index (DI) was calculated

according to Eq. 4-1 and plotted for the individual compounds. Three cases can occur: (i) values equalling 0 indicate a full accordance between the fraction extracted from fresh and freeze-dried sediment, (ii) with values greater 0 the fraction extracted from freeze-dried sediment exceeds that from fresh sediment suggesting an enhanced desorption from the freeze-dried sediment and (iii) with values below 0 a smaller fraction is extracted from freeze-dried sediment assuming a hampered desorption from the dry sediment compared to fresh sediment.

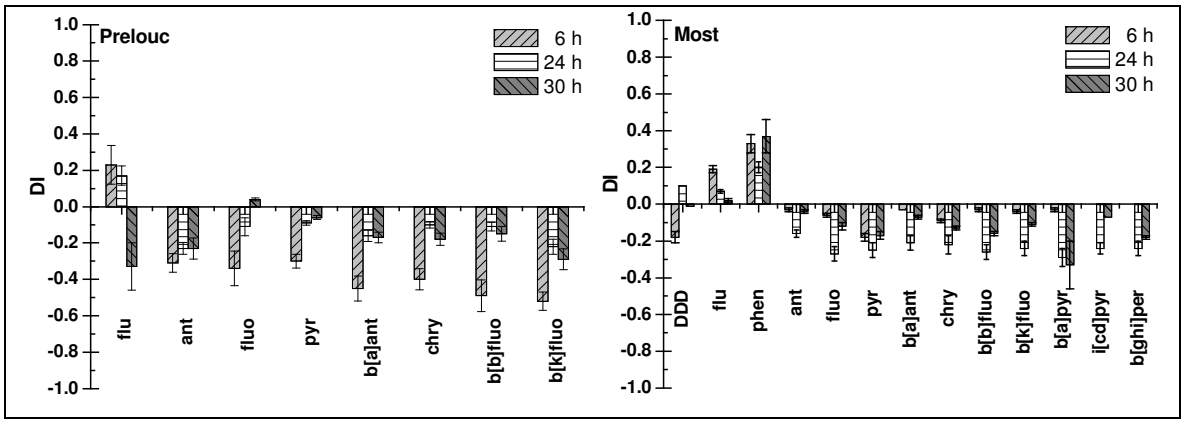

Figure 4-1: *Differences in the desorption behaviour of sediment-associated contaminants of fresh and freeze-dried sediment from Přelouč (left) and Most (right). The Desorption Index (DI) was calculated according to Eq. 4-1. Three different extraction times were applied (6 h, 24 h, 30 h) presenting mean values of three replicates and related standard deviations as error bars.*

For Přelouč the majority of the DI-values was negative with values down to -0.52 indicating a hampered desorption from freeze-dried sediment compared to fresh ones. Greatest deviations were observed for an extraction time of 6 h, whereas DI-values decreased to 0.04 and -0.29 by the elongation of the extraction time. This rule was violated by fluorene with positive values up to 0.23 and the greatest deviation at an extraction time of 30 h. However, the compound volatility and the related risk of losses during sample preparation aggravated the interpretation of these results. DI-values and thus differences in the desorption from fresh and freeze-dried sediment increased from anthracene to benzo[*k*]fluoranthene.

Results for Most sediment were similar to Přelouč DI-values ranging between -0.01 and -0.33. Only for fluorene and phenanthrene DI-values were positive reaching values up to 0.37. Surprisingly, the DI-values indicated greatest deviations at an extraction time of 24 h whereas smallest deviations were observed at an extraction time of 6 h. This rule was

violated by DDD, fluorene and phenanthrene with the smallest deviations at 30 h and 24 h, respectively.

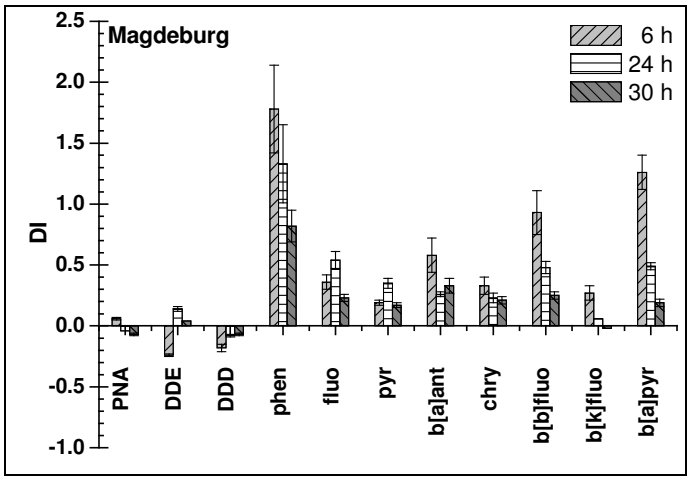

Figure 4-2: *Differences in desorption behaviour of sediment-associated contaminants of fresh and freeze-dried samples from the river Elbe near the city of Magdeburg were investigated applying three different extraction times (6 h, 24 h, 30 h). The mean values of three replicates are shown presenting standard deviations as error bars.*

Desorption behaviour of freeze-dried Magdeburg sediment deviated from that of Přelouč and Most samples since the DI-values were positive in general. The desorption from freeze-dried sediment was elevated compared to desorption from fresh ones. The prolongation of the extraction time up to 30 h lead to values between -0.07 and 0.33 indicating an approximation of the desorbed amounts from freeze-dried and fresh sediments. Phenanthrene followed the same trend, however with a DI-value of 0.82 it exhibited still relatively high deviations at an extraction time of 30 h. According to Eq. 4-1 a DI-value of 0.82 equal a $F_{freeze\text{-}dried}/F_{fresh}$ ratio of 1.82. Thus, the fraction extracted from freeze-dried sediment was twice as much as that desorbing from fresh sediment. For extraction times greater than 6 h DDE, DDD and PNA showed the smallest deviation with DI-values between -0.08 and 0.14.

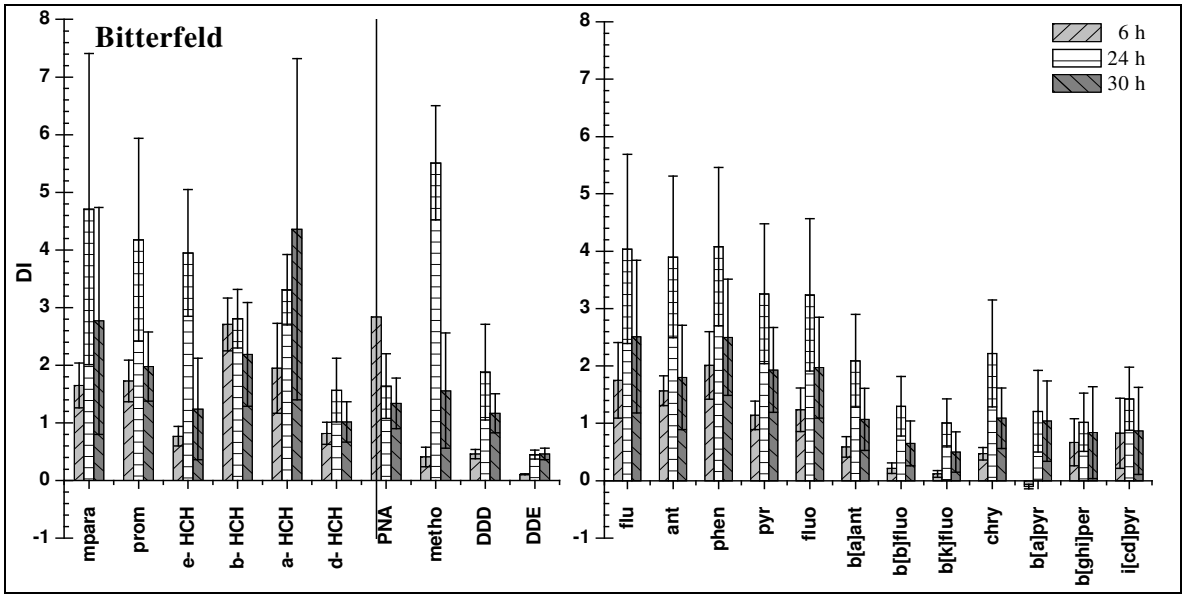

Figure 4-3: *Differences in desorption behaviour of sediment-associated contaminants comparing fresh and freeze-dried samples from the small creak Spittelwasser in Bitterfeld. The mean values of three replicates are shown and standard deviations are given as error bars.*

Bitterfeld sediment showed the greatest differences between the desorption of sediment-associated contaminants from freeze-dried and fresh sediments. The Desorption Index was in general positive reaching maximal values of up to 5.51 (methoxychlor). Similar to the desorption behaviour of Most contaminants, an extraction time of 24 h lead to the greatest deviations, whereas smallest deviations occurred at 6 h extraction time. With the exception of DDE, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene, DIs exceeded a value of 0.2 for all extraction times indicating that the deviations from the fresh sediment exceeded 20 % in most of the cases. For the PAHs a substance dependence could be observed since DI-values decreased with increasing molecular weight and thus lipophilicity.

Whereas standard deviations were commonly smaller than 20 % for the majority of the compounds identified in Přelouč, Most and Magdeburg sediments, the results of Bitterfeld sediment showed much higher standard deviations of up to 60 and 70 %.

4.4 Discussion

Freeze-drying is a common method to avoid microbial as well as chemical degradation processes in sediment samples. The goal of the present study was to investigate the influence of freeze-drying on the desorption behaviour of sediment-associated contaminants as determined with TENAX® extraction method. It was demonstrated that desorption can be elevated (Magdeburg, Bitterfeld) as well as hampered (Přelouč, Most) after freeze-drying being hard to predict. No substance dependent pattern, but an extraction time dependency was observed since the deviations were smallest at an extraction time of 30 h. The presented results are consistent with results of several groups showing unpredictable changes including increased or decreased toxicity or a lack of changes when testing freeze-dried sediment assuming an alteration of the bioaccessibility of sediment-associated contaminants [84, 89].

Figure 4-4 summarizes the results with a special focus on the time dependence of the extracted fraction, whereas location dependent trends should be stressed rather than substance dependent patterns. The solid line included in Figure 4-4 illustrated the ideal case of $F_{freeze\text{-}dried} = F_{fresh}$ implying an identical desorption behaviour of contaminants from fresh and freeze-dried sediment. Symbols above this line indicate an elevated desorption from the freeze-dried sediment, whereas symbols below the solid line mean a hampered desorption. The dotted lines mark a deviation of ± 20 % from the ideal case $F_{freeze\text{-}dried} = F_{fresh}$. With the exception of results from Bitterfeld samples, that deviation reflects the maximal standard deviation observed within these experiments. As sediment of Bitterfeld consists of a compact bulk phase due to its high sand and low water content (Table 6-1), the homogenisation of the sediment and thus the production of equal subsamples is hampered.

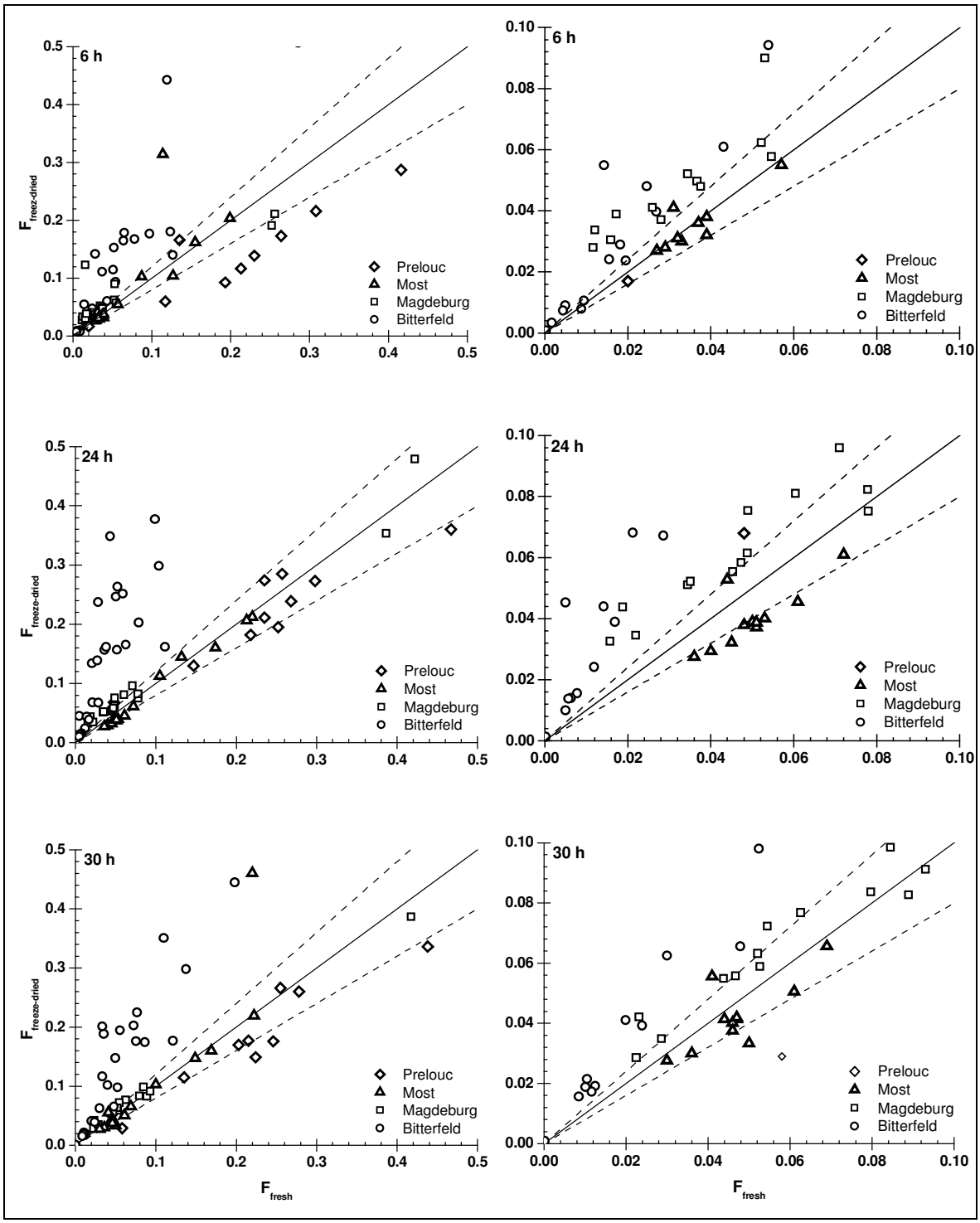

Figure 4-4: *Correlation of the fraction extracted from freeze-dried ($F_{freeze\text{-}dried}$) and fresh (F_{fresh}) sediment. Sediments from Přelouč, Most, Magdeburg and Bitterfeld were extracted with TENAX® for 6 h, 24 h and 30 h. The solid line indicates the ideal case of $F_{freeze\text{-}dried} = F_{fresh}$, whereas the dotted lines mark a deviation of ± 20 % from the ideal case. The graphs in the right column present an enlargement of the range between F= 0 and F= 0.1 of the graphs depicted in the left column.*

At an extraction time of 6 h, only the desorption of Most contaminants was not influenced significantly by the sediment state having almost all data points within the

± 20 % deviation interval. The influence of freeze-drying on the desorption behaviour of sediment contaminants decreased with the extraction time since almost all data points were within the ± 20 % deviation interval prolongating the applied extraction time up to 24 h or 30 h. The sediment of Bitterfeld location behaved special since freeze-drying caused an enormous mobilisation of sediment contaminants. Even for an extraction time of 30 h, $F_{freeze\text{-}dried}$ exceeded F_{fresh} by a factor up to 6.0. Sediment of Bitterfeld is known to contain traces of disposed resins possibly trapping organic compounds. Freeze-drying might destroy the resin structure making formerly trapped compounds easily desorbable and thus accessible for TENAX® extraction.

Several reasons might be responsible for observed changes in the desorption behaviour of sediment associated contaminants due to the freeze-drying procedure involving re-moistening processes of the sediment particles, elevated Dissolved Organic Carbon (DOC) concentrations in the aqueous phase as well as a destruction of sediment aggregates.

Firstly, when remoistening freeze-dried sediment, the particle surface will be coated by a thin liquid film and water enters the pore system initiating the dissolution and partitioning of compounds according to their distribution properties. The time required for these processes might be greater than the time provided by the TENAX® extraction procedure implying moistening and partitioning artefacts rather than real freeze-drying alterations. Therefore, exemplarily freeze-dried sediment of Přelouč was soaked for 6 h and 24 h prior to the TENAX® extraction procedure for 6 h and 24 h (Figure 4-5). Soaking of freeze-dried sediment for 6 h prior to the TENAX® extraction for 6 h as well as 24 h caused a Desorption Index (DI) between -0.81 to 0.23. However, soaking had no influence on the desorption behaviour, neither at an extraction time of 6 h nor at 24 h since DI-values were equal to those of un-soaked sediment. This rule was violated by fluoranthene and benzo[*b*]fluoranthene as well as fluorene and anthracene. Values for the former pair indicated that the differences between fresh and freeze-dried sediments decreased, whereas the deviations are enhanced by the soaking procedure for fluorene and anthracene. No explanation could be found for this behaviour. Soaking freeze-dried sediments for 24 h yields similar results showing no influence on the desorption behaviour of the sediment contaminants. It may be assumed that soaking of freeze-dried sediment prior to the TENAX® extraction has a minor influence on the extracted fraction. Moistening artefacts can thus be excluded as reason for the different desorption behaviour of contaminants originating from freeze-dried sediment.

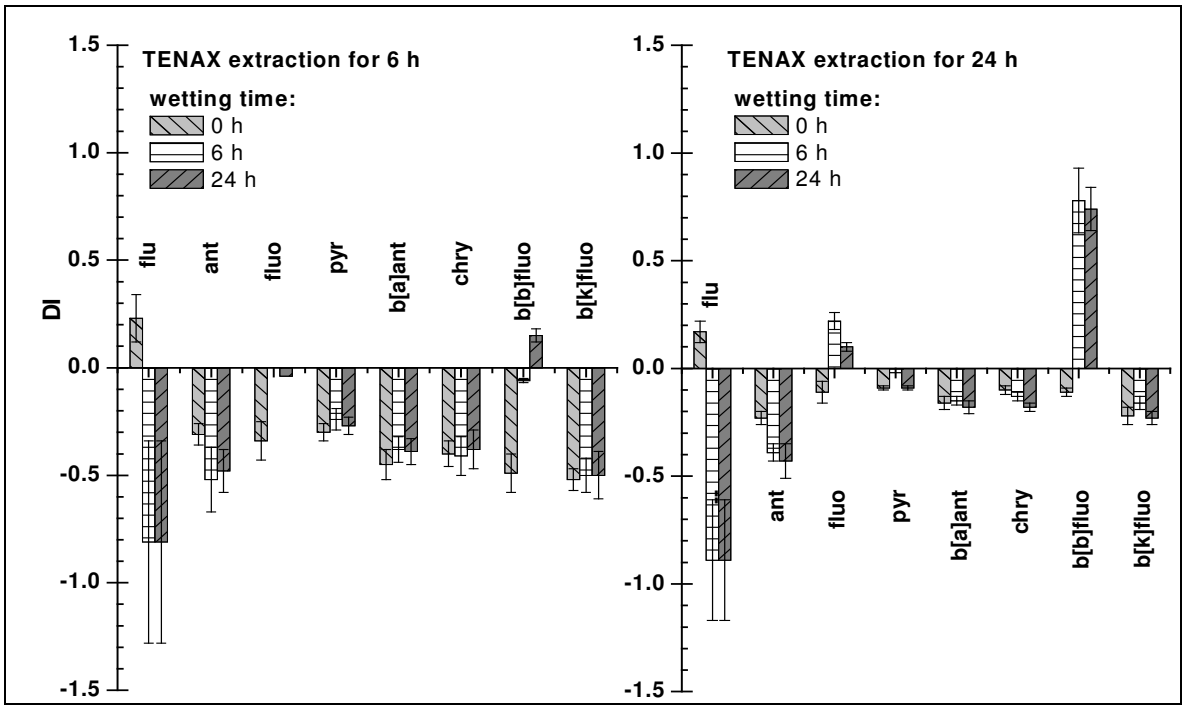

Figure 4-5: *Soaking of freeze-dried sediment from Přelouč for 6 h and 24 h prior to the TENAX® extraction procedure for 6 h and 24 h. Presented are mean values of the Desorption Index (DI) calculated according to Eq. 4-1. Error bars reflect standard deviations of three replicates.*

Secondly, several groups detected elevated DOC levels in elutriates of freeze-dried sediment [92, 93]. But DOC is known to cause a complexation of freely dissolved compounds and thus a reduction of the bioavailable faction [94]. Since the fraction sorbed on TENAX® will be composed of the fraction already dissolved in the water phase (bioavailable) and the rapidly desorbing fraction [72] elevated DOC levels might be an explanation for lower concentrations when investigating freeze-dried sediment.

Thirdly, through freeze-drying, including the previously freezing of the wet sediment, aggregates might be destroyed due to physical forces. This might result in baring of intraparticle sorption sites and thus in shorter diffusion distances for the molecules making formerly slowly desorbing molecules to rapidly desorbing ones. Thus, the destruction of sediment aggregates can ease the transformation from the adsorbed to the desorbed state resulting in an increased leaching of sediment-associated contaminants what was also observed by Geffard et al. [84].

As can be seen in Figure 4-4 F_{fresh} and $F_{freeze\text{-}dried}$ converge with increasing extraction times assuming that the deviations between the fraction extracted from fresh and dried sediments, especially at short extraction times, might be explained by an alteration of

the desorption rate rather than an alteration of the rapidly desorbing fraction itself. Figure 4-6 might clarify that fact presenting the fluoranthene concentration in Přelouč sediment after a 6 h, 24 h and 30 h single TENAX® extraction. The solid lines included in Figure 4-6 were only given to visualize the desorption progress and do not represent fitted curves since a fitting based on only three points was not feasible.

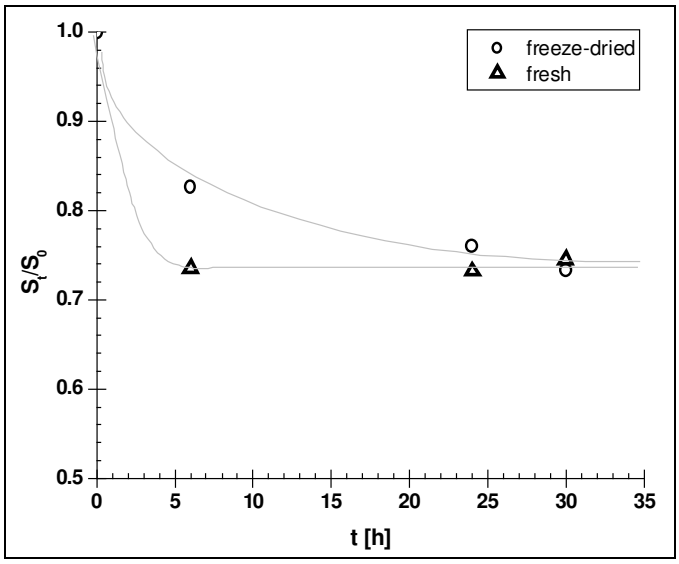

Figure 4-6: *Desorption of fluoranthene measured in fresh and freeze-dried sediment of Přelouč, plotting the sediment concentration S_t normalized to the initial concentration S_0 versus the extraction time [h]. Solid lines are only given to visualize the desorption progress and do not represent fitted functions.*

Figure 4-6 indicated that the concentration of fluoranthene in freeze-dried sediment decreased more slowly compared to fresh sediment, whereas after an extraction time of 30 h the concentration in fresh and freeze-dried sediment was comparable. Since desorption from sediment is characterized by an initial rapid release followed by a slower release of sorbed contaminants, concentrations in sediment will not significantly decrease after depletion of the rapidly desorbing fraction. Thus, through a prolongation of the extraction time up to 30 h differences between desorption rates from fresh and freeze-dried sediments might be compensated. Through a prolongation of the extraction time, the slowly desorbing fraction will contribute to the extracted amount as well. But due to smaller rate constants the contribution of slowly desorbing molecules to the overall trapped fraction will be comparatively small and thus negligible.

Birdwell et al. [95] showed that rate constants depend on numerous factors such as the aqueous diffusivity, the organic carbon content as well as bulk particle density and

porosity. Diffusion processes as main mass transport mechanism had only a minimal influence on the desorption rate. Furthermore, the organic matter fraction as most important component responsible for sorption and desorption was not correlated with the rate constant [95]. The way how rate constants are affected and the reasons behind cannot be defined yet.

Summarizing, freeze-drying might alter either the concentration in the aqueous phase or the desorption properties of sediment-associated contaminants. However, results suggest a change of the desorption rate rather than of the bioaccessible fraction itself. These alterations might be compensated by an adaptation of the extraction time to obtain comparable results for fresh and freeze-dried sediments in the context of a bioaccessibility-directed extraction. No information exists concerning the reasons behind these changes.

4.5 Conclusions

The use of freeze-dried sediment instead of fresh ones can result in an underestimation as well as an overestimation of the desorbing fraction. As TENAX® extraction is known to selectively trap the bioaccessible fraction these results would imply a wrong estimation of the bioaccessibility using freeze-dried instead of fresh sediments. Deviations between the rapidly desorbing fraction of fresh and freeze-dried sediments were shown to be smaller than 20 % if the selected extraction time is long enough. Deviations can be assumed in most cases to be caused by altered desorption rates rather than changes in the bioaccessible fraction. Thus if the extraction time is long enough to compensate the changes in the desorption rate, it might be feasible to use freeze-dried sediment to assess the bioaccessibility of sediment-associated contaminants. The use of freeze-dried sediment poses a possibility to store sediment especially if the test design does not allow to immediately test the sediment or if repeats become necessary at a later date.

However, it was shown at the example of Bitterfeld sediment, that freeze-drying can also cause an enormous mobilization of sediment-associated contaminants e.g. through the destruction of either sediment or resin aggregates. That example raises the question whether such an enormous mobilizations might occur in the environment as well under extreme events such as river channel-dry up in an aridity period or through mechanical forces during flood or dredging events. Thus freeze-drying might be a suitable method to simulate such a “worst-case-scenario” in the laboratory.

5 Application of the large volume TENAX® method as basis of an Effect-Directed Analysis of contaminated sediments

5.1 Introduction

Sediments are often polluted with a broad spectrum of compounds. High concentrations of pesticides, polycyclic aromatic compounds, chlorinated compounds or pharmaceuticals were measured in harbours, lakes and sedimentation zones of rivers [96-99] affecting benthic communities or accumulating in food webs. The Water Framework Directive demands the improvement of the ecological and chemical status of water bodies until 2015. But without any knowledge about the relevant chemical stressors, neither pollution sources can be located nor counteractions be initiated. A linkage of chemical causes to observed effects is thus essential. The EDA procedure, as a combination of physico-chemical fractionation steps, biotesting and chemical analysis, enables the consecutive reduction of the mixture complexity by separating biologically not active compounds from active ones.

EDA studies of sediments are mostly based on total extracts neglecting that only a fraction of sediment contaminants is accessible to biota while a significant and compound-dependent percentage is sequestered and may not desorb from sediment particles within a relevant time frame of hours or days. Kreitinger et al. [100] investigated sediments from manufactured-gas plants concerning toxicity towards *Hyalella azteca*. They could show that effects were unrelated to the total PAH concentration since some samples with high concentrations did not effect growth and survival, during several sediments with significant lower contaminant concentrations inhibited growth and survival of the test organism nearly completely. Similar results were obtained by Paine et al. [101] and West et al. [102] investigating effects towards marine amphipode and aquatic invertebrate assuming a reduced exposure as explanation for the observed lack of toxicity at high contaminant levels. Thus, the introduction of bioaccessibility in EDA may increase the environmental realism of this approach and helps to identify hazards rather than solely the general potential to exhibit effects.

Bioaccessibility is defined on the basis of desorption kinetics and may be seen as one process determining bioavailability of sediment-associated compounds [20]. Bioaccessibility was operationalized by applying mild extraction methods using TENAX® [21, 70], cyclodextrin [61, 62], mild solvents [103] or supercritical fluids [63]. Recent

research showed a good correlation between rapidly desorbing fractions extracted by TENAX® and fractions bioaccumulating in benthic organisms [64, 104], with a lower contribution of the slowly and very slowly desorbing fractions [105]. TENAX® is regarded as an infinite sink that quickly traps lipophilic compounds desorbing from sediment [83] and can be easily separated from sediment suspensions. So far, only for TENAX® a large volume extraction method is available that allows the extraction of sufficient amounts of sediment for a subsequent EDA [106].

The goal of the present study was to determine the influence of bioaccessibility on the outcome of an EDA in terms of the prioritization of hot spots. Therefore, the work was organized in three steps: Firstly, three sediment samples, collected at the river Elbe and two tributaries, were bioaccessibility-directed extracted with the large volume TENAX® method and fractionated on three coupled normal-phase HPLC columns to reduce the complexity of the samples. In the second step, parent extracts, fractions as well as recombined samples were tested towards growth inhibition of green algae *Scenedesmus vacuolatus*. In a third step, effective fractions were chemically analysed to identify and quantify potential toxicants. The sampling locations were ranked according to the toxic potency of fractions and related to toxicant concentrations. To assess the influence of bioaccessibility on the outcome of the EDA and thus prioritization of sampling locations according to risk, results of chapter 2 are comprised as a reference.

5.2 Material and Methods

5.2.1 Bioaccessibility-directed sediment extraction, clean up and fractionation

Fresh sediments from Přelouč (P), Most (M) and Bitterfeld (B) were sieved to 2 mm to remove coarse material. Samples for the TENAX® procedure were stored in dark at 4 °C until required. For more information see chapter 6.1.

The bioaccessibility-directed extraction was performed as described in chapter 6.2.2 using approximately 125 g of sediment (dry weight), 180 g of ASE-cleaned TENAX® and at least 3 L of de-ionised water. The loaded TENAX® was separated from the sediment suspension after 24 h of stirring and extracted with acetone and hexane. To ensure that a sufficient amount of the sample is available for the performance of an EDA, including fractionation, biological testing and chemical analysis, the extraction procedure was performed 5 times for each sediment. TENAX® beads were checked for background toxicity prior to the extraction of sediment samples.

Clean up of TENAX® extracts was performed after solvent exchange to dichloromethane using gel permeation chromatography (see chapter 6.2.3) to remove elemental sulphur and macromolecular matrix components. To avoid a plugging of capillaries samples were filtrated through a glass cartridge equipped with a glass microfibre filter and a PTFE-frit. Cleaned raw extracts are following named parent extract (pe).

To reduce the sample complexitiy and separate toxic from non-toxic components, parent extracts of the three sediment samples were fractionated on three coupled normal-phase HPLC columns including a cyanopropyl silica (CN), nitrophenyl silica (NO) and porous graphitized carbon (PGC) column utilizing the compound polarity, the number of aromatic rings and the chlorination degree, respectively (for a detailed description see chapter 6.2.4).

5.2.2 Toxicity towards green algae *Scenedesmus vacuolatus*

Parent extracts, fractions and recombined samples as well as blanks of GPC and TENAX® were tested towards growth inhibition of green algae *Scenedesmus vacuolatus*. Samples were provided in dimethyl sulphoxide (DMSO) with a constant concentration of 0.1 % in the test system comparing the population growth to that of untreated controls. Geometrical dilution series were prepared to cover effects between 10 and 90 % with at least 4 data points. Exact test conditions are described in chapter 6.3.

Experimental data were fitted applying the Hill model (Eq. 6-4, chapter 6.3) providing an exact mathematical description of the concentration-response relationships. On that basis the concentration evoking an inhibition of 30 % (EC_{30}) can be calculated for a characterization of concentration-response plots.

The influence of the applied extraction method on observed effects was assessed by correlating TENAX®- and ASE-based (chapter 2) toxic potencies. The EC-ratio, calculated on the basis of Eq. 5-1 provided a quantitative measure of the distance between related concentration-response curves of ASE (see chapter 2.3.2) and TENAX® fractions and thus an estimate of the extent of bioaccessibility.

$$EC-ratio=\frac{EC_x^A}{EC_x^T}$$

Eq. 5-1

where ^{A} and ^{T} mark ASE and TENAX® fractions. EC is the effective concentration evoking x % inhibition calculated on the basis of the Hill parameters. The index x ranges from 10 % to 60 % inhibition. Since some curves did not reach an inhibition of 60 %, their effective concentration range was calculated for maximal effects of 40 and 50 % inhibition, respectively.

5.2.3 GC-MS analysis

Compound identification and quantification was performed as described in chapter 6.2.5 applying a gas chromatograph combined with a mass selective detector (GC-MS). The oven was programmed from 50 to 300 °C with 4 °C/min holding this temperature for 6.5 min. Identification of the compounds was conducted in the Total Ion Current (TIC) mode comparing the retention time and mass spectra with that of standard compounds. An external calibration enabled the quantification of the sample components. For that purpose, the GC-MS was operated in the selected ion mode (SIM).

Concentrations of identified compounds were compared to the respective concentrations in ASE fractions (see chapter 2.3.3) calculating the c-ratio:

$$c-ratio = \frac{c^{T}}{c^{A}}$$

Eq. 5-2

whereas c is the concentration [µg/g(sed dw)] in ASE (^{A}) and TENAX® (^{T}) samples, respectively.

5.3 Results

The goal of the present work was to investigate the influence of bioaccessibility on prioritization of fractions with respect to growth inhibition of *Scenedesmus vacuolatus.* TENAX®-derived extracts of three sampling locations were fractionated, biologically and chemically analysed and compared to samples extracted exhaustively gained with Accelerated Solvent Extraction (ASE). ASE results, as already given in chapter 2, are solely presented as reference.

5.3.1 Biological screening of HPLC fractions

Testing HPLC fractions of TENAX® sediment extracts at one concentration identified $P8^T$ to $P11^T$, $P15^T$ as well as $B14^T$ as toxic to green algae (Figure 5-1). Effects of Most fractions did not exceed effects of 30 % when tested at a concentration of 32 g SEq/L(medium). With effects of 84 %, 30 % and 74 %, $P10^T$, $B10^T$ and $B14^T$ exhibited the highest effects of the respective sampling location. For Přelouč and Bitterfeld these effects were greater than effects caused by the parent extract (pe) accounting for 61 % and 51 %, respectively. Effects of Most fractions ($M10^T$) equalled that of the related parent extract (29 %).

Figure 5-1 additionally contained screening results of the ASE-extracts (see chapter 2.3.2) to compare the influence of the bioaccessibility on the toxicity of sediment samples. Přelouč TENAX® fractions exhibited a similar toxicity pattern as related ASE fractions with $P8^T$ to $P11^T$ and $P15^T$ as effective fractions. Except $P8^T$, with a fractional inhibition of only 32 %, effects were comparable to the effects caused by respective ASE fractions tested in same concentrations. For Most and Bitterfeld significant differences between the effects of ASE and TENAX® fractions occurred since tested TENAX® fractions did not cause effects greater than 30 %. This rule was violated by $B14^T$ inhibiting the algal reproduction about 74 % and thus evoking effects comparable to $B14^A$. $P10^T$, $M10^T$ and $B14^T$ were the most toxic fractions of the three sampling sites being identical with the ASE fractions identified as most toxic as well.

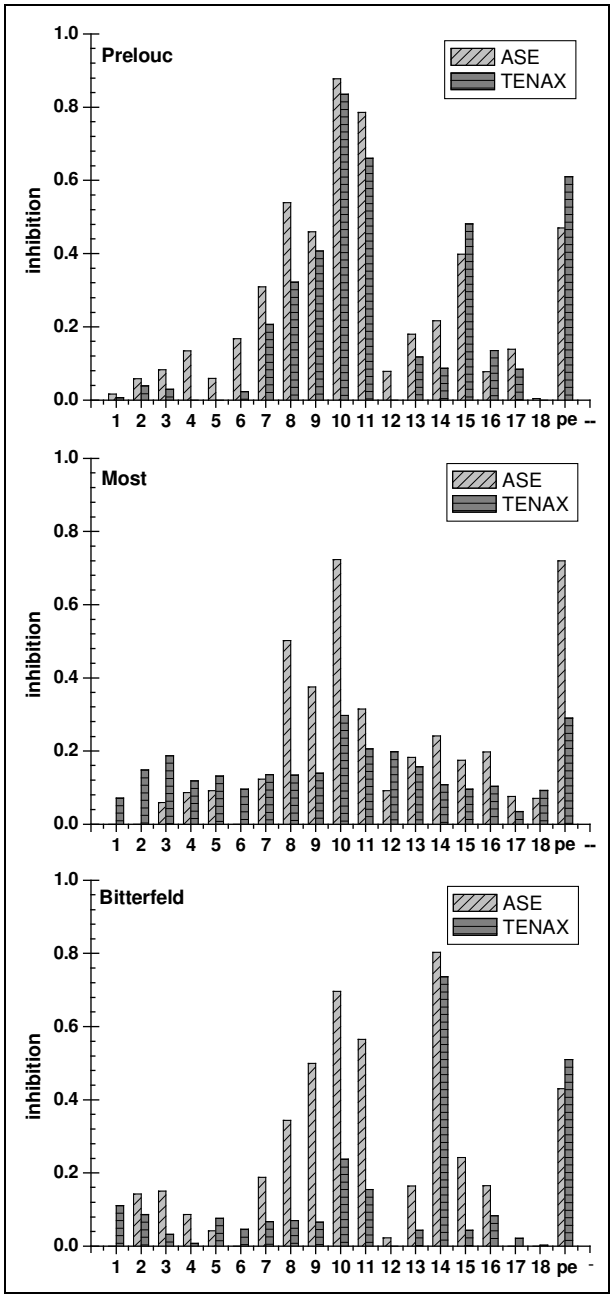

Figure 5-1: *Toxicity patterns of the three sampling locations are presented showing the fractional inhibition of reproduction of Scenedesmus vacuolatus evoked by F1 to F18 and the parent extracts (pe) of ASE and TENAX® samples. Fractions were tested in following concetrations: Přelouč (ASE and TENAX®: 32 g SEq/L(medium)), Most (ASE and TENAX®: 32 g SEq/L(medium)), Bitterfeld (ASE: 156 g SEq/L(medium), TENAX®: 152 g SEq/L(medium)).*

Testing of fractions at one concentration yielded a relative ranking of the fractions with respect to their toxicity. Based on these results, for parent extracts, F8 to F11 of all three sampling locations, P15 as well as B14 complete concentration-response relationships were prepared. Effective fractions were subjected to chemical analysis for an identification and quantification of potential toxicants.

5.3.2 Concentration-response relationships of the parent extracts, recombined samples and the effective fractions

Firstly, parent extracts (pe) of the three sampling locations were tested to gain information about the toxic potency of the bioaccessible fraction preparing complete concentration-response relationships. For clarity of the figures, only fitted functions were presented since the Hill model was proved to accurately describe concentration-response relationships (chapter 2.3.1). To show the influence of the chosen extraction method and thus the accessibility of sediment contaminants on green algae, ASE pe were given as well.

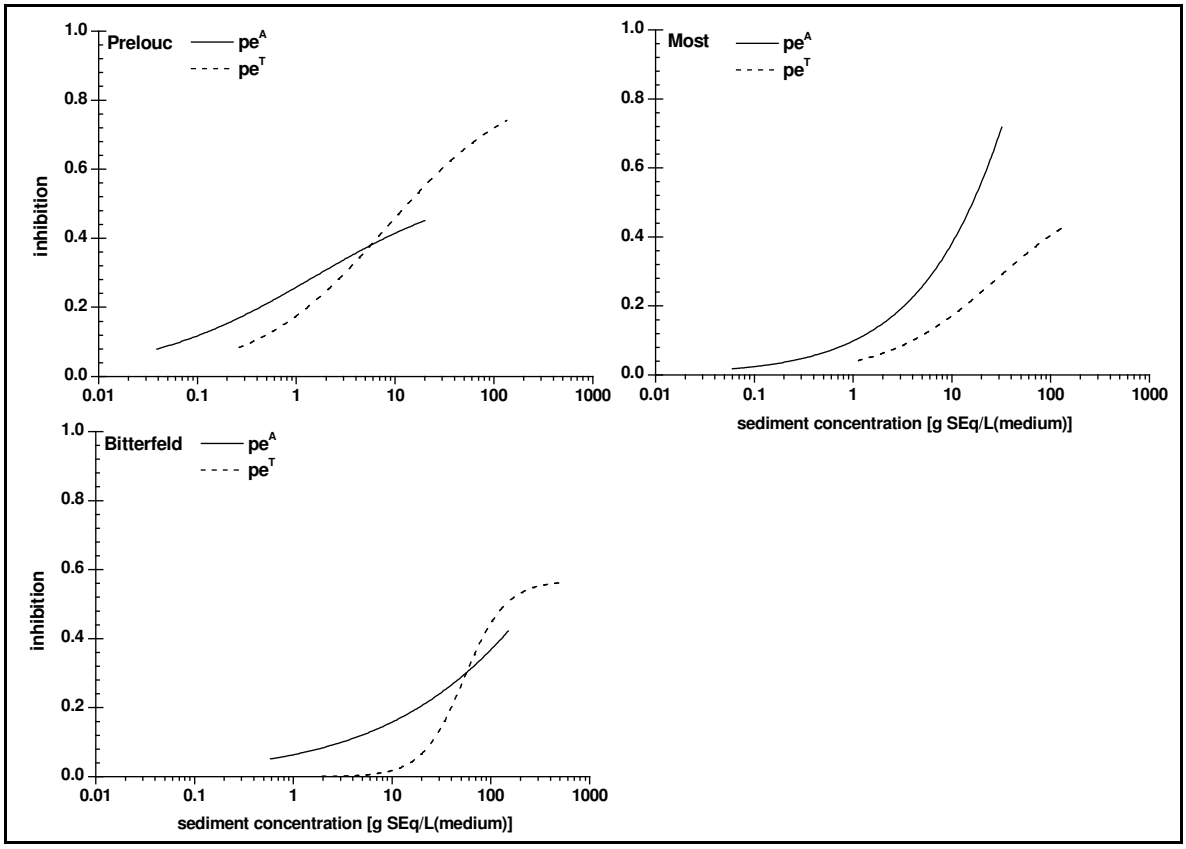

Figure 5-2: *Growth inhibition of Scenedesmus vacuolatus exhibited by ASE- and TENAX® parent extracts (pe^A, pe^T) of Přelouč, Most and Bitterfeld sediment. Only fitted functions are presented.*

TENAX® parent extracts were tested at concentrations between 0.01 and 1000 g SEq/L(medium) (Figure 5-2). The maximum inhibition as achieved by pe was in all cases lower than 100 % in the tested concentration range. In case of Most it only accounted for 43 %. Testing the extracts in higher concentrations was not feasible, since the solubility of pollutants would be exceeded in water as well as in the DMSO phase. To characterize

the sample toxicity, the concentration causing an inhibition of 30 % (EC_{30}) was calculated (Table 5-1). Whereas EC_{30} values of Most and Bitterfeld pe^T were with 34.6 and 56.4 g SEq/L similar, Přelouč pe^T was effective at concentrations one order of magnitude smaller.

Comparing the effects of bioaccessibility- and exhaustively-derived extracts, related parent extracts (pe^A and pe^T) of Přelouč and Bitterfeld sediment had almost similar EC_{30}s, whereas the EC_{30} of Most pe^T is, with a value of 34.58 g SEq/L about 5 times higher than the EC_{30} of the pe^A (6.56 g SEq/L).

For fractions inhibiting population growth by more than 30 % when tested at one concentration (Figure 5-1), complete concentration-response relationships were prepared (Figure 5-3). Experimental data was fitted using the Hill function presenting the calculated parameters in Table 5-1.

All tested TENAX® fractions evoked sigmoid concentration-response curves varying in their position, slope and maximal observed effect. Toxic potencies were compared on the basis of calculated EC_{30} values (Table 5-1). Whereas EC_{30} values of Přelouč TENAX® fractions ranged from 5.66 to 32.73 g SEq/L(medium), the toxic potency of Most and Bitterfeld fractions was lower with EC_{30} values between 66.77 to 254.53 g SEq/L(medium) and 66.71 to 255.12 g SEq/L(medium), respectively. $P10^T$, $M10^T$ and $B14^T$ were identified as most toxic TENAX® fractions of the respective sampling location. The slope of a concentration-response curve was reflected by the p-value of the calculated Hill parameters. Slopes were more consistent than the EC_{30} values since almost all values fell between 0.62 and 1.74 with the exception of $B11^T$ and $B14^T$. With p-values of 2.63 and 4.64, respectively these curves were significantly steeper than the remaining concentration-response curves. Maximal observed effects were quite different varying between 47 % ($M9^T$) and 91 % ($M8^T$).

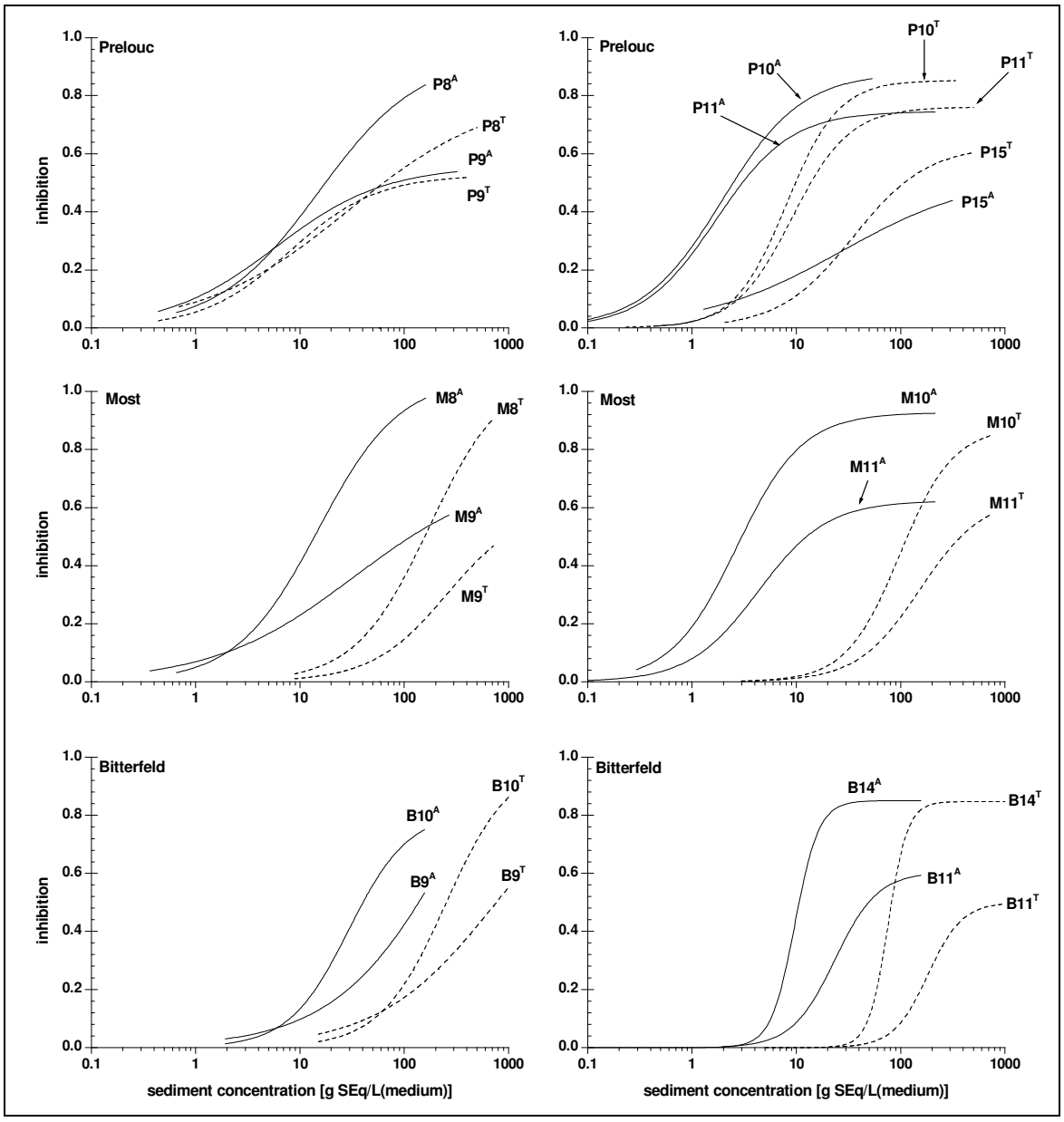

Figure 5-3: *Concentration-response relationships of effective fractions. Solid curves indicate ASE fractions (Fi^A), whereas dotted curves represent TENAX® fractions (Fi^T).*

To clarify the influence of the selected extraction method on growth inhibition of green algae, concentration-response relationships of effective TENAX® fractions were compared to ASE-based effects, described in chapter 2. Therefore, Table 5-1 additionally contained EC_{30} and p values of related ASE fractions (chapter 2.3.2). As for Přelouč and Bitterfeld fractions EC_{30} values of the TENAX® fractions were in the same order of magnitude as related ASE ones deviating by a factor of not more than 6 and 9, respectively, effective concentrations of Most TENAX® fractions were at least one order of magnitude greater than related ASE ones. For $M10^T$ and $M11^T$ the EC_{30} was up to 43

and 35 times higher than that of $M10^A$ and $M11^A$, respectively. Ranking and prioritization of ASE and TENAX® samples identified the same fractions as most toxic (P10, M10, B14) and confirmed the ranking as already achieved by testing only one concentration (Figure 5-1). Corresponding ASE and TENAX® fractions exhibited similar slopes while the TENAX® curves were shifted parallel to higher concentrations compared to those based on ASE. This rule was violated by $P8^T$, $P10^T$ and $M9^T$.

Table 5-1: *Concentration-response relationships of parent extracts (pe,) recombined samples (remix) and effective fractions of the TENAX® (^{T}) samples with respect to algal growth inhibition. Experimental data were fitted using Hill function (Eq. 6-4) showing the square of correlation coefficient (R^2), the concentrations [g SEq/L] inhibiting algal growth about 30 % (EC_{30}), the slope of the curve (p), the maximal inhibition (a) as well as the number of data points (N) and controls (C). Additionally, EC_{30} and p values for ASE fractions (^{A}) are presented.*

	N^T (C^T)	$EC_{30}{}^T$ [g SEq/L]	$EC_{30}{}^A$ [g SEq/L]	p^T	p^A	a^T	$R^{2\,(T)}$
Přelouč							
pe	30 (18)	3.11	1.78	0.65	0.50	0.86	0.93
remix	15 (6)	6.22	2.65	1.07	0.71	0.63	0.94
P8	21 (12)	12.39	6.53	0.62	0.90	0.81	0.93
P9	26 (18)	10.38	7.06	1.04	0.83	0.53	0.94
P10	27 (18)	5.66	1.09	1.74	1.14	0.85	0.98
P11	23 (18)	6.98	1.23	1.58	1.22	0.76	0.98
P15	18 (12)	32.73	41.28	1.22	0.65	0.63	0.97
Most							
pe	24 (12)	34.58	6.56	0.78	0.62	0.56	0.94
remix	18 (12)	58.51	8.72	0.68	1.60	0.56	0.96
M8	15 (12)	80.94	6.56	1.22	1.11	1.07	0.99
M9	15 (12)	254.53	19.37	1.20	0.64	0.60	0.96
M10	17 (12)	66.77	1.57	1.69	1.38	0.88	0.99
M11	17 (12)	141.97	4.08	1.44	1.31	0.64	0.97
Bitterfeld							
pe	27 (5)	56.41	55.89	2.05	0.42	0.57	0.95
remix	13 (6)	256.41	39.47	0.82	1.12	0.34	0.78
B9	15 (12)	255.12	55.04	0.77	0.76	1.00	0.92
B10	15 (12)	137.19	20.69	1.38	1.51	0.99	0.97
B11	15 (12)	213.16	23.28	2.63	2.11	0.50	0.85
B14	17 (12)	66.71	8.27	4.64	3.79	0.85	0.97

F1 to F18 of each sampling location were recombined and tested in algae assay as well ($remix^T$). For the remix of Přelouč and Most fractions the calculated EC_{30} values were greater but not more than by a factor of 2. The deviations were thought to be acceptable suggesting negligible procedural artefacts and losses. While effects of recombined samples

of Přelouč and Most fitted well with the original sample, the concentration-response curve of Bitterfeld remix was shifted to higher concentrations by a factor of 4.55. Toxic artefacts can be excluded since the recombined sample was less toxic than the parent extract. Losses during fractionation procedure and sample preparation might occur but showing no systematically occurring phenomena.

5.3.3 Quantification of key toxicants in effective HPLC-fractions

Effective TENAX® fractions (^{T}) were analysed for the compounds already identified in the related ASE fractions (chapter 2.3.3) suggesting no deviating compound pattern. Table 5-2 contained the compounds of each effective fraction as well as their concentrations. Concentrations in the ASE (^{A}) fractions were given additionally to assess the influence of the extraction method.

Přelouč TENAX® fractions contained polycyclic aromatic compounds in concentrations of 0.025 to about 1.312 µg/g sediment (dry weight) (Table 5-2). Concentrations in the TENAX® fractions were in the same order of magnitude as in ASE fractions or lower by a factor of not more than 2 to 3. No explanation could be found for the high concentration of benzo[*ghi*]fluoranthene in $P9^{T}$, which exceeded the concentration in $P9^{A}$.

Concentrations in Most TENAX® fractions vary between only 0.0003 and 0.129 µg/g sediment (dry weight). Whereas concentrations in Most ASE fractions were quite similar to those in Přelouč, concentrations in Most TENAX® fractions were one to two orders of magnitude lower than in corresponding ASE fractions.

The Bitterfeld fractions contained the smallest concentrations of PAHs with 0.003 to 0.038 µg/g (dry weight) in TENAX® fractions. Concentrations in TENAX® fractions fell below those in ASE fractions by a factor of 3 to 10.

Table 5-2: *Identified compounds and respective concentrations [µg/g] in the effective ASE (^{A}) and TENAX® (^{T}) fractions. Concentrations refer to the dry weight of the sediment.*

		Přelouč		***Most***		***Bitterfeld***	
		c^A [µg/g]	c^T [µg/g]	c^A [µg/g]	c^T [µg/g]	c^A [µg/g]	c^T [µg/g]
F8	**fluoranthene**	1.773	1.312	1.098	0.087	0.136	0.026
	pyrene	0.882	1.054	1.161	0.129	0.117	0.026
	benzo[*b*]naphtho(2,3-*d*)furan	./. [(a)]	./.	0.043	0.002	./.	./.
	11H-benzo[*b*]fluorene	0.236	0.211	0.135	0.019	0.015	0.003
	benzo[*b*]naphtho(1,2-*d*)thiophene	0.051	0.025	0.025	0.002	./.	./.
	benzo[*b*]naphtho(2,3-*d*)thiophene	0.046	./.	0.018	0.0003	0.003	0.0003
F9	**benzo[*ghi*]fluoranthene**	0.044	0.125	0.104	0.012	0.024	0.008
	benzo[*a*]anthracene	0.859	0.323	0.482	0.015	0.060	0.005
	chrysene	1.103	0.459	0.622	0.032	0.077	0.010
F10	**benzo[*b*]fluoranthene**	0.842	0.321	0.475	0.022	0.071	0.011
	benzo[*j*]fluoranthene	0.373	0.235	0.226	0.011	0.029	0.005
	benzo[*k*]fluoranthene	0.406	0.135	0.194	0.006	0.027	0.003
	benzo[*a*]fluoranthene	0.158	0.040	0.104	0.003	0.013	0.001
	benzo[*e*]pyrene	0.644	0.222	0.386	0.018	0.047	0.006
	benzo[*a*]pyrene	0.664	0.215	0.433	0.016	0.054	0.005
	perylene	0.177	0.077	0.172	0.016	0.014	0.002
F11	**indeno[1,2,3-*cd*] fluoranthene**	./.	./.	0.011	0.001	./.	./.
	indeno[1,2,3-*cd*] pyrene	0.442	0.100	0.246	0.007	0.049	0.005
	benzo[*ghi*]perylene	0.516	0.088	0.015	0.002	0.050	0.004
	dibenzo[*ah*]anthracene	./.	./.	0.360	0.012	./.	./.
F14	***N*-phenyl-2-naphthylamine**	./.	./.	./.	./.	0.586	0.038
F15	**7H-benzo[*de*]anthracen-7-one**	1.178	0.895	./.	./.	./.	./.

[(a)] *./. means not detected*

5.4 Discussion

The aim of the present study was to determine the influence of bioaccessibility on the results of an EDA. Therefore, sediments were extracted with TENAX® to selectively trap the bioaccessible fraction. Whereas pollutant patterns were comparable, the degree of bioaccessibility significantly differs for the three sampling locations. A strong reduction of toxicity towards green algae was observed for Most location when applying TENAX® samples. Similar results were obtained by Puglisi et al. [107] extracting sediment exhaustively as well as bioaccessibility-directed with TENAX® and cyclodextrin. A strong reduction of DR-CALUX activity was found when testing TENAX® and cyclodextrin

derived extracts. It was shown that the implementation of ecotoxicologically relevant extraction techniques can have an important influence on the outcome of a risk assessment, especially in the case of very low bioaccessibilities.

Bioaccessibility is an important factor that determines the toxicity of sediment contaminants and thus the potential risk originating from the contaminated sites. TENAX® extraction is a widely accepted tool to yield bioaccessible fractions [21, 60, 64, 106] while ASE was proved to extract total amounts of organic contaminants [108]. Thus, a comparison of fraction-specific toxicity as well as concentrations of identified compounds achieved with both methods has been used to gain information on bioaccessibility. This comparison is visualized in Figure 5-4 showing the EC-ratio, defined as EC_x^A/EC_x^T (Eq. 5-1), as well as the concentration-ratios (c-ratio), calculated from c^T/c^A (Eq. 5-2), of the effective fractions and identified contaminants, respectively. Since EC-ratios can depend on effect levels and c-ratios are compound-specific, ranges rather than single values are given for both ratios.

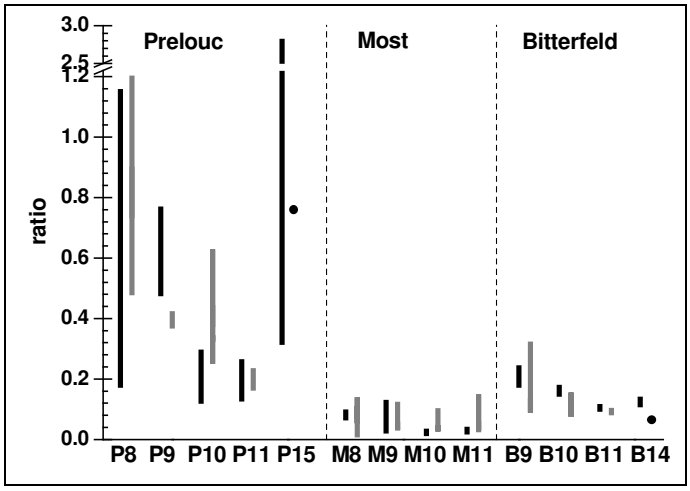

Figure 5-4: *Differences between the bioaccessibility-directed and exhaustive extraction method as indicated by the growth inhibition of Scenedesmus vacuolatus (EC-ratio, black line) and determined concentrations of potential toxicants (c-ratio, grey line).*

The toxicity pattern for Přelouč fractions based on one concentration indicated small differences comparing the inhibition caused by ASE and TENAX® fractions (Figure 5-1). This was confirmed by recording full concentration-response curves (Figure 5-3). The curves for $P8^A$ and $P8^T$ were almost identical at low effect levels, while at higher effect levels ASE-based effects exceeded the inhibition caused by the related TENAX® fraction. Non-parallel behaviour is indicated by big ranges of EC-ratios in Figure 5-4.

Changes in slopes as found for $P8^A$ and $P8^T$ as well as $P15^A$ and $P15^T$ indicate changes in the relative shares of different components. This finding is supported by a great range of the c-ratios that indicates a different bioaccessibility of the components of P8. Reduced concentrations together with a constant relative composition of a fraction should lead to a parallel shift of concentration-response curves towards higher concentrations. P10 and P11 exhibit the expected parallel shift of the related TENAX® concentration-response curves towards higher concentrations (Figure 5-4*)*. For the polar fraction P15 ASE and TENAX®-based fractions were effective in the same concentration range suggesting almost full bioaccessibility in agreement with the polar nature of expected components (7H-benzo[*de*]anthracen-7-one). Similar concentrations of the toxicant in ASE and TENAX® fractions support this assumption. In contrast, the non-parallel shift of the ASE and TENAX® based concentration-response curves suggests an alteration of the sample composition. As already mentioned in chapter 2.4, $P15^A$ is deep red coloured interfering the fluorescence analysis of the algae assay. Using TENAX® to extract the sediment might reduce the amount of fluorescent components assuming a weaker disturbance of the fluorescence analysis rather than an altered sample composition as explanation for the non-parallel curve shift. Biological analysis of $P9^A$ and $P9^T$ indicates a full bioaccessibility in spite of the lipophilic, unpolar nature of the expected components. Contrarily, chemical analysis detected deviating ASE and TENAX®-based concentrations varying about a factor of up to 3. The discrepancy between the high bioaccessiblity and the lower TENAX®-based concentrations might be explained by unidentified and highly accessible components since the confirmation of the identified toxicity detected unresolved toxicity (see chapter 2.3.4).

As indicated by toxicity patterns (Figure 5-1) and confirmed by concentration response curves bioaccessibility of the toxicants in Most is low for all four fractions. While concentration-response curves of TENAX® fractions of M8 and M9 are shifted to about one order of magnitude higher concentrations, for more hydrophobic fractions M10 and M11 the shifts are in the range of about a factor of 30 to 50. This is supported by concentrations although some individual compounds show bioaccessibilities of up to 10 % compared to the total concentration (Table 5-2).

The toxicity pattern of Bitterfeld suggests only a limited bioaccessibility of PAHs in fractions B9 to B11 while B14 with *N*-phenyl-2-naphthylamine as a major toxicant inhibits algal growth in a similar range for both extraction procedures (Figure 5-1). The limited bioaccessibility of PAH fractions is confirmed by shifted concentration-response

curves by a factor of 5 (B9) to 10 (B10) (Figure 5-3). The analysis of the full concentration-response curves of $B14^A$ and $B14^T$ seems to contradict the finding from pattern analysis (Figure 5-1) by a shift of the TENAX® curve to about one order of magnitude higher concentrations. The explanation of this phenomenon is that the tested concentrations of the screening experiment were in the domain of maximal inhibition for both extraction methods that does not allow any discrimination between different toxic potencies. This finding points out the significance of recording concentration-response relationships rather than one-concentration effect measurements. The limited accessibility of *N*-phenyl-2-naphthylamine may be due to specific interactions with sediment components. Bitterfeld sediments are known to contain traces of disposed adsorbent resins being a possible explanation of the low accessibility even of moderately hydrophobic compounds.

Discrepancies between chemical and biological analysis occur as well. For M8, M10, M11, B9 and B10 small EC-ranges are contradictory to great c-ranges meaning parallel concentration-response curves in spite of an altered sample composition. Some of the identified compounds might not be toxic in the considered concentration range and therefore do not contribute to the observed whole toxicity. In order to exclude non-effective compounds from this consideration, the contribution of the individual compounds to the whole toxicity can be determined improving the correlation between biological and chemical findings.

For all three sediments a reduction of bioaccessibility from small PAH-fractions to large PAH-fractions can be observed (F8 to F11). This is consistent with results of Shor et al. [109] investigating the desorption of field aged PAHs by TENAX® extraction. They found that larger, more hydrophobic PAHs less extensively desorb than three-ring PAHs, for example evoking a significant negative correlation between the $\log K_{OW}$ and the desorbed PAH fraction within one day. This can be ascribed to the higher affinity of high molecular weight PAHs to black carbon or organic matter and lower molecular diffusion rates [110]. Large PAHs are mostly of pyrogenic origin. They are formed in parallel with soot and might enter the environment already associated with soot, whereas petrogenic PAHs enter the environment dissolved in water. The low bioaccessibility is therefore caused by entrapping in soot particles rather than a high lipophilicity [78].

PAHs are often identified in environmental samples as they are ubiquitous pollutants. They can arise from anthropogenic as well as from naturally occurring processes, for example the combustion of fossil fuels (pyrolytic origin), slow maturation of

organic matter (petrogenic origin) as well as the degradation of biogenic precursors (diagenic origin) [96]. An (eco)toxicological relevance of that individual substance class arises since PAHs are known to cause carcinogenic and mutagenic effects [111] as well as narcotic and photoinduced toxicity against many organisms, amongst them green algae [46]. Observed adverse effects on fresh water ecosystems and EROD activity were completely or partially attributed to the presence of different mixtures of PAHs [48, 112]. PAHs are highly lipophilic, enter the environment mostly bound on particles and are highly persistent. Due to atmospheric deposition and surficial run-off they enter the aquatic ecosystem and enrich in sediments. A remobilization of contaminants can occur by the disturbance of the equilibrium between sediment and dissolved phase, for example by flood events or dredging actions, whereas the arising (eco)toxic potential and risk is strongly determined by the extent of the bioaccessibility.

The sediment composition [75, 113], the contact time between pollutant and sediment [114, 115] and the compound properties [116, 117] are major factors affecting bioaccessibility. Since PAHs are often associated with soot and black carbon [118, 119] and soot-associated PAHs are known to hardly desorb in relevant time frames [110, 120] it was hypothesized that increasing contents of black carbon may reduce bioaccessibility [75, 121]. However, for our samples the differences can not be predicted from Total Organic Carbon (TOC) contents (6.1 % in Přelouč and 6.4 % in Most, 0.6 % in Bitterfeld, see Table 6-1). This suggests that at least for fresh sediments close to emission sources other factors like the pathway of emission may play a dominant role. If for example at one location pyrogenic PAHs are entered associated with soot particles while at other sites petroleum waste emissions dominate the PAH input, the TOC content appears to be a poor predictor for bioaccessibility. The extent of desorption can neither be estimated solely from compound properties (lipophilicity) nor from sediment chemical and physical properties (organic matter content, porosity, specific surface area) what was shown by Shor et al. [109] due to the multitude of determinant factors and the complexity of their interactions.

In summary, contaminants of Přelouč sediment pose the greatest risk since a great fraction of the PAHs is bioaccessible and thus available for the uptake into organisms. Bioaccessibilities of toxicants in all fractions decrease in the order Přelouč > Bitterfeld > Most. PAHs in Přelouč may be characterised as particularly highly bioaccessible while Most is at the low extreme of bioaccessibility. The prioritization of effective fractions identified P10, M11 and B14 as most toxic fractions independent of the selected extraction method. But at the example of similar total concentrations of toxicants in Přelouč and Most

sediment that result in bioaccessible concentrations in Přelouč that exceed those in Most by a factor of 10 to 20, the introduction of bioaccessibility strongly influences the risk prioritization of fractions and hot spots. The sample composition can be affected by the applied extraction procedure as well due to a substance dependent bioaccessibility as can be seen by non-parallel shifts of related concentration-response curves. These facts might explain why the fractions of the three sampling locations behave biologically quite different in spite of the same identified compounds. Bioaccessibility concepts are not recognized in regulatory processes yet, as they are perceived as “do nothing” or “do less” approaches [122]. But these results stress the influence of bioaccessibility on the prioritization of contaminated sites. The introduction of bioavailability studies in risk assessment strategies might improve the accuracy of risk assessment what was also recommended by Oen et al. [70].

5.5 Conclusions

Effect-Directed Analyses of three sediment samples from different industrial hot spots of the River Elbe basin agreed in the identification of PAHs as major toxicants to green algae while in Bitterfeld and Přelouč additional toxicants such as *N*-phenyl-2-naphthylamine and 7H-benzo[*de*]anthracen-7-one play an important role. Commonly sediment-associated PAHs are considered as hardly bioavailable. The application of bioaccessibility-directed extraction with TENAX® demonstrated that a significant fraction of PAHs in sediment can be bioaccessible and might cause adverse effects e.g. to benthic algae. The bioaccessible fraction of PAHs as well as its composition strongly depend on the specific sample and are hardly predictable by sediment properties. TENAX® extraction with a subsequent EDA proved to be a powerful tool to prioritize contaminants and contaminated sites with respect to hazards of sediment-associated toxicants to algae. Although bioaccessibility affects toxic hazards in a compound-specific way, no pronounced changes in the prioritization of fractions due to different extraction methods has been found in our study. However, it may be questioned whether this is general rule or occurred only in our specific case.

6 Experimental section

6.1 Sediment sampling

Fresh sediments were collected at four locations that are strongly influenced by chemical industry, urban waste waters and relicts of former industrial plants. Figure 6-1 shows a map of the selected hot spots.

One hot spot of the river Elbe is located downstream of the industrial region of Pardubice near the city of Přelouč (Czech Republic). Waste waters originating from the production of different chemicals like pigments and dyes, oxy- and nitrocellulose, diphenylamines, hexogene, inorganic salts and acids, solvents, pharmaceuticals, isocyanates and nitriles [123, 124] and from the Pardubice waste water treatment plant are discharged into the Elbe.

The tributary Bilina is a small river in Northern Bohemia flowing in the Elbe near the city of Ústí nad Labem. Sediments were sampled near the city of Most (Czech Republic) being characterized by a quite complex pollution situation. Discharges from waste water treatment plants, petrochemical, textile as well as chemical industry 8.5 km upstream near Litvinov producing phthalates, acrylates, benzene-, toluene- and xylene-aromates, polyolefines and agrochemicals [125] determine the pollutant input in the river as well as brown coal mining near the city of Litvinov.

The small creek Spittelwasser, as a tributary to the river Mulde, is strongly influenced by the heavily polluted area of Bitterfeld (Germany). The industrial area of Bitterfeld was known for the production of dyes, pesticides, synthetic materials, acids, ion exchanger and chemical weapons [126]. Today, a broad spectra of compounds is still produced like disinfectants, algicides, dyes, chlorine, hydrogen and hydrogenperoxide, methylcellulose, resin lacquers and pharmaceuticals [127].

Additionally, sediment from the Elbe near the city of Magdeburg was sampled. The sampling location was near to the former chemical and pharmaceutical plant Fahlberg-List GmbH producing pharmaceuticals, acids, biocides and herbicides [128] leaving mobilizable relicts like high concentrations of HCH. This sampling location is not influenced anymore by the direct discharge of a production site [129], but rather by discharges of the Mulde and Saale river.

For all locations diffuse input caused by communal and industrial point sources has to be considered as well.

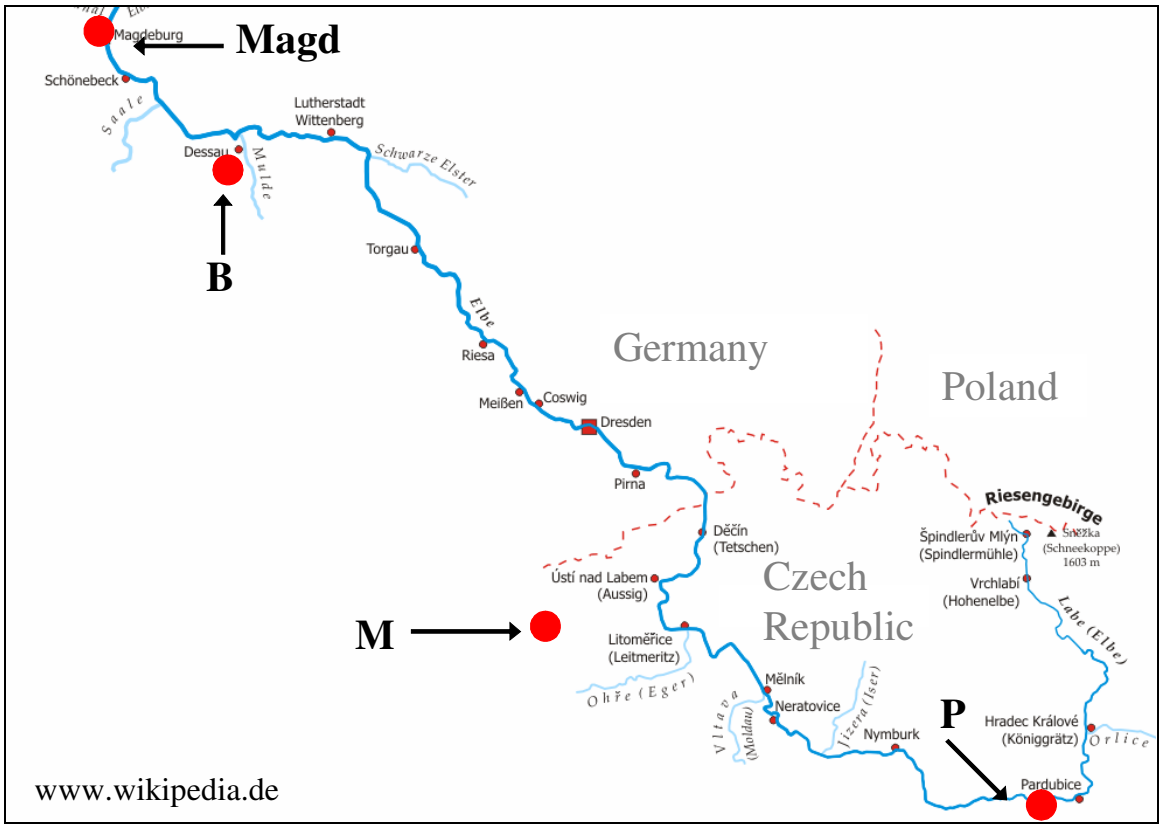

Figure 6-1: *Map of the river Elbe basin showing the selected sampling locations. Magdeburg (Magd) and Přelouč (P) represent locations at the Elbe, whereas samples were also taken from the tributaries Spittelwasser near the city of Bitterfeld (B) as well as Bilina near the city of Most (M).*

The sampling was performed using an Ekman-Birge-grab. Sediments were wet sieved (2 mm) to remove coarse material and stored in amber bottles at 4 °C until required. Prior to experiments, sediments were homogenized. Sediments were characterized by the Total Organic Carbon (TOC) and the water content. The TOC was determined applying an elemental analyzer heating a freeze-dried sub-sample to 580 °C after removal of inorganic carbon with hydrochloric acid. A moisture analyzer Sartorius MA40 (Göttingen, Germany) was used to determine the water content heating a sub-sample to 110 °C and recording the weight until it stays constant. Both measurements (TOC and water) were carried out in triplicate.

Due to the limited storing time of fresh sediment, several batches were sampled, having the same location only differing in the sampling date. Table 6-1 contains information about the sampling date and for what purpose the sediment was used.

Table 6-1: *Sampling locations with the respective abbreviation (abbr), coordinates and the sampling date. Sediment characteristics are given with total organic carbon (TOC) and water (H_2O) content [%]. When several batches were sampled for one location, the use of the respective batch is listed including the chapter number and the page.*

location	abbr	coordinates	sampling date	used for (chapter, page number)	TOC [%]	H_2O [%]
Přelouč (CZ, Elbe)	P	N 50° 02‘ 33.5’’ E 15° 34’ 36.5’’	July 05	freeze-dried (chapter 4, p. 41) kinetic (chapter 3, p. 25)	5.03	72.0
			May 06	ASE (chapter 2, p. 5) TENAX® (chapter 5, p. 53)	6.10	68.6
Magdeburg (D, Elbe)	Magd	N 52° 04‘ 47.5’’ E 11° 40‘ 29.3’’	Nov 04	freeze-dried (chapter 4, p. 41)	7.46	60.9
Most (CZ, Bilina)	M	N 50° 30’ 19.5’’ E 13° 41’ 03.3’’	Apr 05	freeze-dried (chapter 4, p. 41)	7.36	74.1
			Jul 06	ASE (chapter 2, p. 5) TENAX® (chapter 5, p. 53)	6.35	65.6
Bitterfeld (D, Spittelwasser)	B	N 51° 41’ 30.0’’ E 12° 17’ 22.5’’	Apr 05	freeze-dried (chapter 4, p. 41)	5.69	24.5
			Nov 06	ASE (chapter 2, p. 5) TENAX® (chapter 5, p. 53)	0.62	33.3

6.2 Chemical methods

The following chapter contains information about extraction techniques, the sample clean up as well as the fractionation and chemical analysis procedures.

Firstly, to make sediment associated contaminants available for further investigations like chemical or biological analysis, sediments have to be extracted, whereas the asked question determines the selected method, for example the total sediment concentration or just the bioaccessible fraction. Background information and applied conditions are given on the following pages.

After the extraction step, samples have to be cleaned to avoid interferences with chemical and biological analysis due to elemental sulphur as well as matrix components. To reduce the complexity, samples were fractionated according to three different separation principles.

6.2.1 Exhaustive sediment extraction

Exhaustive extraction of sediment makes the total amount of compounds available for further investigations. Thus, Accelerated Solvent Extraction (ASE) was used (Dionex Corp., Sunnyvale, USA) providing a fast extraction method with a relatively low solvent consumption [130]. The difference to commonly applied techniques, like Soxhlet extraction, is that the extraction is performed under an elevated temperature to enhance the extraction kinetics. Since the applied temperature might be above the boiling point of the

solvents an elevated pressure is required to keep the solvent below the boiling point and thus liquid [131]. Figure 6-2 shows a scheme of the mode of operation of an ASE. Briefly, the stainless steel cell containing the sediment is introduced into the oven, filled with solvent and pressurized. The conditions are hold for a specified time periodically flushing the cell with fresh solvent (static cycle). When the extraction is complete, the solvent is removed from the cell by compressed nitrogen completing the static cycle. The static cycle can be repeated several times.

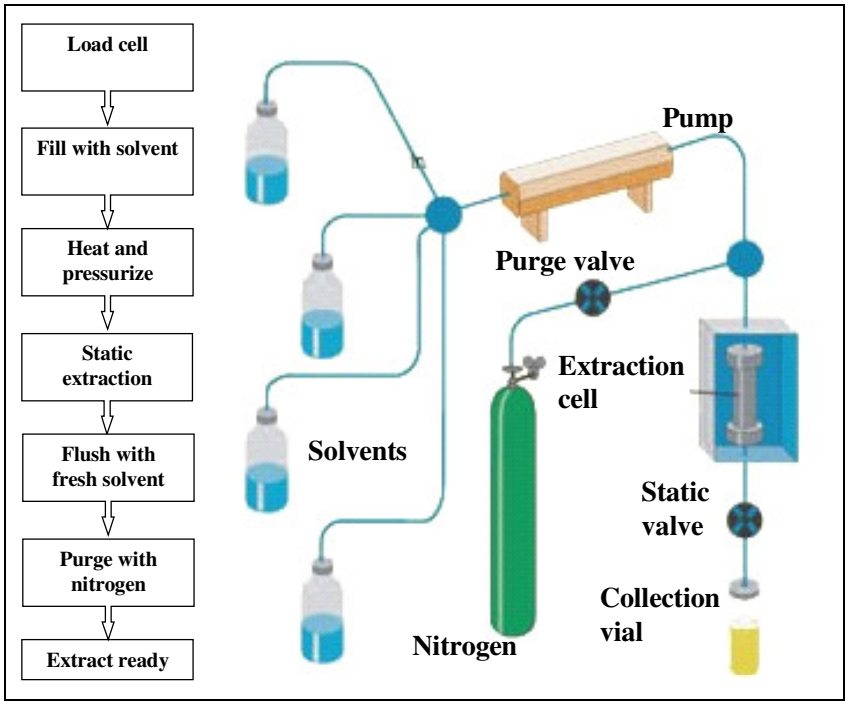

Figure 6-2: *Working principle of the Accelerated Solvent Extraction (copied from [131]).*

Sediments were mixed with Isolute HM-N (IST Ltd., Hengoed, UK) to bind water in case of fresh sediments or to prevent clogging of the ASE cartridge if freeze-dried sediments were used. Three static cycles, each lasting 10 min, were run at 50 °C and 103 bar using acetone and dichloromethane (25:75 v/v) as solvents. The volume of the samples was reduced by the means of rotary evaporation.

For the determination of the desorption kinetics and the validation of the large volume approach, the extraction procedure was slightly modified. The extraction was carried out at 100 °C and 103 bar using a mixture of acetone and hexane 1:1 (v/v).

6.2.2 Bioaccessibility-directed extraction with TENAX®

Background

TENAX® (Figure 6-3) is a polymer sorbent based on 2,6-diphenyl-*p*-phenylene oxide being synthesized by oxidative coupling. The polymer is not cross-linked and partially crystalline as well as glassy with a glass-to-rubber transition temperature of 227 °C [83]. TENAX® beads have an average diameter of 0.5 to 0.6 mm and are more or less homogeneous aggregates of small particles. Figure 6-3 (right side) depicts a schematic configuration of an agglomerated TENAX® bead consisting of mircrospheres and intraparticle pores (copied from Zhao & Pignatello [83]).

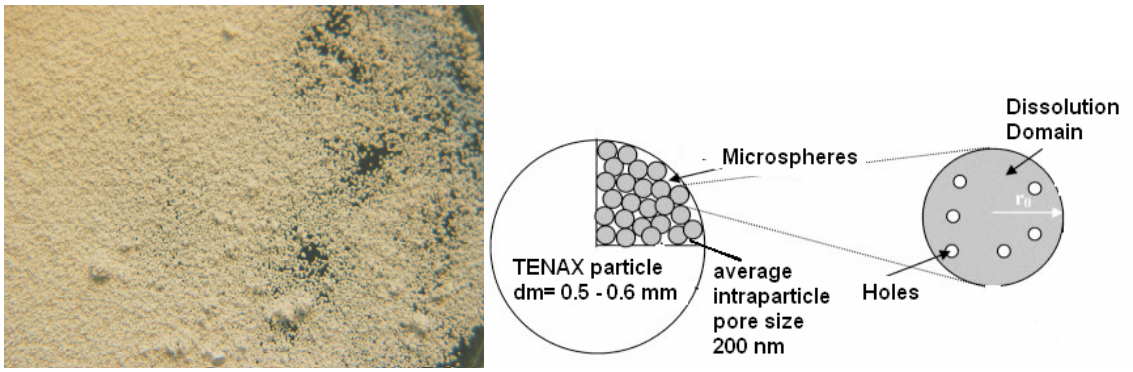

Figure 6-3: *Photo and schematic picture (according to [83]) of TENAX®.*

When TENAX® beads are introduced into a sediment suspension, organic substances dissolved in the pore water will immediately sorb onto TENAX®. By depleting the aqueous phase desorption of organic compounds sorbed on the sediment particles will be stimulated. Only extracting the amount already dissolved in the aqueous phases and actually desorbing molecules, the TENAX® method poses a possibility to characterize the bioaccessible fraction of sediment associated contaminants [60, 65]. Therefore, desorption from the sediment, diffusion through the water phase and sorption on TENAX® beads are the main steps.

Firstly, the desorption from the sediment is determined amongst others by the contact time between sediment and contaminant. At the first contact, molecules will solely adsorb on the outer surface of the sediment particles. But with increasing contact time, molecules are able to diffuse in the pore system of sediment particles to more remote sites and possibly become sequestered within the sediment matrix [132]. In reverse, molecules

will need more time to desorb from sediment particles. The given contact time between sediment and TENAX® determines therefore the extracted amount.

By shaking the sediment TENAX® suspension, the diffusion is eliminated as rate limiting step. Thus, the extraction method solely depends on how the substances desorb from sediment and how they sorb on TENAX® beads.

Sorption of organic compounds on TENAX® beads is characterized by the compound affinity to TENAX®, its capacity and the uptake rate from water into TENAX®. Cornellisen et al. [72] were able to show that the compound affinity to TENAX® equals that of the organic carbon in the sediment for chlorobenzenes, PAHs and PCB. A correlation between the fraction accumulated in biota (BSAF) and the fraction entrapped in TENAX® could be presented for PAHs and PCBs [60] demonstrating the ability of TENAX® to act like a surrogate for living organisms. TENAX® has a very high capacity and an equilibrium between sediment-water-TENAX® will hardly be reached. Thus, TENAX® beads act like an infinitive sink maintaining the concentration gradient between sediment and water phase stimulating the desorption from the sediment.

Using TENAX® for sediment extraction aimed at the investigation of the desorption of organic compounds from sediment, and not the sorption on TENAX® beads. Therefore, it has to be ruled out that the uptake into TENAX® is the rate limiting rather than the desorption from the sediment. Cornelissen et al. [72] was able to show that the rate constants for the extraction of the aqueous phase were greater than the rate constants of the desorption from the sediment by at least one order of magnitude keeping the aqueous phase solute free. Thus, sorption on TENAX® is much more faster than desorption from sediment being the rate limiting step.

One practical requirement is the separation of the TENAX® beads from the sediment suspension to further investigate the extracted fraction. When placed in water, beads are wetted only poorly due to their high hydrophobicity. Some air remains in the resins pores causing that TENAX® will float on the top immediately when the sediment-TENAX® suspension is not shaken anymore. TENAX® can thus be easily separated by the use of a separation funnel since the floated beads stick at the glass wall when discharging the sediment suspension. Figure 6-4 illustrates these circumstances showing a TENAX® layer on the top of the sediment suspension as well as at the glass wall. Sediment particles do not adhere well to TENAX® minimizing the inclusion of sediment particles. An inclusion of sediment particles into TENAX® beads would cause an

erroneous determination of the desorbed fraction since the amount sequestered on the included particles would be extracted as well.

TENAX® preparation

The standard cleaning method of fresh TENAX® beads comprises rinsing them with water, acetone and hexane (each 30 mL/g TENAX®) and drying at 60 °C and 110 °C holding each temperature for 2 hours [105].

Further cleaning steps were undertaken using ASE since the bio assay with green algae detected measurable effects caused by TENAX® blanks. At least 6 static cycles of 10 min each were run at 100 °C and 103 bar. Methanol, acetone, hexane and acetone:hexane 1/1 (v/v) were used as solvents. If extracts were still coloured, extraction with the respective solvent was continued until the extracts were colourless. The cleaned TENAX® was dried in a nitrogen stream at 60 °C, 110 °C and 200 °C holding each temperature for 2 h.

Prior to sediment extraction, TENAX® blanks were prepared and checked for background disturbance.

Consecutive sediment extraction

Investigating the desorption behaviour of sediment associated contaminants the concentration of pollutants in TENAX® will be kept low by a periodically renewing to avoid competitive effects due to saturation of TENAX® beads.

Consecutive desorption was determined at room temperature by means of an extraction method described previously [72]. A mixture of wet sediment (1 g dry weight), TENAX® (0.2 g) and bi-distilled water (70 mL) was shaken vigorously in a 100 mL separation funnel. Hg(II)-acetate was added (1.2 mg) to inhibit microbial activity. TENAX® was refreshed at periodic intervals and replaced by fresh beads. To address the rapid release of contaminants at the beginning of the desorption experiment TENAX® beads were refreshes every 30 min. After 4 h of extraction the intervals increase up to one hour replacing TENAX® after 5 h and 6 h of extraction. Until 8th day of extraction TENAX® was refreshed daily, followed by one refreshing at day 24 and the termination after 41 days. The loaded TENAX® was washed by shaking with bi-distilled water until the water phase was clear and subsequently rinsed in an amber glass bottle using firstly 10 mL of acetone and subsequently the same volume of hexane. A glass cartridge equipped

with a PTFE-frit enabled the separation of the TENAX® from the solvent after it was shaken over night. The volume of the extract was reduced by the means of rotary evaporation. TENAX® can be easily separated from the sediment since it sticks at the glass wall when discharging the sediment suspension from the separation funnel.

After termination of the kinetic studies, the remaining sediment was separated from the water phase and extracted with ASE (see paragraph 6.2.1) to analyze residual organic compounds.

Figure 6-4: *Picture of a separation funnel containing sediment and TENAX®.*

The desorption of sediment associated contaminants was described using a first-order three-compartment model [73] assuming no significant readsorption, independence of modelled fractions and simultaneous desorption of all three compartments:

$$\frac{S_t}{S_0} = F_{rap} \cdot e^{-k_{rap} \cdot t} + F_{slow} \cdot e^{-k_{slow} \cdot t} + F_{veryslow} \cdot e^{-k_{veryslow} \cdot t}$$

Eq. 6-1

Initial sediment-sorbed amounts (t=0) and remaining amounts at a given time t [h] are indicated by S_0 and S_t [µg], respectively. The dimensionless parameters F_{rap}, F_{slow} and $F_{veryslow}$ represent the rapidly, slowly and very slowly desorbing fractions, respectively, with corresponding rate constants k_{rap}, k_{slow} and $k_{veryslow}$ [h^{-1}].

Values for F_{rap}, F_{slow} and $F_{veryslow}$ and their respective rate constants were determined by a non-linear curve fitting on the basis of the Levenberg-Marquardt algorithm using Origin Software (Microcal™ Origin® Working Model, Version 6.0, Copyright© 1991-1999 Microcal Software, Inc.) allowing an accurate mathematical description of the desorption process.

Since desorption occurs simultaneously of the three compartments, F_{rap} can never be extracted individually. The TENAX® methods focuses on the extraction of an amount that is equivalent to F_{rap} (F_{rap_eq}) rather than on the extraction of F_{rap} itself. Thus, on the

basis of Eq. 3-1 and the calculated values for fractions and rate constants, a time t_{rap_eq} was determined at which F_{rap_eq} was extracted setting $S_t/S_0 = (1-F_{rap})$. Solver-function of Texas Instruments calculator TI-85 (Texas Instruments Incorporated, Dallas, USA) was used operating on the basis of a Newton interpolation. The amount extracted equals that contained in F_{rap} but integrates over all three fractions removed at t_{rap_eq}.

A simple example might make this procedure more clear. Given a F_{rap}= 0.4 for a compound (A) the time is calculated after which 40 % of the total sediment load of A is extracted being aware that this might mean for example only 90% of the extracted amount stems from the rapidly desorbing pool, but 9.9 % and 0.1 % originate from the slowly and very slowly desorbing pools, respectively.

Single extraction procedure in the small volume TENAX® approach

Whereas the consecutive extraction of sediment using TENAX® enables the stepwise extraction of sediment and thus the determination of the desorption kinetic of pollutants, it is time consuming and laborious. To solely harvest the fraction desorbing rapidly, the sediment can be extracted without the introduction of fresh TENAX® applying an elevated TENAX® mass [21].

Therefore, 1 g of sediment (dw), 1.5 g of cleaned TENAX® and about 70 mL of bi-distilled water were shaken vigorously in a 100 mL separation funnel for a selected period without introducing fresh TENAX®. The subsequent washing and extraction of the loaded TENAX® was performed as outlined above.

Large volume TENAX® extraction

For the large volume approach 125 g of sediment (dw) of Přelouč location, 180 g of TENAX® and at least 3 L of deionised water were used. A special glass vessel was constructed with following features: volume of 7 L, double-walled (for setting the temperature of the extraction) and a conical outlet (Figure 6-5). A two-propeller stirrer made of stainless steel prevents settling of sediment in the conical part of the vessel. Providing an exchangeable valve (with and without frit) enables both the sediment-extraction procedure using TENAX® (valve without frit) and the following extraction of TENAX® (valve with frit) in one vessel.

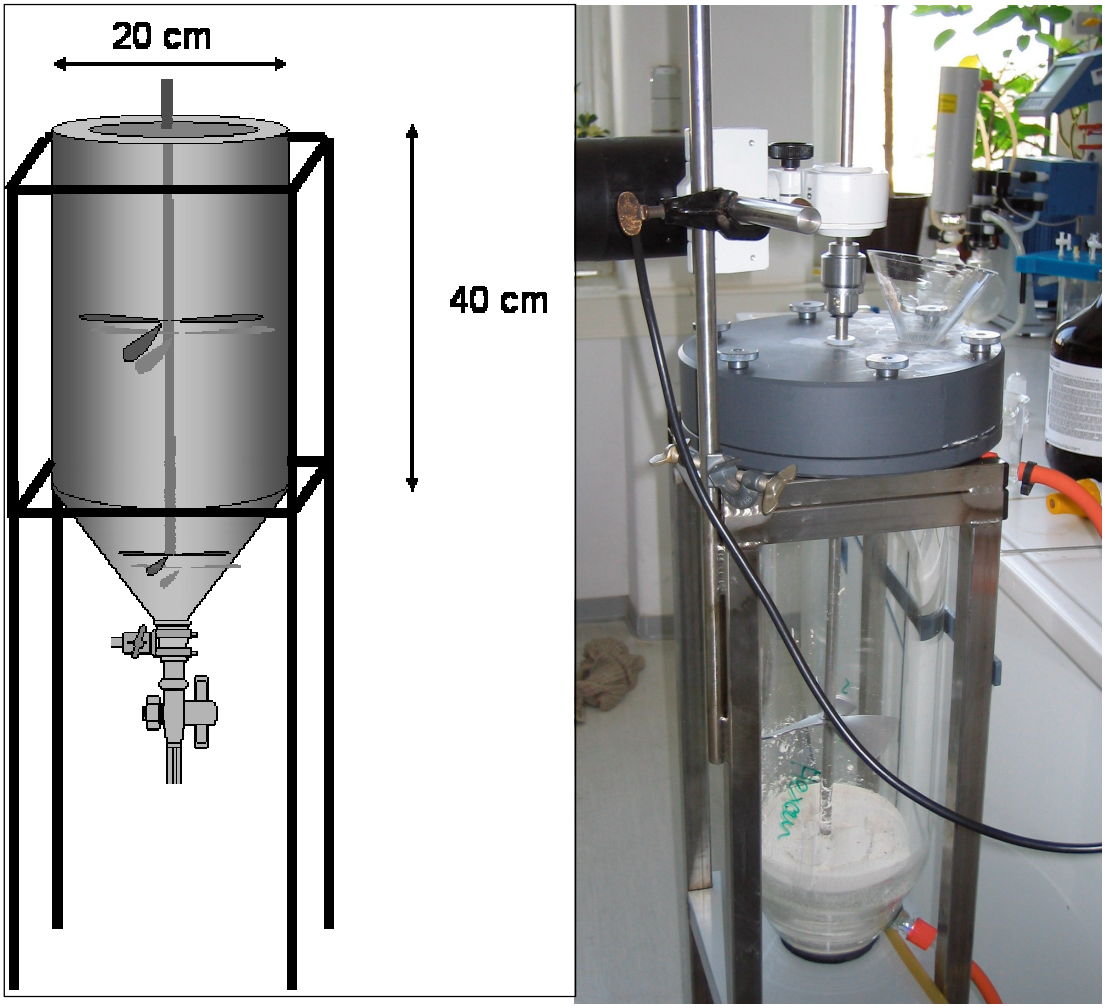

Figure 6-5: *Schematic drawing and a picture of the large volume extraction glass vessel.*

Following vigorous stirring at 20 °C for 24 h, the sediment suspension was isolated from the TENAX®, which forms a well separated layer on top of the suspension. The loaded TENAX® remained in the glass vessel and was washed until the water phase was clear. Bulk water was removed from TENAX® by applying a vacuum pump connected to the bottom outlet of the vessel valve equipped with a frit. TENAX® was extracted in the vessel by vigorous stirring five times with 0.5 L acetone and subsequently five times with 0.5 L of hexane. The volume of the combined acetonic extract (extract 1) was reduced by means of rotary evaporation. Subsequently, the mixture of residual water and acetone was liquid/liquid extracted with dichloromethane and hexane, dried with granular Na_2SO_4 (Merck, Darmstadt, Germany), reduced to dryness and re-dissolved in dichloromethane (extract 1). Hexane extracts (extract 2) were solvent exchanged with dichloromethane and combined with extract 1.

6.2.3 Clean up methods

Gel Permeation Chromatography (GPC)

Elemental sulphur and macromolecular matrix components interfere with the biological as well as with the chemical analysis and were removed taking advantage of their different molecular size. Separation was performed on a porous, highly cross-linked material consisting of small beads. As small molecules like elemental sulphur are able to diffuse into the beads resulting in greater retention times, macromolecular compounds like matrix components are not able to enter the interior of the beads eluting in shorter retentions times. Most sample components of interest will elute between macromolecular components and the elemental sulphur. To determine the elution window of the sediment contaminants, a standard solution containing a mixture of oil, sulphur as well as organic compounds of different molecular sizes was separated prior to the cleaning of sediment extracts.

The clean up was performed applying an automated Gel Permeation Chromatography (GPC) system (AccuPrep MPS™, Antec GmbH, Sindelsdorf, Germany). The chromatography column (3.5 * 38 cm) was filled with BioBeads S-X3 (200-400 mesh, J2 Scientific, Missouri, USA) and dichloromethane served as eluant. Chromatograms were detected at a wavelength of 254 nm. Prior to the clean up procedure, samples were evaporated to dryness in a gentle nitrogen stream and re-dissolved in dichloromethane. Extracts were filtrated using a glass cartridge containing a combination of glass microfibre filters and PTFE-frits to remove solid particles. Before cleaning of extracts, blanks were run and checked for background disturbance of the algae assay.

Activated copper

To remove elemental sulphur, samples were evaporated to dryness in a gentle nitrogen stream, re-dissolved in dichloromethane and shaken with an amount of activated copper covering the tip of a spatula over night. The copper was separated from the sample by using a glass cartridge equipped with a glass microfibre and a PTFE-frit. Since TENAX® extracts contained residual water, glass cartridges additionally contained $NaSO_4$.

Powdered copper needed to be activated prior to the cleaning procedure by rinsing it with 10 mL of concentrated hydrochloric acid (Merck, Darmstadt, Germany), 100 mL bi-distilled water and 100 mL acetone.

Adsorption chromatography

Adsorption chromatography using Alumina, Silica and Florisil (Merck, Darmstadt, Germany) formed the basis for the removal of pigments and macromolecular soluble sediment components. Van der-Waals or dipole-dipole-interactions cause a retention of the sample components evoking a stacked elution of pigments, matrix components and contaminants. Alumina as well as Silica was activated at 150 °C over night. Prior to the clean up, Alumina was deactivated with 10 % bi-distilled water and homogenised for 2 h. Florisil was activated by rising with methanol, DCM and following heated at 100 °C and 180 °C holding the temperature for 1 h and 4 h, respectively. The column was firstly filled with Alumina, followed by Silica and Florisil. Samples were eluted with hexane and a mixture of hexane:dichloromethane 1:1 (v/v).

6.2.4 Fractionation

To reduce the complexity of the extracts, samples were separated according to (i) compound polarity, (ii) non-polar compounds by the size of the aromatic system and (iii) chlorinated diaromatic compounds by their planarity and chlorination degree on three coupled and automatically switched normal-phase HPLC columns [33]. The separation system consists of a cyanopropyl silica (CN) column, where retention is determined by hydrogen bond abilities and dipole-dipole interactions between the stationary phase and the analyte, a nitrophenyl silica (NO) column, where interactions between the electron-depleted nitrophenyl group and the electron-rich π-electron systems of polycyclic compounds determine the compound retention and a porous graphitized carbon (PGC) column, where the separation is based on sterically eased or hindered interactions between the electron-enriched aromatic system of the stationary phase surface and the electron-depleted aromatic systems of chlorinated polycyclic compounds. 18 fractions (F) are obtained coeluting with polychlorinated naphthalenes (PCNs) and biphenyls (PCBs), dibenzo-*p*-dioxins/furans (PCDD/Fs), polycyclic aromatic hydrocarbons (PAHs) and heterocyclic compounds containing sulphur or oxygen in the aromatic system, quinones, nitro- and keto-PAHs. Table 6-2 shows an overview of fractionation windows and associated substance classes.

Table 6-2: *Fraction (F) number and associated substance classes including model substances in brackets are presented, whereas Cl means chlorine atoms. The related columns and eluents are given additionally.*

F	compound classes	column[(a)]	eluent[(b)]
1	no model compound identified	PGC + NO + CN	HX
2	PCBs, eluting in order of chlorination in ortho-position and chlorination degree, PCNs with 3 Cl		
3			
4	co-planar PCBs without chlorination in ortho-position, PCNs with 3 to 6 Cl	PGC	DCM:toluene
5	PCDDs/Fs. PCNs with ≥6 Cl		
6	small PAHs such as acenaphthylene with more than two aromatic rings	NO	HX:DCM
7	PAHs with three aromatic rings (anthracene)		
8	PAHs with four aromatic rings (pyrene)		
9	PAHs with four aromatic rings (chrysene)		
10	PAHs with five aromatic rings (benzo[*a*]pyrene)		
11	PAHs with six aromatic rings (indeno[1,2,3-*cd*]pyrene)		
12	PAHs with seven aromatic rings (coronene)		
13	mainly mononitro-PAHs	CN	DCM:ACN
14	(hydroxy-)quinones, keto-, dinitro-, hydroxy-PAHs, N-heterocycles with rising polarity		
15			
16			
17			
18			

(a) PGC (porous graphitized carbon), NO (nitrophenyl silica), CN (cyanopropyl silica)
(b) HX (hexane), DCM (dichloromethane), ACN (acetonitrile)

Separations were performed on an HPLC system composed of three high pressure pumps (Pro Star 410, Varian Inc., Palo Alto, USA), a fraction collector (fraction collector 701, Varian Inc.) and a DAD detector (Pro Star PDA detector 330, Varian Inc.) operated from 200 to 400 nm. As stationary phases following preparative stainless steel columns were used: (i) cyanopropyl (CN) silica (21 * 125 mm, 5 µm Nucleosil 100-5 CN, Macherey & Nagel, Düren, Germany), (ii) nitrophenyl (NO) silica (21 * 250 mm, 5 µm Nucleosil 100-5 NO_2, Macherey & Nagel), and (iii) porous graphitized carbon (PGC, Hypersil PGC, 10 * 50 mm, 7 µm, ThermoFisher Scientific Inc., Waltham, USA).

For the elution of the sample the following solvents and mixtures were used: (i) hexane:dichloromethane (95:5 v/v), 100 % dichloromethane, 100 % acetonitrile to elute CN phase, (ii) hexane:dichloromethane (95:5 v/v) and 100 % hexane to elute NO phase and (iii) hexane:toluene (60:40 v/v) and 100 % toluene for the elution of PGC column.

6.2.5 GC-MS measurement

Analysis was performed applying a gas chromatograph (HP 6890 Series GC system, Agilent Technologies, Waldbronn, Germany) equipped with a split/splitless injector and autosampler (HP 7682 Series Auto sampler, Agilent Technologies). Separation was carried out on a capillary column (HP-5MS, 30 m * 0.25 mm I.D., 0.25 µm film thickness, Agilent Technologies) after a splitless injection at 250 °C with a constant flow of 1.2 mL/min using helium as carrier gas. After separation, the sample was analysed using a mass selective detector (HP 5973 Mass Selective Detector, Agilent Technologies) with an electron impact energy of 70 eV and a source temperature of 230 °C. The transfer line was held at 280 °C.

The samples were firstly measured in the Total Ion Current (TIC) mode to record complete mass spectra of the compounds contained. To identify the compounds, the retention time and the mass spectra were compared to that of the standard compound.

Quantification was performed on the basis of an external calibration providing a dilution series of at least five concentrations. The GC-MS was operated in the selected ion mode (SIM) on the basis of the molecular ion of the respective substances.

6.3 Biological analysis

Synchronous cultures of the unicellular green algae *Scenedesmus vacuolatus* Shih et Krauss (strain 211/15, culture collection Pringsheim, Göttingen, Germany) were used as test system. Synchronisation was achieved by a light dark change of 14:10 h and a periodical dilution to a standard cell density before the onset of the light phase. The use of uniform test organisms provides a maximal sensitivity of the test system.

The inhibition of the population growth during one generation cycle lasting 24 h served as endpoint. The data analysis was performed on the basis of chlorophyll *a* fluorescence measurement at test begin (t_0) and after 24 h (t_{24h}) (Backscat Fluorometer, Haardt, Kiel, Germany). The inhibition of population growth is expressed as the fraction of the fluorescence increase in the samples and the control cultures according to Eq. 6-2. Samples without algae were measured as well to correct for the background fluorescence of the tested samples.

$$inhibition = 1 - \frac{PG^{sample}}{PG^{control}}$$

Eq. 6-2

whereas PG means the population growth of the sample and the control, respectively. PG is calculated according to Eq. 6-3 on the basis of the fluorescence values at the test beginning (f_{t0}) and after one reproduction cycle at 24 h (f_{24h}).

$$PG = \frac{f_{24h}}{f_{t0}}$$

Eq. 6-3

The culture was grown photoautotrophically at 28 ± 0.5 °C in a medium according to Grimme & Boardmann [133], modified by Faust et al. [134]. Test organisms were illuminated using a combination of two types of white fluorescent tubes (Osram L36W/41, Osram L36W/11 Daylight, Osram, München, Germany). The photon flux density in the test vessels accounted for 300 to 380 µmol photons/(m^2·s) (LI-250 Light Meter, Heinz Walz GmbH, Effeltrich, Germany). Carbon supply was provided by the addition of an aqueous solution of $NaHCO_3$ with a final concentration of 1.45 mmol/L. Glass tubes with a nominal volume of approximately 10 mL and capped with a gas tight screw cap served as test vessels. Teflon coated magnetic stirrer bars, stirring every 30 s for 30 s with 200 rounds per minute, prevented precipitation of the cells over the test duration. The fluorescence at test beginning should range between 0.3 and 0.5. The pH in the test system accounted for 6.8 ± 0.2 and was randomly checked.

Samples were provided at t_0 dissolved in dimethyl sulphoxide (DMSO) acting as a co-solvent. DMSO did not exceed a final concentration of 0.1 % in the test system since this concentration proved to cause no measurable effects. Geometrical dilution series were prepared having at least between 4 data points between 10 % and the maximal observed inhibition.

Experimental data of the growth inhibition of *Scenedesmus vacuolatus* was fitted using a three parameter Hill function (Eq. 6-4), which assumes a logistic distribution of the data.

$$E = \frac{a}{1 + \left(\frac{x_{50}}{c}\right)^p}$$

Eq. 6-4

E denotes the fractional effect ($0 \leq E \leq 1$) and c the concentration (g SEq/L(medium)). The parameters of the models (a, x_{50}, p) were estimated using

SigmaPlot 10.0 software (Systat Software Inc., San José, CA, USA). The fitting was based on the Marquardt-Levenberg algorithm.

To characterize concentration-response relationships, the effective concentration responsible for an inhibition of 30 % (EC_{30}) was calculated. Therefore, Eq. 6-4 was rearranged to obtain the concentration c setting the effect E to 0.3 and inserting the estimated parameters a, x_{50}, p of the Hill function.

7 Summary

On behalf of the Water Framework Directive (WFD) the chemical and ecological status of European water bodies should be improved until 2015 requiring the assignment of chemical stressors to observed effects. This comprises the identification of toxicants present and their contribution to observed effects. That is what is normally done in an Effect-Directed Analysis (EDA). But total concentrations, as basis of most EDA studies, are a poor predictor of hazard and risk since a certain fraction of sediment contaminants is bound at the sediment and thus not available for the uptake into organisms. A large volume method is needed to selectively extract the bioaccessible fraction of sediment contaminants to enable further investigations such as fractionation, biotesting and chemical analysis. That method poses the possibility to introduce the concept of bioaccessibility into an EDA study. In some cases it might become necessary to store sediment requiring specific conditions to avoid degradation processes and changes in the bioaccessibility.

The first chapter aimed at the identification of major algal toxicants at selected hot spots in the river Elbe basin through fractionation, biotesting and chemical analysis of sediment extracts. Polycyclic aromatic hydrocarbons, heterocycles containing oxygen and sulphur as well as *N*-phenyl-2-naphthylamine and 7H-benzo[*de*]anthracen-7-one were identified as major algal toxicants. The last step of an EDA comprises the confirmation of the identified compounds and was based on artificial mixtures and ICQ values. The interpretation and evaluation of the ICQ values was based the overlap of 95 % confidence intervals of related concentration-response curves an eases the decision from which distance deviations gain in importance. This resulted in an ICQ range of 0.5 to 2 where confidence intervals of the original fraction overlap with that of the artificial mixture for most samples.

The TENAX® extraction method is commonly used to trap the bioaccessible fraction of sediment-associated contaminants. Chapter 3 deals with the question whether the existing method can be enlarged to extract sufficient amounts of sediment for a subsequent EDA. The time was calculated when the rapidly desorbing fraction was extracted and applied to a single extraction procedure. But the selected extraction time can only be a compromise between the complete extraction of the rapidly desorbing fraction of highly lipophilic compounds and a limited overestimation of less hydrophobic compounds. An extraction apparatus and a corresponding protocol were developed allowing the extraction of larger amounts of sediment. The transfer of the small volume approach

conditions to the large volume extraction procedure was shown to be successful on the basis of the good agreement between extraction properties of these two approaches. In the context of an EDA, blanks have to be checked for background toxicity to avoid false-positive results.

Approaches that focus on the investigation of fresh sediment such as a bioaccessibility-directed extraction or sediment contact tests are faced with the problem that sediments might alter with the storage time due to biological or chemical degradation processes. Having freeze-drying as one possibility for sediment conservation, this method is suspected to change the bioaccessibility of sediment contaminants. The fourth chapter aimed at the investigation of freeze-dried sediment with respect to the desorption behaviour of sediment-associated contaminants as determined with TENAX® extraction method. The fraction extracted from freeze-dried sediment was related to that of fresh sediment. Desorption was shown to be enhanced as well as hindered compared to the desorption from fresh sediments. As the deviations decrease with increasing extraction time the alterations are assumed to be caused by changes in the desorption rate rather than a change of the bioaccessible fraction itself. If the extraction time is selected sufficiently long, freeze-dried sediments can be used for bioaccessibility-directed investigations.

The total concentration of sediment contaminants was shown to be a poor predictor of risk since no correlation between the total concentration and observed effects exists. Chapter 5 aimed at the investigation whether the introduction of the concept of bioaccessibility into an EDA substantially influences the prioritization of contaminated sites and key toxicants. Three sediment samples were bioaccessibility-directed extracted with the large volume TENAX® method and subjected to an EDA consisting of a chromatographically fractionation, biotesting with green algae *Scenedesmus vacuolatus* as test organism and a chemical analysis of the effective fractions to provide information about the identity and quantity of toxicants. A prioritization of sampling locations according to posed risk was possible since the bioaccessibility of toxicants was significantly different for the selected hot spots. Similar total concentration at two sampling locations results in bioaccessible concentrations that significantly deviate. No pronounced changes in the prioritization of fractions due to different extraction methods have been found.

In conclusion, a large volume extraction method was developed to introduce the concept of bioaccessibility into an Effect-Directed Analysis of sediments. The risk assessment on the basis of a bioaccessibility-directed extraction yielded a prioritization of

sampling locations according to hazard rather than to total concentrations. Sediment can be freeze-dried to avoid degradation processes, but with respect to a bioaccessibility-directed extraction, an adaptation of the extraction conditions, such as the extraction time, might become necessary.

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9 Appendix

Used solvents and chemicals:

	MW[(a)]	supplier	purity	CAS
acetone		Merck		67-64-1
acetonitrile		Merck		75-05-8
dichloromethane		Merck		75-09-2
hexane		Merck		110-54-3
methanol		Merck		67-56-1
toluene		Merck		108-88-3
DMSO		Merck		67-68-5
fluoranthene	202	Aldrich	99 %	206-44-0
pyrene	202	Fluka	99 %	129-00-0
benzo[*b*]naphtho[1,2-*d*]furan	218	Chiron		239-50-5
benzo[*b*]naphtho[2,3-*d*]furan	218	Chiron		243-42-5
11H-benzo[*b*]fluorene	216	Chiron		243-17-4
benzo[*ghi*]fluoranthene	226	BCR ref.mat 139		203-12-3
benzo[*b*]naphtho[1,2-*d*]thiophen	234	Promochem	99.8 %	205-43-6
benzo[*b*]naphtho[2,3-*d*]thiophen	234	Promochem	99.2 %	243-46-9
benzo[*a*]anthracene	228	Fluka	98 %	56-55-3
chrysene	228	Riedel-de Haen	97 %	218-01-9
benzo[*b*]fluoranthene	252	Promochem	99.9 %	205-99-2
benzo[*j*]fluoranthene	252	BCR ref.mat 49		205-83-3
benzo[*k*]fluoranthene	252	Fluka	99 %	207-08-9
benzo[*a*]fluoranthene	252	BCR ref.mat 97		192-97-2
benzo[*e*]pyrene	252	Aldrich	99 %	50-32-8
benzo[*a*]pyrene	252	Riedel-de Haen		198-55-0
perylene	252	Fluka	99 %	193-43-1
indeno[1,2,3-*cd*]fluoranthene	276	Promochem		193-39-5
indeno[1,2,3-*cd*]pyrene	276	BCR ref.mat. No 53		53-70-3
dibenzo[*ah*]anthracene	278	Fluka	98 %	191-24-2
***N*-phenyl-2-naphthylamine**	219	Aldrich		135-88-6
7H-benzo[*de*]anthracen-7-one	230	Fluka		82-05-3

(a) molecular weight

Lebenslauf

Persönliche Angaben

Geburtsdatum:	3. Oktober 1980
Geburtsort:	Markranstädt

Schulbildung

1987 – 1992	86. Polytechnische Oberschule Leipzig
1992 – 1999	Georg-Christoph-Lichtenberg Schule/Gymnasium der Stadt Leipzig
1999	Allgemeine Hochschulreife (Note: 1,6)

Universität

1999 – 2004	Chemiestudium an der Universität Leipzig
2003/ 2004	Diplomarbeit im Arbeitskreis Bioanalytische Ökotoxikologie am Helmholtz-Zentrum für Umweltforschung Leipzig-Halle (UFZ)
2004	Studienabschluss (Note: sehr gut)

Promotion

2004 – 2008	Doktorandin im Department Wirkungsorientierte Analytik am Helmholtz-Zentrum für Umweltforschung Leipzig-Halle (UFZ)

Veröffentlichungen

Schwab K, Brack W. 2007. Large volume TENAX® extraction of the bioaccessible fraction of sediment-associated organic compounds for a subsequent effect-directed analysis. *J Soil & Sediments* 7: 178-186.

Präsentationen und Poster

Präsentationen

Schwab K, Brack W. Toxizitätsidentifikation in kontaminiertem Sediment basierend auf bioverfügbarkeitsorientierter Extraktion mit TENAX®. 2006. SETAC GLB

Poster

Schwab K, Brack W, Altenburger R. Effect analysis of *N*-phenyl-2-naphthylamine and structurally similar compounds in unicellular algae –employing mixture toxicity analysis. 2005. SETAC Europe

Schwab K, Brack W. Toxicity assessment and identification in contaminated sediments based on bioavailability-directed extraction with TENAX®. 2006. SETAC Europe

Schwab K, Streck G, Lübcke-von Varel U, Altenburger R, Brack W. Toxicity pattern of key toxicants in an Effect-Directed Analysis using TENAX® method to extract sediment- comparison to results of total extracts. 2007. SETAC Europe

Schwab K, Seiler TB, Streck G, Schulze T, Hollert H, Brack W. Comparison of different membrane based extraction and clean up techniques for risk assessment of contaminated sediments. 2007. SETAC Europe

Erklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig und ohne fremde Hilfe verfasst habe. Ich habe ausschließlich die angegebenen Quellen und Hilfsmittel benutzt und die den Werken wörtlich und inhaltlich entnommenen Stellen als solche kenntlich gemacht. Ich versichere, dass ich die Arbeit erstmalig und nur an der MLU eingereicht habe.

Leipzig, 15. Juli 2008